Gadofullerenes and Gadonanotubes: A New Paradigm for High-Performance Magnetic Resonance Imaging Contrast Agent Probes

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In this review, the physicochemical properties and biomedical applications of Gd@C₆₀ (gadofullerenes) and Gd@Ultra-short Single-walled carbon nanotubes (gadonanotubes) are discussed, especially in regard to the unique benefits of this novel class of materials for Magnetic Resonance Imaging (MRI). The introduction of carbon nanotechnology into biomaterial science has created great opportunity for improving medical diagnostics and therapeutics. In this regard, derivatized gadofullerenes and gadonanotubes offer a new nanoscale paradigm for the design of high-performance MRI contrast agent (CA) probes with efficacies up to 100 times greater than current clinical CAs. Studies show that such CAs are well suited for both passive (magnetic labels for cells) and active (pH-sensitive probes) MRI-based molecular imaging. This capability might lead, for example, to the detection of molecular components or processes that are at the cause of disease. The various important physicochemical factors or parameters discussed in this work provide valuable insight into the development of not only other carbon nanostructure-based MRI contrast agents, but for any fullerene-based biomedical application.

Keywords: Gd@C₆₀, Gadofullerenes, Single-Walled Carbon Nanotube, US-Tubes, Gadonanotubes, Molecular Imaging, NMRD, Contrast Agents, Magnetic Resonance Imaging (MRI), Biomedical Applications.

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1. INTRODUCTION TO MAGNETIC RESONANCE IMAGING (MRI)

Contrast agents (CAs) play a prominent role for magnetic resonance imaging (MRI) in medicine. This chapterreviews and projects two new Gd@{(carbon nanostructures)}, gadofullerenes and gadonanotubes, as nanoscale paradigms for the design of high-performance MRI CA probes with efficacies up to 100 times greater than current clinical CAs. Figures 1(a and b) display schematic representations of the basic gadofullerene (Gd@C₆₀) and short- ened gadonanotube (Gd@US-tube) structures. For both structures, MRI-active Gd³⁺ ions are contained within highly-ordered carbon atom networks approximately one nanometer in diameter.

The last three decades have seen the evolution of MRI into one of the most powerful and central techniques in diagnostic medicine and biomedical research.¹ MRI is one of the most non-invasive modes of examination, and it is often superior to other medical imaging techniques. Primarily, MR images produce 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 use of MRI scanners is growing...
at an explosive rate. Since 1997, the number of scanners worldwide has more than doubled to 22,000.

MRI is an extension of nuclear magnetic resonance (NMR) spectroscopy used extensively in chemistry, and it involves the manipulation of hydrogen atoms (i.e., protons) in the human body. All protons have a property called spin. These nuclear “spins” are like magnetic dipoles, and without an external magnetic field, the orientation of these spins is random. For a single proton (1H) spin system with a nuclear spin number $\frac{1}{2}$, when an external magnetic field is applied, two different spin states arise: a low-energy spin state (the ground state with the spin aligned with the magnetic field) and a quantized high-energy spin state, with the spin aligned against the magnetic field state (the excited state). For a large number of protons the number of spins in each state will be determined by their Boltzmann distribution. A larger number of 1H spins in the ground state aligned with a magnetic field, $B_0$, results in a net magnetization (Fig. 2(a)). These proton spins precess about the applied magnetic field, $B_0$, that is along the z-axis (Fig. 2(b)). Inside a clinical MRI instrument, the patient lies along the direction of the z-axis, also known as the longitudinal axis, while the direction perpendicular to the magnetic field (x, y) is known as the transverse axis (Fig. 2(c)). The frequency ($\omega$) of the precession about the z-axis, known as the Larmor frequency, is proportional to the applied field and is given by $\omega = \gamma B_0$ ($\gamma$ is the gyromagnetic ratio which is a characteristic of each different NMR-active isotope of an element). An applied RF pulse at the Larmor frequency excites the proton spin state from the low-energy ground state to the higher-energy excited state. The time required for the excited-state spin to return to its equilibrium state is known as the relaxation time. This relaxation time has two components: a longitudinal relaxation time $T_1$ (along the applied magnetic field) and a transverse relaxation time $T_2$ (perpendicular to the applied

