Preclinical In Vitro and In Vivo Assessment of a Novel Graphene Based MRI Contrast Agent

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Target Audience: Scientists and clinicians with research interests in MRI contrast agents (CAs)

Purpose: In the US, approximately 43% of the 27.5 million clinical magnetic resonance imaging (MRI) procedures use Gd^{3+}-based T_1 contrast agents (GBCAs) to improve disease detection by increasing sensitivity and diagnostic confidence. However, the recent discovery and association with nephrogenic systemic fibrosis (NSF) in some patients has fostered concern, and led to restrictions on their clinical use. Thus, there is a need for a T_1 MRI CA that is safer and efficacious, than clinical GBCAs. Such a CA can allow the same clinical MRI performance at substantially lower dosages. Further, their lower toxicity and higher relaxivity profile could allow their development as extended-residence-intravascular, tissue (organ)-specific, and molecular imaging CAs. We have developed a novel high-performance carbon nanostructure-based MRI CA by synthesizing graphene nanoplatelets (GNPs; small stacks of graphene with thickness = 3-4 nm, diameter ~ 40 nm) that are monodispersed, water-soluble and intercalated (insertion of chemical species within the voids between two graphene sheets), with trace amounts of manganese (~0.06 wt%). The graphene sheets sequester the manganese to prevent its leakage and amplify its relaxivity. In this work, we report their pre-clinical evaluation *in vitro* and *in vivo.*

Materials and Methods: Graphene nanoplatelets (GNP) were prepared and functionalized with dextran (Dex) (GNP-Dex) according to the method described elsewhere.[1,2] Mannitol was added to control osmolality and viscosity. The following key physicochemical parameters at GNP-Dex concentrations between 1-200 mg/ml (chosen to mimic the expected bolus or equilibrium blood concentration) were assessed: osmolality, viscosity, thermal stability, partition coefficient, protein binding, and histamine release. Acute toxicity study was performed in rats at the dosages 1-500 mg/kg of GNP-Dex and monitored for 30 days for any adverse effects. Nephrotoxicity study was performed in a renal compromised rat model at potential therapeutic dosages between 1-50 mg/kg of GNP-Dex and monitored for 15 days for any adverse effects. T_1 weighted small animal MRI was performed at 1-25 mg/kg dose of GNP-Dex using a 1.5 Tesla Siemens scanner and compared with Magnevist and Ablavar at clinical dose.

Results and Discussion: The table summarizes the major findings**.** The GNP-Dex solutions show similar osmolality and viscosity values as blood. The lower partition coefficient value indicates that the GNP-Dex formulations are hydrophilic. Protein binding and histamine release studies show very little or no biological interaction. The GNP-Dex showed relaxivity of $r_1 = 92\ mM^{-1}s^{-1}$ ~15-20 fold greater than clinical Gd^{3+}- and Mn^{2+}-chelate based MRI CAs. The small animal safety studies indicate that the LD_{50} value of the GNP-Dex formulations is greater than 500 mg/kg dose, can safely administered as bolus injections up to 100 mg/kg doses, and do not show nephrotoxicity at potential therapeutic dosages. *In vivo* MRI showed significant contrast enhancement compared to Magnevist and Ablavar at potential therapeutic dosages.

Parameters	Results	Condition
Osmolality	290 mOsm/kg	100 mg/ml
Viscosity	2.09 cP	100mg/ml 37°C
Partition Coefficient	-0.18	100 mg/ml
Protein Binding	<LOD(0.001mg/ml)	0.1,1,10 mg/ml
Release of Mn^{2+} ions	< LOD (0.01μM)	100 mg/ml 37°C
Histamine Release	<19.6 ng/l (control)	0.1,1,10 mg/ml
In vitro relaxivity r_1	92 $mM^{-1}s^{-1}$	B – 0.47 T
LD_{50} (rats)	> 500 mg/kg	Studied for 30 days
Nephrotoxicity	No symptoms of NSF	5/6 nephrex rat model, 15 days

Conclusion: Pre-clinical *in vitro* and *in vivo* small animal studies indicate that GNP-Dex formulations are safe and efficacious over large range of dosages and show potential for development as a novel clinical MRI CA.

References: **1.** Paratala BS, Jacobson BD, Kanakia S, Francis LD, Sitharaman B. Physicochemical characterization, and relaxometry studies of micro-graphite oxide, graphene nanoplatelets, and nanoribbons. *PloS one.* 2012;7(6):e38185.
2. Kanakia S, Toussaint J, Mullick Chowdhury S, et al. Physicochemical Characterization of A Novel Graphene-Based Magnetic Resonance Imaging Contrast Agent. *Nanomedicine: Nanotechnology, Biology and Medicine (in preparation).* 2012.