# Gd@(Carbon Nanostructures) as Nanoprobes for Cellular Magnetic Resonance Imaging

Sitharaman, B [1], Tran LA [1], Bolskar, RD [2], Muthupillai, R [3,4], Flamm, SD [4], Mikos, AG [1], Wilson LJ [1].
[1] Rice University, Houston, TX, USA; [2] TDA Research inc., Wheat Ridge, CO, USA; [3] Philips Medical Systems, Cleveland, OH, USA; [4] Baylor College of Medicine, Houston, TX, USA.

**Introduction:** Carbon nanostructures containing paramagnetic gadolinium offer several advantages as Magnetic resonance (MR) contrast agents (CAs) compared to commercially available $Gd^{3+}$ chelated compounds currently used today.[1, 2] These compounds exhibit unusually large proton relaxivities (efficacies), do not disassociate under physiologic environment, and therefore, may be useful for cellular imaging. Our prior work shows that derivatized $Gd@C_{60}$ metallofullerenes (gadofullerenes) and Gd@US-tubes (gadonanotubes) nanomaterials **(**Fig. 1**)** can serve as high-performance MRI CA probes with efficacies up to 100 times greater than currently used commercial clinical CAs. [1, 2] Recent studies have shown that, both fullerenes as well as

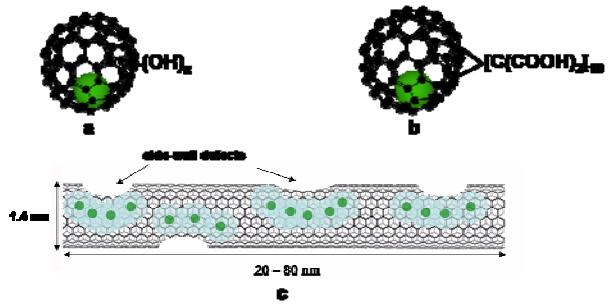


**Figure 1.** Depiction of (a) $Gd@C_{60}(OH)_x$, (b) $Gd@C_{60}[C(COOH)_2]_{10}$ and (c) a single Ultra short tube (US-tube) loaded with hydrated $Gd^{3+}$ ions ($Gd^{3+}{}_n@$US-tubes). In the case of US-tubes $Gd^{3+}$-ion loading is likely through side-wall defects created by cutting full-length nanotubes to produce US-tubes (not to scale and Cl- anions and atoms attached to dangling C bonds not shown).

single-walled carbon nanotubes can translocate into the interior of cells with minimal cytotoxicity, implying a potential application as an intracellular MRI probe.[3-5] In this paper, we report the first MRI phantom studies on these Gd@(Carbon Nanostructures) as a first step to examine their ability as nanoprobes for cellular MRI.

**Methods:** *Samples:* Vials of $GdCl_3$, gadofullerene ($Gd@C_{60}[C(COOH)_2]_{10}$ ), and gadonanotubes $Gd@C_{60}(OH)_x$, at 0.4 mM and 0.04 mM gadolinium concentrations were prepared as has been described previously.[1,2] Additional vials containing pure water, and 1% Sodium dodecyl sulphonate (SDBS) solutions were also included in the imaging section as a reference. *MRI Acquisition:* The samples were imaged on a 1.5T Philips MR imager. A quadrature head coil was used for signal reception. Following an inversion pulse, a series of gradient echoes were obtained at different inversion delay times, to map the regrowth of longitudinal magnetization. The imaging parameters of the segmented gradient echo readout were as follows: repetition time (TR)/echo time (TE)/flip angle: 10 msec/2.3msec/10°; the field-of-view and matrix sizes were chosen to yield an acquired voxel size of : 2 x 2 x 6 $mm^3$, that was reconstructed as a 1 x 1 x 6 $mm^3$ voxels using zero-filled reconstruction. The inversion pulse was repeated every 3580 msec, and the longitudinal recovery was sampled at 28 msec temporal resolution (128 time points). In addition to conventional magnitude reconstruction, phase corrected real images were also reconstructed.

**Results / Discussion:** Representative $T_1$ weighted MRI images of the vials is shown in Fig. 2. At 0.4 mM, and 0.04 mM concentrations, gadonfullerenes and gadonanotubes showed significant enhancements compared to $GdCl_3$ at the same concentrations.

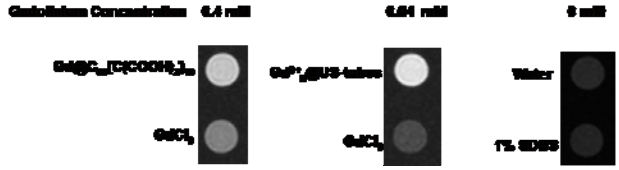

**Figure 2.** $T_1$ weighted MRI phantoms of the (a) $Gd@C_{60}[C(COOH)_2]_{10}$ and (b) $Gd^{3+}{}_n@$US-tubes solutions at 0.4 mM and 0.04 mM gadolinium concentrations, respectively, with a 1.5 T Philips MR imager. For comparative purposes MRI phantoms of pure water and 1% SDBS solutions are also shown.

Thus, accumulation of CA probes derived from these materials within targeted cells could further boost MRI signal strength. For example, we estimate that each bundled (10 nm x 100 nm) gadonanotube probe with relaxivity $r_1 = 170\ mM^{-1}s^{-1}$ per $Gd^{3+}$ at clinical fields contains about a hundred $Gd^{3+}$ ions to give an effective $r_1 = 17{,}000\ mM^{-1}s^{-1}$ per probe. If only one thousand such probes were to accumulate within a single cell, the $r_1$ of the cell would be $17{,}000{,}000\ mM^{-1}s^{-1}$ (!) which should easily permit single-cell imaging. Early detection of metastasized cancer cells would be one very desirable application of such a cellular imaging capability.

**Conclusions:** In conclusion, MRI phantom studies on the Gd@(Carbon Nanostructures) show extremely large signal enhancement with intensities up to 100-150 times larger than pure water at modest concentrations of gadolinium. The ability of the nanoprobes to be internalized by cells could allow the first $Gd^{3+}$-based magnetic labeling of individual cells. This is an ongoing area of research at our laboratory.

**References:**

1.Toth, E. JACS. 2005; 127:799-805.
2. Sitharaman, B. Chem. Commun. 2005;31:3915-3917.
3. Dugan, L. L. PNAS. 1997; 94: 9434 - 9439.
4. Kam, N. W. S. JACS. 2004 126;22: 6850 - 6851.
5. Pantarotto, D. Chem. Commun. 2004; 1 16 - 17.