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**Fig. 1.** Depiction of (a) a gadofullerene (Gd@C$_{60}$) and (b) an ultra-short gadonanotube (Gd@US-tube)

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Lon J. Wilson has been a Professor of Chemistry at Rice University for over 30 years. He has held NSF and NIH-sponsored fellowships and has published over 150 manuscripts and book chapters. Professor Wilson’s research program involves bringing carbon nanotechnology to the fields of biology and medicine. The nanoparticle “building blocks” of this program are fullerenes (C$_{60}$), endohedral metallofullerenes (M@C$_{60}$), and ultra-short (20 nm long) single-walled carbon nanotube capsules (US-tubes). Externally, these carbon nanostructures are being chemically derivatized to make them biocompatible and cell-specific through peptide and antibody targeting. Internally, the nanostructures are being loaded with materials of medical interest for diagnostic and therapeutic medicine. Materials of interest include Fe$_3$O$_4$ and Gd$^{3+}$ ions for MRI, I$_{131}$ for X-ray CT and alpha-particle radionuclides (At-211) for alpha-radiotherapy against cancer. Nanoengineered materials promise great advances in medicine, and, working with colleagues at various medical centers, Wilson’s goal is to bring key, high-performance materials to the clinic as soon as possible.
magnetic field. Precessing spins cause a change in flux (F) in a transverse receiving coil. This flux change induces a voltage across the coil which produces an MR signal. In MRI, the intensity of this MR signal depends on three important intrinsic tissue factors: the proton density, $T_1$, and $T_2$. Thus, various mathematical techniques have been developed to highlight the differences in $T_1$ or $T_2$ to obtain good contrast, i.e., 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 the body.

2. MRI CONTRAST AGENTS

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. These include 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 sixty million MRI procedures are performed worldwide, with 30% of these procedures using MRI CAs.

CAs used in clinical MRI procedures operate by changing the proton nuclear spin relaxation times of water molecules in their vicinity.2–4 Thus, the most commonly used clinical CAs decrease $T_1$ relaxation times of water protons in living tissue in the vicinity of the paramagnetic CA. $T_1$ relaxation is sometimes referred to as spin-lattice relaxation. 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 mM$^{-1}$ sec$^{-1}$.

The high-spin paramagnetic Gd$^{3+}$ metal ion is the most effective $T_1$ relaxation agent for the following reasons:

1. It has seven unpaired f-electrons, the greatest number of unpaired electrons exhibited by any atom or ion. The water proton relaxation rate is directly proportional to the electron-spin quantum number of the CA. Thus, the more unpaired electrons, the larger the spin quantum number and the greater the (proton spin)-(electron spin) interaction.

2. It has a large magnetic moment ($\mu_1 = 63\mu_B$, where $\mu_B$ is the Bohr Magneton). The water proton relaxation rate is directly proportional to the square of the magnetic moment of a paramagnetic CA.

3. It has a highly-symmetrical, slowly-relaxing ground state ($^7S$-state). A slow electronic relaxation rate makes the Gd$^{3+}$ ion compatible with necessary proton-relaxation frequencies. Frequency components of the fluctuating local magnetic field close to the proton larmor frequency are...
3. THE RATIONAL FOR DEVELOPING GADOFULLERENE- AND GADONANOTUBE-BASED MRI CONTRAST AGENTS

Gadofullerenes and gadonanotubes offer some fundamental advantages over conventional chelate-based MRI CAs such as [Gd(DTPA)(H$_2$O)$_2$]$^{3-}$ (Fig. 3) that include the following considerations:

1. The Gd$^{3+}$ ion is trapped within a biologically-stable fullerene cage (Fig. 1(a)) such that the toxic, free Gd$^{3+}$ ion is never released in vivo.$^1$ In contrast, even the best-designed chelates (like DTPA)$^{−}$ in Fig. 3) are susceptible to some thermodynamic instability and metabolic-process-induced metal-ion release.$^6$ This clear advantage for Gd@C$_{60}$ and other gadofullerene structures is especially important for CAs with longer retention times in vivo such as those for blood-pool imaging.

2. The external carbon sheath of the gadofullerenes and gadonanotubes (Fig. 1(b)) can be chemically modified with medically-useful materials (peptides, drugs, antibodies, etc.) without compromising the paramagnetic function of the internal Gd$^{3+}$ ion(s). These chemical modifications can be used to control toxicity and to direct or target the CAs to specific desired cells or tissues.

