The First Magnetic-Nanoparticle-Free Carbon-Based Contrast Agent of Magnetic-Resonance Imaging-Fluorinated Graphene Oxide

*Yun Hang Hu**

Graphene, a single layer of sp^2-bonded carbon atoms arranged in a honeycomb lattice, is the first two-dimensional material in a suspended form.[1] As a rapidly rising star, graphene has attracted much attention in science and engineering because of its unusual properties, such as fast carrier transport, excellent optical transparency, high elasticity, and large surface area.[2,3] The biomedicine applications of graphene and its derivatives are under intense research to develop photo-therapies of cancer, biosensors, and nanocarriers for drug delivery.[4–6] Furthermore, graphene and graphene-oxide (GO) are efficient carriers of fluorescent agents and novel near-infra-red (NIR) probes for cell imaging.[7,8] Recently, Mani, Ajayan and co-workers have made a breakthrough, in which fluorinated graphene oxide (FGO) was demonstrated as the first carbon-based MRI contrast agent without magnetic nanoparticles.[9]

As a unique diagnostic tool without utilizing ionizing radiation, MRI can generate 3D images of opaque and soft tissues due to the variation of nuclear magnetic resonance (NMR) parameters of water in different chemical environments. However, because MRI has a poor inherent sensitivity, contrast agents (CAs) are usually necessary to enhance the image contrast in MRI. Two types of widely used MRI CAs are paramagnetic transition metal ion chelates (mainly Gd chelates) and superparamagnetic iron oxide nanoparticles (SPIONs).[10] The paramagnetic transition metal ion chelates increase the signal intensity by decreasing the longitudinal (or spin-lattice) relaxation time (T_1) of H_2O protons, generating bright positive signal intensity in images.[11] In contrast, the SPIONs decrease the signal intensity by shortening transverse relaxation time (T_2) of H_2O protons, leading to dark negative signal intensity in the images that have high signal intensity.[11] The development of new MRI CAs with an enhanced sensitivity and advanced functionalities is currently needed.

Although carbon materials (such as graphene-oxide) were combined with super-paramagnetic iron oxide (Fe_3O_4) nanoparticles as a composite MRI contrast agent, carbon materials without the incorporation of magnetic nanoparticles cannot confer MRI contrast. Very recently, however, this situation has been change by Mani, Ajayan and co-workers, who revealed that fluorinated graphene oxide (FGO) without magnetic nanoparticles is an efficient MRI contrast agent.[9] To create dipolar C-F bonds for the introduction of paramagnetic centers, they synthesized fluorinated graphene oxide (FGO) via oxidation of graphite fluorinated polymer using the modified Hummer's method.[12] The superconducting quantum interference device (SQUID) characterization of the synthesized FGO showed a linear dependency to the magnetic field, indicating its paramagnetic behavior (**Figure 1**A).[9] To evaluate the feasibility of FGO being an MRI contrast agent, MRI phantoms were scanned on a

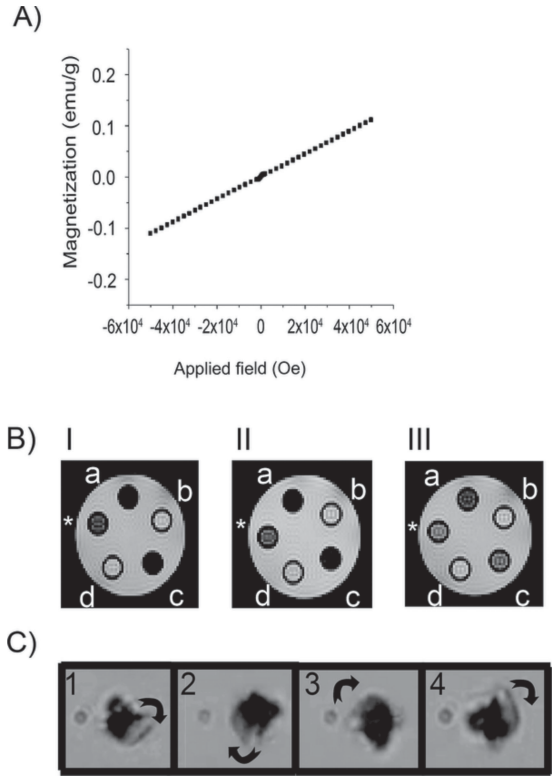

Figure 1. FGO Magnetic properties. A) SQUID magnetization curve. B) Spin-spin (T_2) relaxation measurements obtained on a 4.7 T MRI for FGO (I: a = 625, c = 500; II: a = 313, c = 250; III: a = 156, c = 125 μg mL^{-1}) and GO (I: b = 625, d = 500; II: b = 313, d = 250; III: b = 156, d = 125 μg mL^{-1}) with the positive control (*) consisting of diluted magnevist (0.5 mg mL^{-1}). C) Clockwise rotational magnetic field snapshots of FGO under a field of 12 mT (Reproduced with permission.[9] 2013, Wiley)

Prof. Y. H. Hu
Department of Materials Science and Engineering
Michigan Technological University
Houghton, Michigan, 49931, USA
E-mail: yunhangh@mtu.edu

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4.7 T Biospec system. As predicted, FGO exhibited contrast in T_2 mode, whereas GO did not (Figure 1B). This indicates that the paramagnetic behavior in FGO is due to the presence of C–F bonds. Furthermore, rotational magnetic field experiments showed that FGO can be magnetically triggered, even with a small electromagnetic-system at a magnetic field of 12 mT (Figure 1C). This demonstrates that the small amount of F in the GO lattice could cause a dramatic change in its magnetic properties. In addition, as a MRI promising contrast agent, FGO would have a higher signal to noise ratio due to its scarce distribution of ^{19}F as opposed to protons (^{1}H) in the human body. To the best of our knowledge, FGO would be the first non-magnetic-nanoparticle carbon-based MRI contrast agent.

The ability of FGO as an ultrasound detection agent was also examined.[9] When FGO was imaged at a 40 MHz center frequency, appreciable acoustic backscattering was generated. In contrast, pure agar without added scatterers exhibited an anechoic image. Furthermore, a strong backscatter signal was observed using FGO. Those results promise FGO being an ultrasound contrast agent.

FGO was further subjected to the evaluation of hyperthermic ability under NIR laser irradiation at a wavelength of 800 nm and a power of 1.6 W.[9] The irradiation of 1 min increased the temperature of the tissue culture sample containing FGO to 62.3 °C, whereas the temperature of culture media without FGO remained at ≤25.0 °C. Furthermore, the cells containing FGO exhibited much higher cell death than those without FGO after the NIR laser irradiation. This demonstrates that the heat generated on FGO from NIR laser beam can induce cell death. Therefore, FGO can be employed to increase the local temperature for inducing cancer cell death without damaging nearby healthy tissues.

In summary, FGO is a novel carbon material with clinically translatable multimodal capabilities. It is not only the first carbon-based MRI contrast agent without magnetic nanoparticles, but also a promising ultrasound contrast agent. Furthermore, FGO can convert its absorbed NIR laser energy into heat for increasing the local temperature, constituting its ability as an efficient therapeutic agent against temperature-sensitive cancer cells. The future *in vivo* evaluation of FGO multimodal capabilities would promote its clinical application. Investigation to reveal a relationship between size (structure, shape or composition) of FGO and its properties is expected to establish a scientific base to optimize its biomedicine performances. Furthermore, more attention should be given to the introduction of multifunctional groups into a graphene sheet to create its desired multicapabilities.

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