Photo-destruction of cancer cells by NIR irradiation and graphene nano-sheets

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ABSTRACT

The photo-thermal therapy using nano-materials has attracted great attention as an efficient strategy for the next generation of cancer treatments. Recently, photo-thermal therapy based on nano-materials that can be activated by a skin-penetrating NIR (Near Infra Red) irradiation has been suggested as a noninvasive, harmless, and highly efficient therapeutic technique. Graphene nano-layers synthesized by a bio-compatible method, with reduced toxicity, will be a suitable candidate for the photo-thermal therapeutic agent. A significant amount of heat is generated upon excitation with near-infrared light (NIR, 700-1100nm) which is transparent to biological species including skins. In this paper, we demonstrate *destruction* of solid malignant cells (colon cancer cells) by the combined treatments of bio-compatible graphene nano-layers and NIR irradiation. MTT methods show that more than 85% of the cells which held with autoclaved graphene solution remained alive after 24hr. In addition, NIR laser exposure with a 250 mw diode laser (780 nm) to colon cancer cells (without graphene inclusion) for 15 min kills less than 9% of colon cancer cells but exposing the NIR beam to the graphene-added cells for 25 min kills more than 66% of the cells.

Keywords: graphene, bio compatible, near IR, cancer cell, photo destruction.

1 INTRODUCTION

Graphene, as ‘‘the thinnest material in our universe’’, is a single-atom-thick sheet of sp^2-bonded carbon atoms in a hexagonal two-dimensional lattice which promises unique potential applications in technological fields such as ultrasensitive gas sensors, composite materials, transparent conductive films, gas storage media, and next generation of electronic devices [1]. In addition Because of their unique physicochemical properties, graphene and its derivatives have attracted great attention in biomedical areas [2]. In particular, graphene-based materials are promising potential candidate for biological applications, such as drug delivery and bio-analysis [2]. For instance, it was found that graphene oxide could deliver doxorubicin into cancer cells for the therapeutic purposes [2]. Graphene-paper offers an ideal platform for cell culture experiments owing to the ease of handling [3]. Recently, cancer photo-thermal therapy based on nano-materials that can be activated by a skin-penetrating NIR (Near Infra Red) irradiation has been suggested as a noninvasive, harmless, and highly efficient therapeutic technique. Single wall carbon nanotubes have been widely experienced for cancer cell photo-thermal therapy and because of their well absorbance in NIR wavelength they show promising results in cancer cell destruction [4].

In this paper, we have investigated graphene nano-layers synthesized by melatoni (a bio-compatible material) which enjoys reduced toxicity and will be a highly favorable candidate for the photo-thermal therapeutic. Similar to CNTs, graphene absorbs near-IR (700-1100nm) radiation and as a result a significant amount of heat is generated. Since most biological species including skin are transparent to NIR-irradiation, only graphene-holding species are destroyed. In this line of work, we demonstrate destruction of solid malignant cells (colon cancer cells) by the combined treatments of bio-compatible graphene nano-layers and NIR irradiation. These results show an observable photo-destructive effect of NIR-exposed graphene added cancer cells.

2 EXPERIMENTALS

2.1 Preparation of graphene-oxide and RGO nanosheets:

To chemically reduce the GO suspension, 5 mM MLT solution (MLT powder, N-acetyl-5-methoxytryptamine, C13H16N2O2, 444300-1GM, Merck,) was added to the GO suspension with a concentration of 0.1 mg/mL. To prevent aggregation of the reduced GO in the suspension and to promote the reduction rate, pH of the GO-MLT suspension was adjusted to the range of 9–10 by using ammonia solution (~2 μL for each mL of the suspension). Then, the prepared suspension was sonicated for 30 min. Finally, the

GO-MLT suspensions are heated at temperature of 80 ºC for 3 h.

2.2 Ex Vitro Measurement of Heating a graphene Solution by NIR Radiation

A bio-compatibly synthesized graphene solution (graphene concentration of 100 mg/liter) was irradiated by the 780-nm laser at 3.3W/cm^2. The solution temperature was then measured at 20-s intervals with a thermocouple placed inside the solution for a total duration of 2 min. Care should be taken to avoid exposure of the thermocouple in the beam path to minimize any direct heating of the thermocouple by the laser beam. The result shows an increment of graphene solution temperature to about 57^{o}C after 2 min of NIR exposure. Without graphene, an aqueous solution is transparent without heating under the same radiation conditions.

2.3 Colon cancer Cell Culture

A HT29 cell line was obtained from the National Cell Bank of Iran, Pasteur Institute. Cells were maintained at 37 °C (5% CO_2, 95% air) in RPMI-1640 medium (Sigma 8758) supplemented with 5% fetal bovine serum (Gibco), and 1% penicillin/streptomycin (Gibco). The fresh medium was replaced every other day. HT29 cells were harvested with 0.25% trypsin-EDTA solution (Invitrogen) and the resulting suspended cells with augmented medium were used for further processings.

2.4 Cellular Incubation in graphene Solutions

The incubations of graphene nano-sheets solution with HT29 cells were carried out in 12-well plates, with the cells been seeded for 24 h before incubation. Graphene solution was added to each well (5× 10^3 cells per well) at a final concentration of 3% wt. The incubations were carried out at 37°C and in 5% CO_2 atmosphere for 6h. Figure 1 shows a photograph of the experimental setup used in this study.