3. Gadofullerenes and gadonanotube species have recently been shown to exhibit $T_1$ relaxities up to 100 times greater than those of present CAs in clinical use. Thus, these new nanoscale materials are the highest-performing MRI CA materials known, which may allow their use as new MR molecular-imaging probes.

4. Finally, empty fullerene, gadofullerenes and single-walled carbon nanotube as well as gadofullerenes materials have been shown to translocate across cell membranes and to accumulate within cells without significant toxicity.$^{8-15}$ The gadonanotube materials can be expected to do likewise, potentially becoming a systematic family of intracellular MRI CAs. Once targeted to desired cells with appropriate targeting agents (peptides, antibodies, etc.), and internalized within the cells, intracellular CAs may concentrate sufficiently within cells to enable high-resolution molecular imaging.

3.1. Gadofullerenes

Gd$^{3+}$-based metallofullerenes constitute a promising new class of paramagnetic MRI CAs.$^{16-19}$ Polyhydroxyl derivatives of gadofullerenes (Gd@C$_{60}$) were the first to be proposed as potential MRI contrast agents by several groups. The published longitudinal proton relaxivities for these gadofullerenols are remarkably high, while showing a large variation. For example, Wilson et al. found $r_1 = 20$ mM$^{-1}$ s$^{-1}$ (0.47 T, 40 °C)$^{20}$ and Shinohara et al. measured a particularly high longitudinal proton relaxivity for Gd@C$_{60}$OH$_{80}$ (81 mM$^{-1}$ s$^{-1}$ at 25 °C, B = 1.0 T).$^{16}$ Endohedral metallofullerenols with a series of lanthanide metal ions have also been reported for M@C$_{60}$OH$_{n}$ with M$^{3+}$ = La, Ce, Gd, Dy and Er.$^{17}$ The metallofullerenols containing low-magnetic-moment ions (La$^{3+}$ and Ce$^{3+}$) were shown to have very small proton relaxivities (0.8 mM$^{-1}$ s$^{-1}$ @ 20 MHz/20 °C). Those containing high-magnetic-moment ions, such as Gd$^{3+}$, Dy$^{3+}$ and Er$^{3+}$, exhibited higher proton relaxivities, with Gd@C$_{60}$OH$_{80}$ being the most efficient relaxation agent by far, with both longitudinal and transverse proton relaxivities significantly higher than for their corresponding [M(DTPA)]$^{2-}$ metal chelate compounds. However, a complete physicochemical evaluation of the relaxation mechanism of Gd@C$_{60}$OH$_{80}$...
using the same experimental techniques that are usually applied to traditional Gd\(^{3+}\) chelate CAs, were not performed in the study. Such evaluation studies have been limited by the quantities of gadofullerenes required for the battery of necessary experiments. M@C\(_{82}\) belongs to a minority class of endohedral metallofullerenes generated by the carbon-arc process.31,32 Its separation requires labor-intensive and expensive multi-step HPLC purification,22 a situation that hampered metallofullerene-based research for many years.

In contrast to Gd@C\(_{82}\), Gd@C\(_{60}\) belongs to the most abundantly-produced class of endohedral metallofullerenes generated by the carbon-arc process. Previously, the more abundant Gd@C\(_{60}\) class of endohedrals (comprised mostly of Gd@C\(_{59}\) and Gd@C\(_{60}\)) and Gd@C\(_{70}\), and hereafter designated as “Gd@C\(_{60}\)” were thought to be unusable in practical applications due to their insolubility and air sensitivity. However, recent breakthroughs in derivatization, solubilization and stabilization of these Gd@C\(_{60}\) species have overcome these limitations,33–35 and gram quantities of these materials are now readily available. As a result, water-soluble Gd@C\(_{60}\) species have opened new opportunities for extensive in vitro and in vivo studies of gadofullerenes. The Gd,N@C\(_{60}\) metallofullerenes14 are another high-yielding species of gadofullerene, but its development as a potential MRI contrast agent has seemingly been hampered by its relative lack of chemical reactivity.

