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# Evaluation of the sonosensitizing properties of nano-graphene oxide in comparison with iron oxide and gold nanoparticles

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## HIGHLIGHTS

- Sonosensitizing effects of nano-graphene oxide is reported for the first time.
- Effects of nano-graphene oxide on ultrasound heat generation are compared to gold and iron oxide nanoparticles.
- Ultrasound heat generation in the presence of nanoparticles depends on ultrasound power and nanoparticle concentration.
- A novel hyperthermia method using ultrasonic waves and nanotechnology is proposed.

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## ABSTRACT

In cancer hyperthermia, ultrasound is considered as an appropriate source of energy to achieve desired therapeutic levels of heating. It is assumed that such a heating is targeted to cancer cells by using nanoparticles as sonosensitization agents. Here, we report the sonosensitizing effects of Nano-Graphene Oxide (NGO) and compare them with gold nanoparticles (AuNPs), Iron Oxide nanoparticles (IONPs).

Experiments were conducted to explore the effects of nanoparticle type and concentration, as well as ultrasound power, on transient heating up of the solutions exposed by 1 MHz ultrasound. Nanoparticles concentration was selected from 0.25 to 2.5 mg/ml and the solutions were exposed by ultrasound powers from 1 to 8 W. Real time temperature monitoring was done by a thermocouple and obtained data was analyzed.

Temperature profiles of various nanoparticle solutions showed the higher heating rates, in comparison to water. Heating rise was strongly depended on nanoparticles concentration and ultrasound power. AuNPs showed a superior efficiency in heat generation enhancement in comparison to IONPs and NGO.

Our result supports the idea of sonosensitizing capabilities of AuNPs, IONPs, and NGO. Targeted hyperthermia may be achievable by preferential loading of tumor with nanoparticles and subsequent ultrasound irradiation.



## 1. Introduction

Cancer is one of the major causes of mortality worldwide. Among the well-established methods of cancer treatment, hyperthermia has been recently proposed as an adjuvant therapy with acceptable safety in humans [1–4]. Depending on treatment purposes, the malignant tissue is exposed to high temperatures so that cancer cells get directly destroyed or become more susceptible to other treatment modalities. Hyperthermia mitigates tissue hypoxia and could be concomitantly applied with radiotherapy or chemotherapy to intensify their cytotoxic effects on the tumor [3,5–8].

In conventional methods of hyperthermia, there is no thermal discrimination between target and surrounding normal tissue [9]. Thus, beside the intention of tumor heating, some undesired side effects may be occurred in healthy tissue, due to loss of energy around the tumor. Currently, the continuing efforts are in progress to achieve a specific hyperthermia method so that the tumor receives the maximum thermal dose and surrounding normal tissue would be kept at the safe temperature ranges [1,10].

Recently, nanotechnology based hyperthermia has been introduced as a promising alternative to conventional hyperthermia methods. Nanoparticles can absorb the energy originated from

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external source and enhance the effects of hyperthermia [11,12]. In this kind of hyperthermia, nanoparticles focus the energy of external source on the tumor to induce localized thermal damages while minimize the adverse effects of collateral healthy tissues [13–15].

Different energy sources including microwave, radiofrequency, laser and ultrasound are currently utilized to heat the tumor [16]. Depending on the kind of external energy source, different mechanisms are involved in heat generation in the presence of nanoparticles [17–19]. In comparison to electromagnetic radiations, the possible mechanisms of enhancement of ultrasound heat generation in the presence of nanoparticles are still unclear.

Ultrasound is considered as an external source of energy to heat up cancer cells and it offers some intrinsic advantages over the other heating sources [20]. Heat induced by ultrasound can be remotely focused on any depth of the body. As a result, localized heating of a tumor with reduced thermal damages to the surrounding healthy tissues may be highly achievable by ultrasound [21].

