



Short communication

Graphene-based contrast agents for photoacoustic and thermoacoustic tomography

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ABSTRACT

In this work, graphene nanoribbons and nanoplatelets were investigated as contrast agents for photoacoustic and thermoacoustic tomography (PAT and TAT). We show that oxidized single- and multi-walled graphene oxide nanoribbons (O-SWGNRs, O-MWGNRs) exhibit approximately 5–10 fold signal enhancement for PAT in comparison to blood at the wavelength of 755 nm, and approximately 10–28% signal enhancement for TAT in comparison to deionized (DI) water at 3 GHz. Oxidized graphite microparticles (O-GMPs) and exfoliated graphene oxide nanoplatelets (O-GNPs) show no significant signal enhancement for PAT, and approximately 12–29% signal enhancement for TAT. These results indicate that O-GNRs show promise as multi-modal PAT and TAT contrast agents, and that O-GNPs are suitable contrast agents for TAT.

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1. Introduction

Hybrid imaging modalities, such as photoacoustic (PA) tomography (PAT) [1] and thermoacoustic (TA) tomography (TAT) [2], have been developed for different applications. PAT/TAT combines advantages of pure ultrasound and pure optical imaging/radio frequency (rf), providing good spatial resolution, great penetration depth, and high soft-tissue contrast. These imaging modalities are based on detection of acoustic waves from an object that absorbs electromagnetic (EM) energy (laser in PAT and microwave in TAT). Endogenous molecules, such as hemoglobin, melanin, and water/ion, can absorb EM energy, producing acoustic waves. High resolution PAT and/or TAT enable functional brain imaging [3],

breast cancer detection [4], melanoma detection [5], tumor angiogenesis [6], and functional molecular imaging [2]. However, in cases when endogenous molecules are insufficient, exogenous contrast agents (CAs) are developed and administered. Contrast-enhanced PAT has been applied in lymph node mapping [7], multiscale imaging of tissue engineering scaffolds [8,9], and molecular, cellular, and functional imaging [10,11]. A variety of CAs for PAT have been reported, such as, carbon nanoparticles [7,12–14], metallic nanoparticles [11,15–17], and organic dyes [18]. In comparison to PAT, fewer reports have focused on development of CAs for TAT. Superparamagnetic iron oxide nanoparticles, single- and multi-walled carbon nanotubes (SWCNT and MWCNT), and air-filled microbubbles have been investigated as CAs for TAT [2,13,19,20].

In this work, we investigate efficacy of graphene nanoparticles, prepared by two widely used methods ((1): longitudinal unzipping method [21], (2): modified Hummer's method of oxidation [22]) as CAs for PAT and TAT. We compare PA and TA signal amplitudes of oxidized single- and multi-walled graphene oxide nanoribbons (O-SWGNRs and O-MWGNRs), and oxidized graphene nanoplatelets (O-GNPs) to pristine SWCNTs, pristine MWCNTs, pristine graphite microparticles (GMPs), and oxidized graphite microparticles (O-GMP).

2. Results and discussions

O-SWGNRs, O-MWGNRs, and O-GNPs were synthesized as reported previously [22,23]. Pristine SWCNTs, MWCNTs, and GMPs

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² PAT and TAT.

³ Graphene contrast agents.