Figures 4(a and b) depict the structures of two Gd@C\(_{60}\)-based CAs, Gd@C\(_{60}\)(OH), and Gd@C\(_{60}\)(C(COOH))\(_{10}\), respectively. These water-soluble gadofullerene derivatives exhibit exceptionally large proton relaxivities ranging from 20 mM\(^{-1}\) s\(^{-1}\) to 100 mM\(^{-1}\) s\(^{-1}\) or approximately 5–20 times larger than clinical agents such as [Gd(DTPA)]\(^{2-}\) (Fig. 3) with \(r_1 \sim 4\) mM\(^{-1}\) s\(^{-1}\).

3.2. Factors Influencing Gadofullerene Relaxivity and Their Characterization

Proton relaxivities are influenced by a number of physical chemical factors. There are two main contributions to the paramagnetic relaxation rate enhancement (PRE) and hence the overall proton relaxivity. The first of these is the inner-sphere contribution where the interaction between the Gd\(^{3+}\) electron spins and the water protons in the first coordination sphere of the metal ion are transmitted to the paramagnetic relaxation rate enhancement (PRE) and the rotational correlation time, and the relaxation rate of the Gd\(^{3+}\) electron spin. The relationship between PRE and the various macroscopic properties is described by theories developed by Solomon, Bloembergen, Morgan and others.23–29

To explain the high proton relaxivities measured in aqueous solution for the gadofullerene derivatives, the large number of water molecules on or around the fullerene surface has been generally invoked as being important.22 In such a relaxation process, the gadofullerene might simultaneously relax the protons of many hydrogen-bonded water molecules on the ca. 200 Å\(^2\) paramagnetic metallofullerene surface. The efficient proton exchange of these water molecules with bulk water molecules could then transfer the paramagnetic effect of the gadofullerene to bulk water. Longitudinal and transverse \(^{17}\)O relaxation rates and chemical shifts at variable temperature and multiple magnetic fields have supported an “outer sphere” relaxation mechanism.30 This is indeed, expected for water molecules not directly bonded to the paramagnetic Gd\(^{3+}\) center. However, this effect is 10 times larger than that observed for Gd\(^{3+}\) chelate compounds without inner-sphere water molecules such as [Gd(TETA)]\(^{3+}\) where \(H_4\)TETA = 1,4,8,11-tetrazacyclotetradecane-1,4,8,11-tetraacetic acid.

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. Figure 5 show...
the NMRD profiles (B = 0.01 to 400 MHz) for aqueous solutions of Gd@C_{60}[C(COOH)_{2}]_{10} and Gd@C_{60}(OH)_{3}. For both Gd@C_{60}(OH)_{3} and Gd@C_{60}[C(COOH)_{2}]_{10} at all temperatures, the NMRD profiles show high-field maxima centered around 40 MHz. This profile feature suggests that the relaxivity maxima might arise from an increase in the rotational correlation time, $\tau_{R}$, (on the order of nanosecond), of the CA. An increase in $\tau_{R}$ reflects slower-tumbling CA molecules in solution, potentially from an increase in molecular weight by aggregation. Laser-light scattering (LLS) experiments and cryo-Transmission Electron Microscopy (cryo-TEM) on aqueous solutions of these two Gd@C_{60} CAs corroborated this conclusion. 

The temperature dependence of the proton relaxivities of Gd^{3+}-based CAs gives useful information about the two most important factors that limit proton relaxivity, the proton exchange rate and the molecular reorientation rate, which have opposite temperature dependencies. Surprisingly, the high-field (>10 MHz) relaxivities of Gd@C_{60}(OH)_{3} are independent of the temperature over the large temperature range studied (278–335 K), whereas they are somewhat temperature dependent at lower frequencies. Similarly, a temperature-independent proton relaxivity was also reported for Gd@C_{60}(OH)_{3} at 200 MHz between 288 and 328 K. In contrast, the relaxivities for Gd@C_{60}[C(COOH)_{2}]_{10} decrease with increasing temperature at all fields, clearly indicating that slow proton exchange is not relaxivity limiting in this case. 

