Graphene- Based MRI Contrast Agents: Synthesis, Characterization and *In vitro* MRI

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SUMMARY:

Carbon nanomaterial-based MRI contrast agents (CAs) are currently being pursued for cellular and molecular MRI. Synthetic strategies in the development of these MRI CAs have mainly focused on encapsulation of the lanthanoid, Gadolinium ion (Gd^{3+}) inside a carbon nanomaterial cage such as carbon nanotubes and fullerenes as well as covalent or non-covalent functionalization[1] of external surface of these carbon nanomaterials with Gd^{3+}- chelate complexes.[2] Recently, another carbon nanomaterial, graphene has shown potential for electronic, optoelectronic and biomedical applications. In this work, we present the synthesis, characterization (structure, magnetism and relaxometry) and *in vitro* MRI of graphene oxide nanoribbon (GONR)-based MRI CAs.

DESCRIPTION:

The GONRs are obtained from multiwalled carbon nanotubes (MWCNTs) by a chemical protocol wherein, the MWCNTs are "unzipped" along their long axis using oxidizing agents- sulfuric acid and potassium permanganate ($KMnO_4$).[3] High Resolution TEM (JEOL JEM2100F Analytical HR-TEM) at 200kV was used to characterize the morphology and nanostructure of the GONRs. Raman spectroscopy (Thermo Scientific, DXR, 780nm laser) was carried out to confirm characteristic spectral peaks of graphene oxide. In order to understand the magnetic nature of the GONRs at room temperature, superconducting quantum interference device(SQUID) magnetometer-based studies were carried out at varying temperatures(10, 150 and 300K) and magnetic fields (-50,000 to 50,000Oe). Nuclear magnetic resonance relaxometry measurements were performed at 0.5T field strength and 21.42MHz. Inductively coupled plasma optical emission spectroscopy (ICPOES) was performed on the samples used for relaxometry measurements to obtain the concentration of manganese for calculation of relaxivities using the equation: $r_{1,2} = (R_{1,2} - R_0)/[Mn^{2+}]$ where $R_{1,2}$ and R_0 are the longitudinal or transverse relaxation rates of the samples and 1% Pluronic F127 surfactant solution respectively. T_1- and T_2- weighted phantom MRI images were obtained using a 3T Trio Siemens MRI for GONR samples with Manganese chloride ($MnCl_2$), a widely used preclinical MRI CA, and water as controls.

RESULTS and DISCUSSION:

HRTEM of the GONR (Fig1) displayed nanoribbons with a width of ~100nm and 0.6 - 2μm length. Raman spectra of the GONR samples exhibited characteristic G ($1591cm^{-1}$) and D ($1313cm^{-1}$) bands (Fig2) confirming graphene oxide.[4] SQUID magnetometer measurements indicated room temperature superparamagnetism (Fig3) with a blocking temperature above 300K and a coercive field of 250Oe at 10K. Single point relaxometry measurements of GONR samples dispersed in 1% Pluronic F 127 solution showed high relaxivities with $r_1 = 45mM^{-1}s^{-1}$ and $r_2 = 234mM^{-1}s^{-1}$ which is nearly 16 times higher for r_1 and 63 times higher for r_2 compared to Mn-DPDP (Teslascan, clinical Mn- based contrast agent, $r_1 = 2.8mM^{-1}s^{-1}$ and $r_2 = 3.7\ mM^{-1}s^{-1}$ in aqueous solutions at 20MHz),[5] respectively. Representative T_1- (Fig4) and T_2-weighted MRI phantom images of GONR solutions showed significant signal difference when compared to $MnCl_2$ ($r_1 = 9.2mM^{-1}s^{-1}$; $r_2 = 93mM^{-1}s^{-1}$) and water (Table 1). Our results taken together suggest that the presence of Mn^{2+} ions in GONR, and the inherent room temperature magnetic properties of graphene are responsible for high r_1 and r_2 relaxivities and thus, the enhanced MRI contrast.

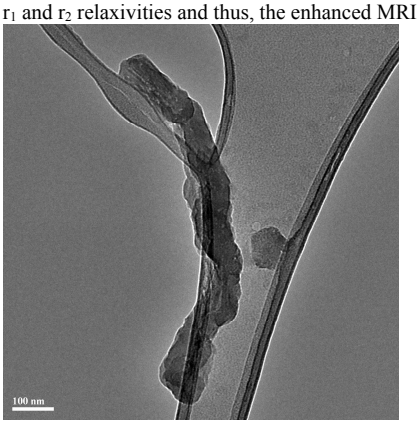


Fig1. Representative HRTEM at 200kV of a ~100nm wide and 2000 nm long GONR

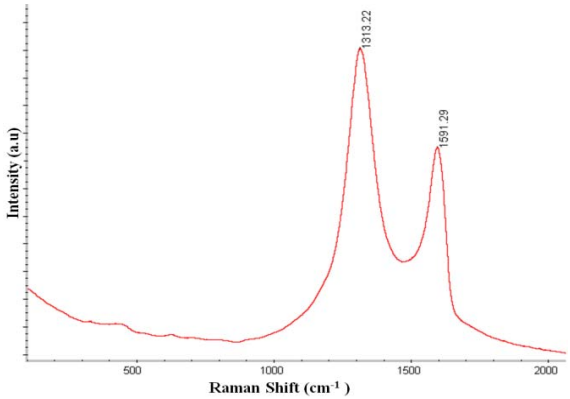
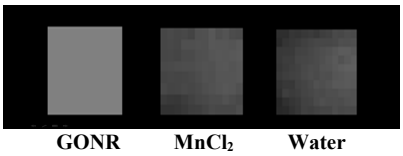


Fig2. Raman spectrum of GONR showing characteristic D ($1313cm^{-1}$) and G ($1591cm^{-1}$) bands



SAMPLE	T_1image intensity ratio to water	T_2 image intensity ratio to water
GONR	1.465	5.05
$MnCl_2$	0.9999	2.272

Fig4. T_1-weighted MRI phantom of GONR compared with $MnCl_2$ and water; **Table1.** Comparison of mean signal intensities ratios from T_1- and T_2- phantom images with respect to water

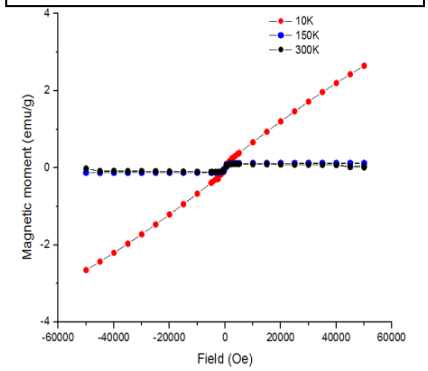


Fig3. Magnetization versus Field plot of the GONR. The shape of the profile indicates room temperature superparamagnetism

CONCLUSION:

We have synthesized and characterized a novel graphene-based MRI CA that exhibits room temperature superparamagnetism, and high relaxivities that significantly enhances the T_1 and T_2 MRI contrast *in vitro*. Further *in vivo* MRI and toxicity studies are currently being performed on these nanoparticles towards their development as advanced MRI CAs.

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