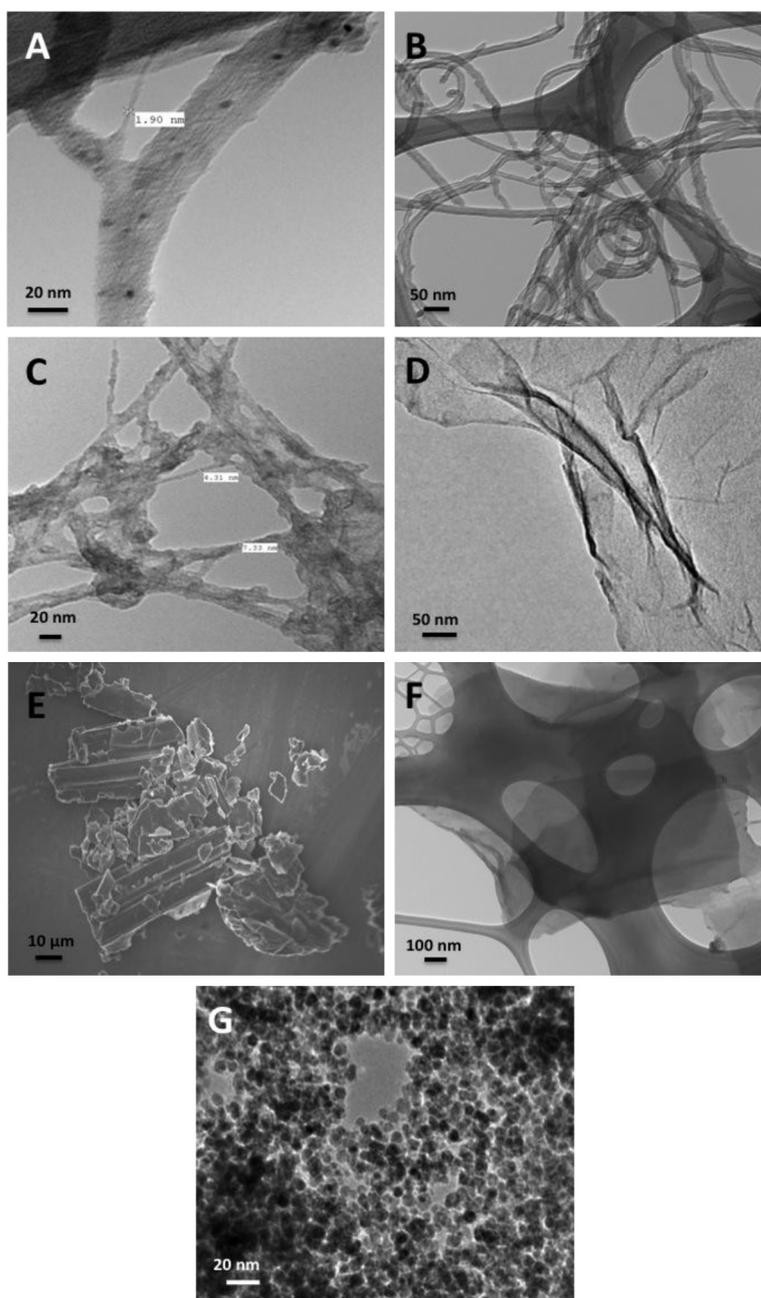


Fig. 1. Representative transmission electron microscopy images of (A) single-walled carbon nanotubes (SWCNTs), (B) multi-walled carbon nanotubes (MWCNTs), (C) oxidized single-walled graphene nanoribbons (O-SWGNRs), (D) oxidized multi-walled graphene nanoribbons (O-MWGNRs), (F) oxidized graphite microparticles (O-GMP), and (G) exfoliated graphene nanoplatelets (O-GNP). Image (E) is a scanning electron micrograph of pristine GMPs.

were used as starting materials in the preparation of O-SWGNRs, O-MWGNRs, and O-GNPs, respectively. O-GMPs are intermediate product formed during the synthesis of O-GNPs. These nanomaterials were characterized by Raman spectroscopy and electron microscopy (EM). Raman spectroscopic characterization of SWCNTs, MWCNTs, O-SWGNRs, O-MWGNRs, GMPs, O-GMPs, and O-GNPs has been reported previously [22,24–26]. Table 1 lists the size distribution of various nanomaterials. Fig. 1 shows representative transmission EM (TEM) images of all nanomaterials used in the study (scanning EM (SEM) for GMPs). SWCNTs (Fig. 1A) and MWCNTs (Fig. 1B) were nanotubes of lengths ≈ 3 – $30 \mu\text{m}$ and 0.5 – $200 \mu\text{m}$, and diameters ≈ 1 – 2 nm and ≈ 20 – 30 nm , respectively. O-SWGNRs (Fig. 1C) and O-MWGNRs (Fig. 1D) possessed lengths ≈ 0.5 – $1 \mu\text{m}$ and 0.5 – $1.5 \mu\text{m}$, and diameters of ≈ 3 – 6 nm