The proton relaxivities also display a remarkable pH-dependency, increasing dramatically with decreasing pH. Figure 6(a) shows the proton relaxivities measured at 60 MHz for Gd@C_{60}(OH)_{3} and Gd@C_{60}[C(COOH)_{2}]_{10} as a function of the pH over a large pH range (pH = 2–12). For both Gd@C_{60} derivatives, the relaxivities increase considerably with decreasing pH (by a factor of 2.6 for Gd@C_{60}(OH)_{3} and 3.8 for Gd@C_{60}[C(COOH)_{2}]_{10}) until pH ≈ 3 where precipitation initiates. The pH might influence either the proton exchange rate or the molecular reorientation (rotation) rate, the two parameters that can limit proton relaxivity. For both Gd@C_{60}(OH)_{3} and Gd@C_{60}[C(COOH)_{2}]_{10}, the molecular rotation rate was found to be dominant. The pH highly affects the proton exchange rate by both compounds as a result of the pH-dependent molecular aggregation, since the increased aggregation size at low pH (Fig. 6(b)) undoubtedly leads to slower molecular tumbling. 

In order to comprehensively examine the behavior of Gd@C_{60}[C(COOH)_{2}]_{10} and Gd@C_{60}(OH)_{3} with respect to their CA applications, studies were performed in the presence of phosphate-buffered saline (PBS, pH = 7.4) at 60 MHz in order to mimic physiological conditions in vitro and to assess the influence of PBS on the relaxivities. Surprisingly, the relaxivity decreases strongly in PBS in comparison to salt-free solutions ($r_{1} = 14.8$ vs. $83.2$ mM$^{-1}$ s$^{-1}$ for Gd@C_{60}(OH)_{3}; $r_{1} = 6.4$ vs. $20.6$ mM$^{-1}$ s$^{-1}$ for Gd@C_{60}[C(COOH)_{2}]_{10}). This decrease in relaxivity has been attributed to disaggregation of the metallofullerene aggregates.

Disaggregation of the water-soluble gadofullerenes leads to smaller and more rapidly tumbling entities, which will directly translate into lower relaxivities. On increasing PBS concentration, the relaxivity, indeed, decreases dramatically as shown in Figure 7(a). Figure 7(b) shows the variation in hydrodynamic diameter of Gd@C_{60}(OH)_{3} and Gd@C_{60}[C(COOH)_{2}]_{10} in aqueous solutions at variable NaCl concentrations and at a 10 mM phosphate-buffer concentration. The plot clearly demonstrates that variation in ionic strength leads to aggregate disruption. The plot further shows that a 10 mM phosphate solution is much more effective at disaggregation than is a 150 mM sodium chloride solution. Thus, disaggregation is not exclusively related to ionic strength. Although the disaggregation mechanism remains unclear, the specific effects of phosphate might be related to the intercalation of $HPO_4^{2-}$ and $HPO_3^{2-}$ ions (pH 7.4) into the hydrogen-bonding network around the malonate or hydroxyl groups of the gadofullerenes. Phosphate has a tendency to create

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For a single non-aggregated Gd@C_{60} molecule, $D_h \sim 1.0$ nm.
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Fig. 7. (a) 1H relaxivity of Gd@C_{60}(OH)_{x} at variable PBS concentration; pH 7.4, 37 °C and 60 MHz (c_{Gd} = 0.5 mM). The curved line is to guide the eye. (b) Hydrodynamic diameters of (1) Gd@C_{60}(OH)_{x} (c_{Gd} = 0.5 mM) and (2) Gd@C_{60}[C(COOH)_{2}]_{10} (c_{Gd} = 0.4 mM) in aqueous solution at variable NaCl concentrations and at a 10 mM phosphate buffer (PB) concentration; pH 7.4, 37 °C.

Strong hydrogen bonds. If the metallofullerene aggregation is mainly due to hydrophobic forces, the intercalation of phosphate ions may help separate the molecules sufficiently to inhibit such interactions.

The disaggregation half-lives have been estimated as 30 and 45 min for Gd@C_{60}(OH)_{x} in PBS and serum, respectively, and as 25 min for Gd@C_{60}[C(COOH)_{2}]_{10} (in both media). These long half-lives imply that a gadofullerene CA injected into the blood stream will be mainly present in an aggregated (high relaxivity) state during the time of a typical MRI examination, since the phosphate ion concentration in human blood plasma is only 0.38 mM or much lower than that used in the above study.