Recent studies showed that nanoparticles can play the sonosensitizing role and enable to absorb the energy of ultrasound to heat up a medium. Wen et al. investigated the synergistic effects of ultrasound and 10–300 nm colloidal gold nanoparticles (AuNPs) on heating rate of a medium. Their thermometry studies were conducted using a thermocouple which measures bulk temperature rise of the medium. Their results showed that AuNPs can significantly increase the heating rate of the base fluid. For example, the heating rate for base fluid was changed from 0.4 °C/s to 1.6 °C/s when AuNPs were added (concentration per gold: 150 μmol/L) [22]. Another study done by Sviridov showed that porous silicon nanoparticle (PSi NP) can act as a sonosensitizer agent to enhance thermal effects of ultrasound waves. The transient temperature rise of water, induced by ultrasound irradiation (1 MHz; 20 W) increased from $2 \pm 0.2$ °C to $3 \pm 0.2$ °C, due to the presence of PSi NPs [23]. Shakeri-Zadeh et al. demonstrated that the heat induced by ultrasound in a colon tumor can be enhanced through administration of magnetic nanoparticles. They measured temperature rise of the tumors exposed to ultrasound [3 MHz; 1 W/cm$^2$, 10 min]. Their experiments showed that temperature of a nanoparticles loaded tumor is increased 3 °C higher than a nanoparticles free tumor when both tumors are similarly sonicated [24].

In this study we investigated the sonosensitizing potentials of Nano-Graphene Oxide (NGO) and compared them with Gold NanoParticles (AuNPs) and Iron Oxide NanoParticles (IONPs), for the first time. NGO is a single layer carbon based nanostructure with outstanding near-infrared (NIR) optical absorbance. Despite of the wide studies performed on optical properties of NGO [25,26], the acoustic behavior of this nanomaterial has not been investigated so far.

## 2. Materials and methods

### 2.1. Materials

AuNPs, IONPs and NGO were purchased from Nanobon Company (Tehran, Iran).

#### 2.1.1. Characterization of nanoparticles

The morphological investigation of nanoparticles was performed using Zeiss LEO 906 transmission electron microscope (TEM) and Phenom ProX desktop scanning electron microscope (SEM). The effective diameters of nanoparticles were measured by dynamic light scattering (DLS, Malvern Zetasizer Nano ZS-90).

### 2.2. Experimental procedure

#### 2.2.1. Calibration of ultrasound generator

To investigate the heating effects of nanoparticles in the presence of ultrasound, different nanoparticle solutions were exposed by a plane circular transducer with an area of 4 cm$^2$ operating at 1 MHz in the continuous wave mode (ultrasound generator: Phyaction 190i, Germany). Power output of transducer was measured using the acoustic radiation force method [24,27]. A linear relationship between nominal intensity ($I_1$) and measured intensity ($I_2$) was obtained as demonstrated below:

$$I_1 = 0.942\, I_2 + 0.185, \quad R^2 = 0.974$$

The ratio of peak intensity to average intensity ($I_p/I_a$) was calculated using the dye paper method and Kossoff approximation method which was found to be approximately 5 [24,27].

#### 2.2.2. Details of experiments

Our experiments were designed and conducted in three sections. The effects of (i) concentration of nanoparticles, (ii) ultrasound power and (iii) type of nanoparticles on the amount of heat generation were separately investigated. Tables 1–3 summarize the experimental protocols considered in each experiment.

Various concentrations of nanoparticles dispersed in deionized water were selected. A container made of Plexiglas with a volume of 4 cc was designed and fabricated. The container was filled with solution of nanoparticles. A thermocouple (Hanyoung, Korea) with sensitivity of $\pm 0.1$ °C was embedded at the center of container to monitor temperature rise of the medium during ultrasound exposure. Fig. 1 presents the schematic illustration of experimental setup.

### 2.3. Statistical methods

All experiments were conducted in triplicate. Statistical analysis was performed by one way ANOVA test and we used the Tukey test at 95% confidence level as a post-test. Statistical analysis was performed by using SPSS software (version 11). A value of $P < 0.05$ was considered statistically significant.

## 3. Theory

The presence of nanoparticles in the acoustic field affects both thermal and mechanical interactions of ultrasound and may lead to enhancement of both physical effects of ultrasound:

(1) ultrasound induced heating,
(2) ultrasound induced cavitation.

### 3.1. Thermal interactions

#### 3.1.1. Effects on attenuation coefficient

Through absorption and scattering of ultrasound waves,

**Table 1**
Details of experiments conducted to evaluate the effects of nanoparticles concentration on heating rise of a medium.

| Effect | Nanoparticles | Concentration [mg/ml] | Exposure time [min] | Ultrasound power [Watt] |
|---|---|---|---|---|
| Nanoparticle concentration | IONPs | 0.25 | | |
| | NGO | 1<br>2.5 | 10 | 2 |