and ≈ 60 – 90 nm , respectively, confirming complete unzipping of SWCNTs and MWCNTs (π^* diameter). Pristine GMPs were $< 45 \mu\text{m}$ in size (Fig. 1E). O-GMPs (Fig. 1F) were loosely arranged sheets of a few layered graphene (≈ 8 sheets, size $> 1 \mu\text{m}$) whereas O-GNPs (Fig. 1G) had ≈ 2 – 4 graphene sheets and diameters of ≈ 5 – 15 nm .

We have estimated that future *in vivo* preclinical safety (acute toxicity) studies to establish the therapeutic dosages of graphene would require their administration at a range of dosages; from 50 mg/kg upto possibly $\geq 500 \text{ mg/kg}$ body weight of the small animal [27]. If the graphene formulations are injected at a dose of 50 or 500 mg/kg body weight of a 250 g rat (total circulating blood volume 12 – 13 ml), its steady state blood concentration after the first pass would be ~ 1 or 10 mg/ml , respectively. Thus, a median concentration of 5 mg/ml was chosen for this study. Since

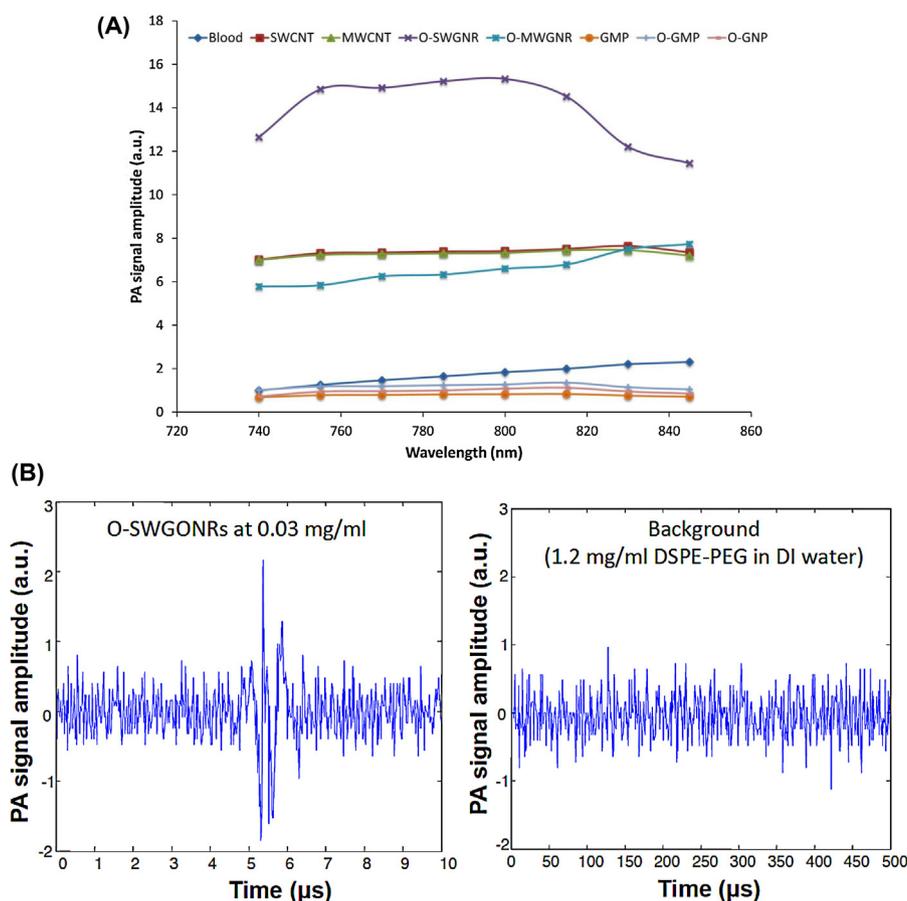


Fig. 2. (A) Photoacoustic spectral amplitudes of blood, single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs), oxidized single-walled graphene nanoribbons (O-SWGNRs), oxidized multi-walled graphene nanoribbons (O-MWGNRs), micro-graphite flakes (GMPs), oxidized graphite microparticles (O-GMPs), and exfoliated graphene nanoplatelets (O-GNPs). PA signal amplitudes are normalized to that of blood at 740 nm. (B) PA signal amplitude of O-SWGNRs at 0.03 mg/ml concentration compared to background (1.2 mg/ml of DSPE-PEG solution).