Also, the different synthetic methods used to prepare the gadofullerenol samples in the literature likely involve various amounts of salts which may remain in the sample even after purification. This could greatly influence the observed relaxivities and explain the diversity of $r_1$ values reported in the literature. The above disaggregation studies have important implications not only for the development of gadofullerene-based MRI CAs, but for any biomedical application of a fullerene or metallofullerene derivative since phosphate buffers (widely used for in vitro tests to mimic biological conditions) will influence the degree of fullerene aggregation, in general. Moreover, all real biological fluids contain some salt which can modify the behavior of fullerene aggregation.

3.3. Gadonanotubes

Single-walled carbon nanotubes (SWNTs) possess unique characteristics that make them desirable for biomedical applications. The ideal length for medical applications is uncertain, but ultra-short nanotubes (20–100 nm) or US-tubes 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 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.

Recently the successful nanoscale loading and confinement of aquated Gd^{3+}-ion clusters within ultra-short single-walled carbon nanotubes (US-tubes) (Figs. 8(a, b and c)) was reported. These Gd^{3+}@US-tube species would likely be used to create a new class of MRI contrast agents that offer advantages over existing materials.


Fig. 8. (a) Depiction of a single US-tube loaded with hydrated Gd^{3+} ions. Gd^{3+}-ion loading is likely through side-wall defects created by cutting full-length nanotubes to produce US-tubes (not to scale and Cl\textsuperscript{-} anions and atoms attached to dangling C bonds not shown). (b) HRTEM image of bundled Gd^{3+}@US-tubes showing the 2–5 nm Gd^{3+} clusters (arrows) formed within US-tubes as confirmed by EDS measurements. (c) Cryo-TEM image of Gd^{3+}@US-tubes from a 1% SDBS surfactant solution. Dark spots are the internally-loaded superparamagnetic Gd^{3+} clusters.
are linear superparamagnetic molecular magnets with Magnetic Resonance Imaging (MRI) efficacies 40 to 90 times larger than any current Gd\(^{3+}\)-based CA in clinical use. As such, gadonanotubes, with their embedded 2–5 nm superparamagnetic Gd\(^{12+}\)-ion clusters, demonstrate potential as radically new synthons for the development of high-performance MRI CAs.

Relaxation rate measurements and resulting relaxivities for Gd\(^{12+}\)@US-tube samples are given in Table I. It is seen from the table that the Gd\(^{12+}\)@US-tube samples significantly enhance the relaxation rates relative to pure surfactant solution or unladen US-tubes. Comparing the relaxivity values of the Gd\(^{12+}\)@US-tube sample with [Gd(H\(_2\)O)]\(^{3+}\), it is interesting to note that \(r_1\) of aquated Gd\(^{3+}\) is 20 times lower at 60 MHz/40 °C than for the Gd\(^{12+}\)@US-tube sample. Thus, the relaxivity obtained for the Gd\(^{12+}\)@US-tube sample of \(r_1 \sim 170 \text{ mM}^{-1} \text{s}^{-1}\) is nearly 40 times greater than any current Gd\(^{3+}\)-based oral or ECF CA. Small variability was observed in the relaxivity values of different batches of Gd\(^{12+}\)@US-tube samples and different surfactants used, but the order of magnitude reported in Table I was always the same (\(r_1 = 159 \text{ mM}^{-1} \text{s}^{-1} \) to 179 \(\text{ mM}^{-1} \text{s}^{-1}\)).

The NMRD profile (B = 5 \(\times\) 10\(^{-4}\) to 9.4 T) for an aqueous solution of a Gd\(^{12+}\)@US-tube sample in 1% SDBS (sodium dodecylbenzene sulphonate, a surfactant employed to suspend the highly lipophilic SWNTs in water) solution at 37 °C is presented in Figure 9. Also presented, for comparative purposes, is data for one of the commercially-available MRI CAs, [Gd(DTPA)(H\(_2\)O)]\(^{3+}\).