hemoglobin is a dominant optical absorber producing strong PA signal in human tissue, efficacy of these nanomaterials was compared with blood in the NIR wavelength window. Fig. 2A shows PA signal amplitudes obtained from a tygon tube (I.D. 250 μm , O.D. 500 μm) filled with SWCNT, MWCNT, O-SWGNR, O-MWGNR, micro-graphite flakes, O-GMP, O-GNP and lysed bovine blood (905–250, Quad Five), respectively. The signals were normalized to that for blood at 740 nm. At 755 nm excitation wavelength, peak-to-peak PA signal amplitudes obtained from micro-graphite flakes, O-GMPs, and O-GNPs were comparable to that from blood alone. In contrast, those from SWCNTs, MWCNTs, O-SWGNRs and O-MWGNRs were more than 5 times stronger than that from blood, in which, O-SWGNRs showed ~ 14 times stronger signal. At 5 mg/ml concentration, PA signal intensities obtained from gold nanoparticles were 3 times greater, and methylene blue dye were similar, compared to blood [28,29]. We detected a very high signal-to-noise ratio (SNR; ratio of the average signal to the

standard deviation of the background) of O-SWGNRs at 5 mg/ml. The SNR was >170 and suggested that the concentration of the O-SWGNRs can be as low as 0.03 mg/ml using PAT. At this low O-SWGNR concentration, a 2-fold increase in PA signal was measured compared to background (1.2 mg/ml DSPE-PEG in DI water) (Fig. 2B). These results suggest that minimum detectable concentration of O-SWGNRs will be comparable to other PA contrast agents such as gold nanoparticles [17,30]. Furthermore, the results showed that PA signal obtained from these nanomaterials exceeded inherent blood signal over the investigated NIR bandwidth, suggesting their utility for *in vivo* imaging.

Water and ions are two well-known sources of microwave absorbers in human tissue, and they generate strong TA signals. Therefore, to show that nanomaterials can function as CAs for TAT, we compared TA signal of nanomaterials to that of DI water. Fig. 3B shows TA signals obtained from a low-density polyethylene (LDPE) vial (I.D. = 6 mm and 1.5 cc volume) filled with DI water, SWCNTs,

Table 1
Size distribution of various nanomaterials.

Nanomaterial	Length	Diameter
Single-walled carbon nanotubes (SWCNTs)	3–30 μm	1–2 nm
Multi-walled carbon nanotubes (MWCNTs)	0.5–200 μm	20–30 nm
Oxidized single-walled graphene nanoribbons (O-SWGNRs)	0.5–1 μm	3–6 nm
Oxidized multi-walled graphene nanoribbons (O-MWGNRs)	0.5–1.5 μm	60–90 nm
Pristine graphite microparticles (GMPs)	–	$<45 \mu\text{m}$
Oxidized graphite microparticles (O-GMPs)	–	$>1 \mu\text{m}$
Oxidized graphene nanoplatelets (O-GNP)	–	5–15 nm

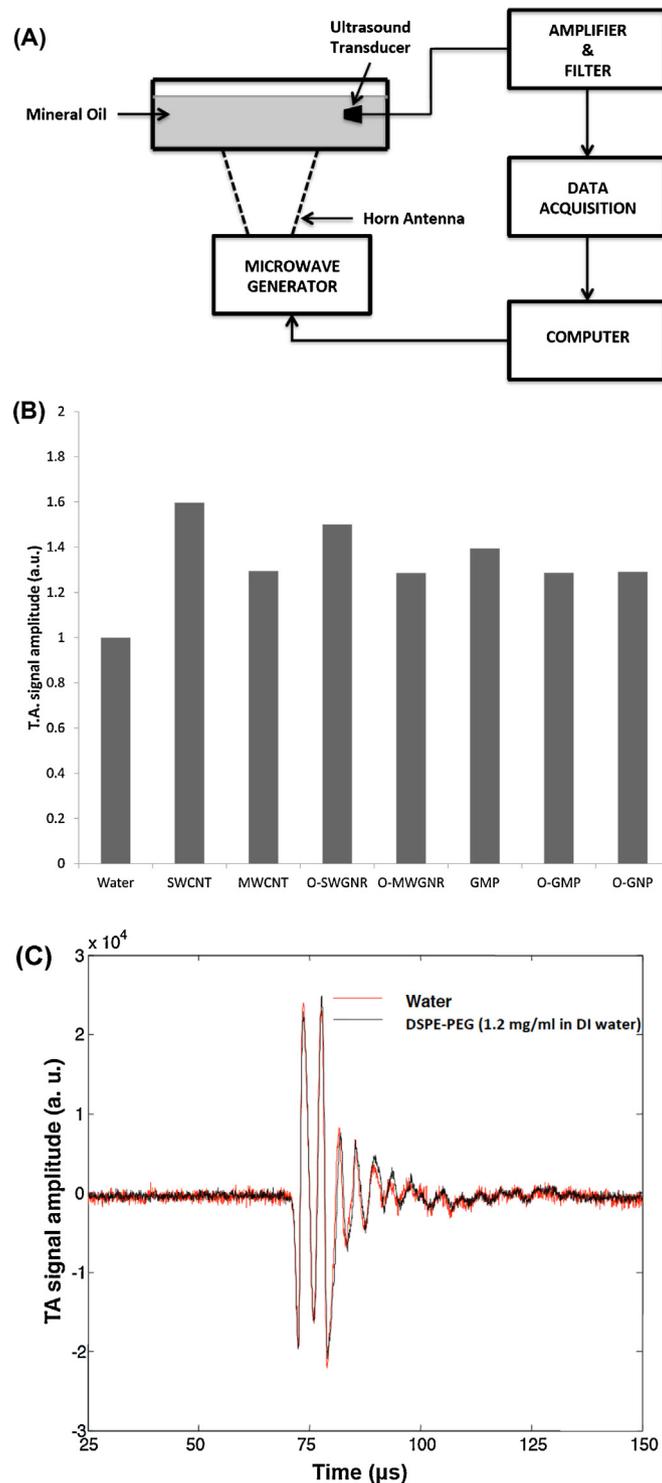


Fig. 3. (A) Schematic depiction of the experimental setup for thermoacoustic signal measurements. (B) Thermoacoustic signal amplitudes of water, single-walled carbon nanotubes (SWCNTs), multi-walled carbon nanotubes (MWCNTs), oxidized single-walled graphene nanoribbons (O-SWGNRs), oxidized multi-walled graphene nanoribbons (O-MWGNRs), micro-graphite flakes (GMPs), oxidized graphite microparticles (O-GMP), and exfoliated graphene nanoplatelets (O-GNP) at 3 GHz. TA signals are normalized to that of water at 3 GHz. (C) TA signal amplitude of DSPE-PEG compared to DI water.

MWCNTs, O-SWGNRs, O-MWGNRs, GMPs, O-GMPs, and O-GNPs, respectively. The signal amplitudes were normalized to DI water. Additionally, TA signal amplitude of DSPE-PEG was comparable to DI water (Fig. 3C), and LDPE vial does not generate any measurable TA signal [13]. At 3 GHz, the SNR of the nanomaterials was >170 , and the nanomaterials exhibited ≈ 10 –28% TA signal enhancement compared to DI water.