For any magnetic field in Figure 9, the relaxivity for the Gd\(^{12+}\)@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 mM\(^{-1} \text{s}^{-1}\) vs. 4.0 mM\(^{-1} \text{s}^{-1}\)), but is even more pronounced (nearly 90 times larger!) at very low fields such as 0.01 MHz (635 mM\(^{-1} \text{s}^{-1}\) vs. 7.0 mM\(^{-1} \text{s}^{-1}\)). In this regard, microtesla MRI imaging technologies\(^{35}\) would especially benefit from low-field, high-efficacy contrast agents derived from gadonanotube synthons. In the case of gadofullerenes, the increase in relaxivity (\(\sim 80 \text{ mM}^{-1} \text{s}^{-1}\)) results mainly from aggregation and the subsequent three-order-of-magnitude increase in \(\tau_\text{e}\), the rotational correlation time.\(^{30,32}\) In the Gd\(^{12+}\)@US-tubes case, however, aggregation is apparently not a contributing factor, since DLS measurements on the NMRD sample solution showed the hydrodynamic diameter of Gd\(^{12+}\)@US-tubes to be 20–80 nm, in good agreement with the Cryo-TEM images of Figure 8(c). Furthermore, the gadolinium centers in Gd\(^{12+}\)@US-tubes have access to water molecules (for Gd\(^{12+}\)-OH\(_2\) bonding), since SWNTs are known to be good transporters of water\(^{46–49}\) and proteins,\(^{11,12}\) whereas the centers in gadofullerenes or other metallofullerenes do not have this access. From a practical point of view, the rate of proton exchange is especially important, since it contributes to the proton relaxivity.\(^{3}\) The present gadonanotubes, with their Gd\(^{12+}\) clusters, are the first gadolinium CA materials where superparamagnetic metal centers have access to many coordinated/exchanging water molecules per Gd\(^{12+}\) ion. This unique situation could underlie the unprecedented large proton relaxivities exhibited by the Gd\(^{12+}\)@US-tubes. Indeed, these large relaxivities argue convincingly for confined, internally-loaded Gd\(^{12+}\)-ion clusters, since a highly-unusual metal-ion environment must be presumed to produce such extreme relaxivities.

In addition to the exceptionally large relaxivity values obtained for the gadonanotubes, the shape of the NMRD curve as shown in Figure 9 is also considerably different from that reported so far for any other Gd\(^{12+}\)-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.

### Table I. Proton relaxivities, \(r_1\) (mM\(^{-1}\) s\(^{-1}\)) of various sample solutions at 60 MHz and 40 °C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>(C_{Gd}/\text{ppm})</th>
<th>(C_{Gd}/\text{mM})</th>
<th>(T_1/\mu\text{s})</th>
<th>(R_1/\text{s}^{-1})</th>
<th>(R_1\text{n}\text{mM}^{-1}\text{s}^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gd(^{12+})@US-tubes in 1% SDBS surfactant solution</td>
<td>7</td>
<td>0.044</td>
<td>127.3</td>
<td>7.85</td>
<td>0.25</td>
</tr>
<tr>
<td>Gd(^{12+})@US-tubes in 1% pluronic F98 surfactant solution</td>
<td>7.8</td>
<td>0.049</td>
<td>120.6</td>
<td>8.29</td>
<td>0.24</td>
</tr>
<tr>
<td>US-tubes in 1% SDBS surfactant solution</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>[Gd(H(_2)O)](^{3+})</td>
<td>313</td>
<td>1.99</td>
<td>59.0</td>
<td>16.95</td>
<td>0.24</td>
</tr>
</tbody>
</table>

**Fig. 9.** NMRD profile measured for Gd\(^{12+}\)@US-tubes in a 1% SDBS solution \((c_{Gd} = 0.044 \text{ mM}, T = 37 \text{ °C})\) (black). For comparative purposes, data for specific CA, [Gd(DTPA)(H\(_2\)O)]\(^{3+}\) (orange), is also shown.
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 Gd³⁺ CAs. This phenomenon is particularly important, given the current tendency to develop MRI scanners of higher and higher fields, where the contrast enhancing effect of traditional contrast agents drops off. Currently, the most efficient T₁ agents show a typical high-field relaxivity peak centered around 30–40 MHz,² characteristic of slow rotation with maximum relaxivities of 40–50 mM⁻¹ s⁻¹. Above this frequency, the relaxivity quickly vanishes to very small values. In this respect, gadonanotubes may represent a significant breakthrough in contrast agent design for high-field imaging.

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 other nanoscalar MRI CA materials, as well.