To the best of our knowledge, this is the first study exploring and comparing efficacy of graphene nanoparticles prepared via longitudinal “unzipping” method and Hummer’s method as CAs for multimodal PAT and TAT. These results indicate that O-GNPs could be used for multimodal PAT and TAT applications, and O-GNPs are suitable CAs for TAT. Bulk of the work performed towards developing CAs for PAT has been focused on metallic nanoparticles,

organic dye molecules, and carbon nanotubes. In comparison to those CAs, graphene possesses several benefits: (1) Compared to carbon nanotubes, graphene possesses larger surface area, lower aspect ratio, and better dispersibility in most biological media. These properties are important, for most *in vivo* applications. Furthermore, colloidal dispersions (with high stability and less aggregation) of graphene sheets can be achieved without impurities that may be harmful in biological systems [31,32]. (2) The sp^2 bonded carbon sheets of graphene can be directly functionalized for targeting and drug delivery [33]. For other PAT/TAT CAs, such as gold nanoparticles and organic dye molecules, to disperse and stabilize gold nanoparticles in solution or embed organic dye molecules, functionalization is performed on the biocompatible coating/capping agent. (3) O-GNPs and O-GNRs have been reported as CAs for other whole-body imaging applications such as magnetic resonance imaging [22] and nuclear imaging [34]. Therefore, they can be developed as multimodal CAs that provide complementary information at micro- to macroscopic length scales. (4) Graphene can be developed as therapeutic (simultaneous therapy and diagnostics) agent combining PAT/TAT molecular imaging and NIR-induced hyperthermia [33]. Due to these unique features, graphene may serve as a platform for the design of multi-modal imaging and multi-therapeutic approaches. Indeed, several *in vitro* and *in vivo* safety and efficacy studies on these graphene nanoparticles have been reported for various biomedical applications [23,35].

3. Materials and methods

3.1. Synthesis and characterization of nanomaterials

SWCNTs (Cheap Tubes Inc., VT, USA) and MWCNTs (Sigma Aldrich, NY, USA) were used as received. O-SWGNRs, O-MWGNRs, O-GMPs, and O-GNPs were synthesized and characterized as reported previously [22–24]. All nanomaterials were dispersed at 5 mg/ml in DSPE-PEG for PA and TA measurements.

3.2. Photoacoustic (PA) imaging

A deep reflection-mode PA imaging system was used (Scheme 1 in Ref. [36]) for PA tests of graphene samples. A tunable Ti:sapphire laser (LT-2211A; Lotis TII, Minsk, Belarus) pumped by a Q-switched Nd:YAG (LS-2137; Lotis TII) laser was used for PA excitation (pulse width: 5 ns, pulse repetition rate: 10 Hz). A 5-MHz central frequency, spherically focused ultrasonic transducer (V308; Panametrics-NDT, Waltham, MA, USA), low-noise amplifier (5072PR; Panametrics-NDT), a digital oscilloscope (TDS 5054; Tektronix, Beaverton, OR, USA) were used to acquire, amplify, and record signals. The reported PA signal amplitudes have been normalized for laser fluence at their corresponding wavelengths.

3.3. Thermoacoustic (TA) imaging

Fig. 3A is a schematic depiction of the experimental setup for TA measurements. TA results were obtained from a TAT system with a 3.0-GHz microwave generator (pulse width = 0.6 μ s, repetition rate = 10 Hz) and a 20 dB amplifier. The pulses (average power density = 4.5 mW/cm², within safety standard) were guided toward the target through a horn antenna (11 cm \times 7 cm) [37]. A 1-MHz spherically focused transducer with a bandwidth of 70% (V314, Panametrics, Olympus) was used to receive TA signals from samples placed in a plastic tank filled with mineral oil for ultrasound coupling. The received TA signals were amplified and stored by a data-acquisition (DAQ) card (CS 14200; Gage Applied, IL) [38]. The microwave generator simultaneously triggered data acquisition.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgments

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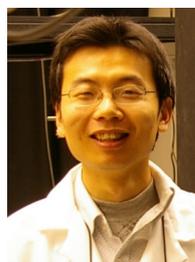
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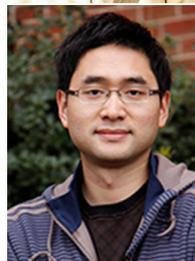
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