4. FUTURE APPLICATIONS

4.1. Smart Probes

Smart MRI CAs use some variable (pH, temperature, enzymes) in their immediate environment as a stimulus to generate a signal. The strong pH-dependency of their proton relaxivities make gadofullerenes excellent candidates for pH-responsive MRI CA applications. Although several CAs based on Gd³⁺-chelate compounds have been reported to have pH-dependent relaxivities,⁵²,⁵³ and so far have proved efficient enough for in vivo imaging.

In clinical MRI, there is an increasing demand for pH-responsive contrast agents. The in vivo pH-mapping of tissues could be of importance in future tumor diagnosis since the pH of solid tumors is approximately 0.4 units lower than for healthy tissue. This decrease in pH is attributed to the increased production of lactic acid by tumor cells.⁵⁴–⁵⁶ Due to its high spatial resolution, MRI is particularly well suited to studying pH variations over small tissue volumes, and thus, pH-responsive smart MRI CAs such as the gadofullerenes are of special interest for the characterization of solid tumors.

4.2. Blood-Pool Probes

It has been previously established that non-covalent linkages between paramagnetic Gd³⁺-chelate compounds and a protein can enhance relaxivities.³ The interaction of proteins has been shown to lead to slower molecular rotation and a subsequent relaxivity increase. Since these proteins are confined to the blood pool, most studies have focused on serum albumin, whose concentration in blood is by far the highest of any protein. Two important factors that enhance the binding of a CA to proteins are the hydrophobicity of the CA and the number of negative charges on the CA. Gd@C₆₀⁻ is strongly hydrophobic, even if derivatized extensively with watersolubilizing groups. Gd@C₆₀[COOH]₁₀ also possesses a large number of negative charges at neutral or basic pH. This suggests that aggregated Gd@C₆₀[COOH]₁₀ may show long residency times in the blood pool for non-invasive angiographic MR imaging as a substitute for standard invasive angiographic methods that employ ionizing radiation. Gd³⁺-chelate-based angiographic MRI CAs also are known,⁴ but gadofullerenes are synthetically more flexible for customizing agents with variable blood-pool residency times.

4.3. 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 gadofullerenes and 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). The T₁ relaxivities of the gadofullerenes and gadonanotubes, (Figs. 5 and 9) could provide sufficient signal/noise to image cell-surface receptor sites in the nanomolar range. For example, for a clinical CA with r₁ = 4.0 mM⁻¹ s⁻¹ at clinical field strengths, the minimum detectable concentration of a Gd³⁺ is 10⁻⁷ M. The increase in relaxivity provided by the gadonanotube changes this minimum concentration to 2 nM.

(c) Recently, both fullerenes (such as the neuroprotective fullerene drug, C₆₀)⁹,¹⁰ metallofullerenes (gadofullerenes),¹¹,¹² and SWNTs¹³–¹⁵ have been shown to translocate into the interior of cells with minimal cytotoxicity. Thus, CA probes derived from these materials could also accumulate within targeted cells to further boost MRI signal strength. For example, for the gadonanotubes, we have estimated that each bundled (10 nm × 100 nm) gadonanotube probe with r₁ = 170 mM⁻¹ s⁻¹ per Gd³⁺ at clinical fields contains about a hundred Gd³⁺ ions to give an effective r₁ = 17000 mM⁻¹ s⁻¹ per probe. If only one thousand such probes were to accumulate
within a single cell, the relaxivity of the cell would be 17,000,000 mM\(^{-1}\) s\(^{-1}\) (!) which should easily permit single-cell imaging. Early detection of metastized cancer cells would be one very desirable application of such an intracellular molecular-imaging capability.

### 4.4. Guided-Therapy Probes

The superparamagnetic gadonanotubes also show promise for 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 partial or complete destruction can be accomplished by several methods,\(^{58}\) the least invasive 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 to ferromagnetic materials.\(^{57}\) The limitation of hyperthermia for human treatment is the failure to generate appreciable heat at safe magnetic fields. However, recent results with Ultra-Short Iron Oxide Particles (USPIOs) have shown promise for the first human trials.\(^{58}\) The gadonanotubes may make ideal MRI-delivery capsules for cancer imaging/therapy by hyperthermia due to their nanoscale size, superparamagnetism, external derivatization potential and cellular-translocating abilities. Furthermore, non-spherical particles such as the gadonanotubes are yet to 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.\(^{59}\)

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### References and Notes

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