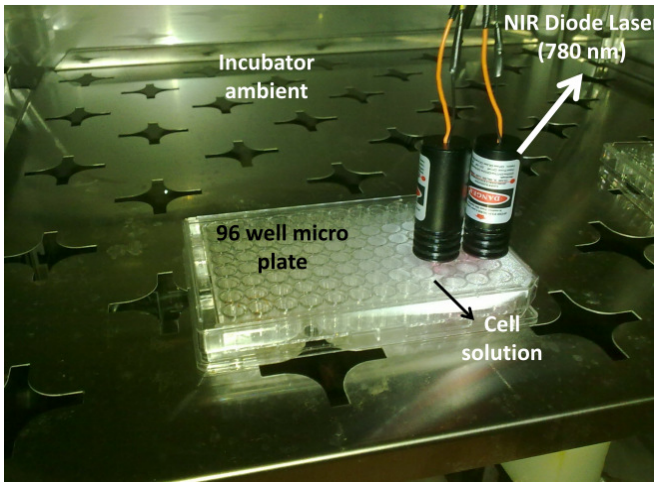

Fig 1. The Photograph of our experiment setup. The cell solutions and the laser sources are placed in an incubator.

2.4 NIR irradiation

some of the wells which contain graphene-added cell solution and cell solution without graphene were exposed to a 780 nm diode laser source for different period of times. The exposure times were ranged from 5 to 25 min. A photograph of the laser ablation setup to cells, holding 96 micro-plates in a cell incubator is shown in figure1. After the exposure process is carried out inside the incubator for 12hr, the cell medium was removed from the well, and the cells were washed and detached from the surface by the addition of trypsin-EDTA solution (Invitrogen) for various characterizations like MTT and flow cytometry.

3 RESULTS AND DISCUSSION

3.1 Characterization of graphene nanosheet:

AFM images of the graphene platelets have been shown in figure 2. It is seen that the films are consisting of overlapping graphene oxide and graphene platelets so that any specific region might comprise of one layer or overlapped layers with typically two or three platelets. The graphene oxide and graphene platelets deposited on the surface showed a relative smooth planar structure. The as-deposited graphene oxide thin film presented in this figure depicts several overlapped graphene platelets. The bottom part in this figure shows the height of a non overlapping layer where two arrows point out the height on top of the graphene layer with respect to the background base-plate.

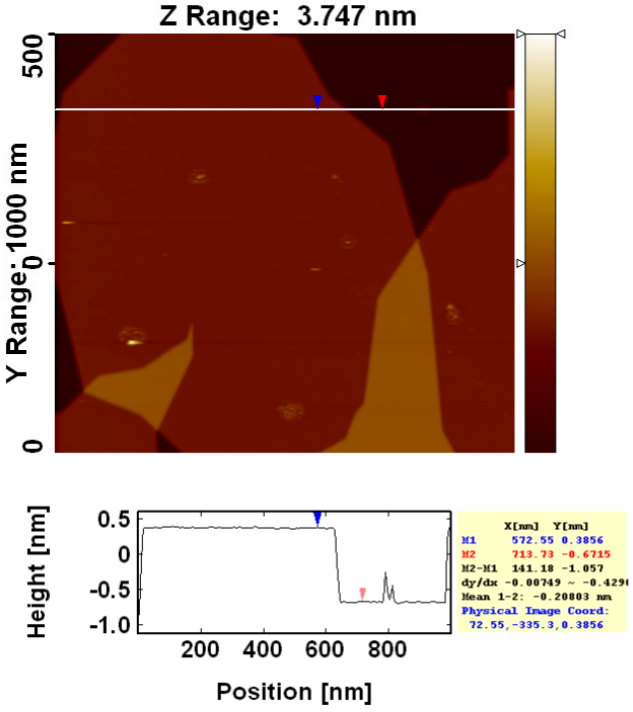

Fig 2. AFM image of reduced graphene oxide several layers. The hight of one layer is about 1 nm.

Raman spectroscopy is a powerful nondestructive technique to study carbonaceous materials such as graphene, especially for examining the ordered and disordered crystal structures and also distinguishing the single-, bi-, and multilayer characteristics of graphene and/or graphene oxide layers. Figure 3 shows Raman spectrum of the reduced graphene oxide prepared by melatonin which is a biocompatible redox agent. Typical features in the Raman spectra of carbon materials are G-line (1580 cm^{-1}) which is usually assigned to the E2g phonon of C sp^2 atoms and the D line (1350 cm^{-1}) as a breathing mode of j-point phonons of A1g symmetry [5] which is assigned to local defects and disorders especially at the edges of graphene and graphite platelets.

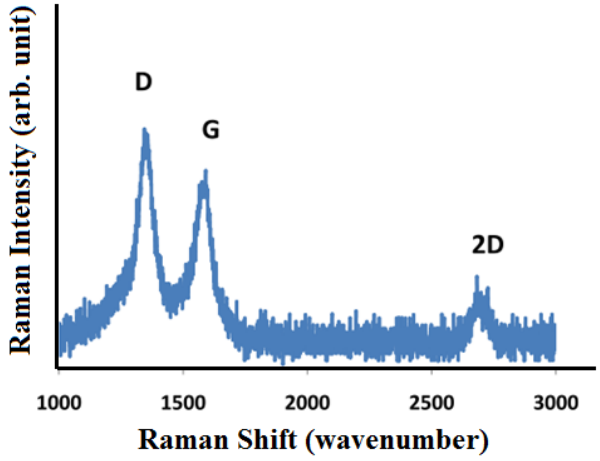

Fig. 3. Raman spectrum of as prepared graphene oxide layers.

The Raman spectrum in Figure 3 displays the G line at 1583 cm^{-1} and the D line at 1342 cm^{-1}. It was previously shown that the peak frequency of the G band of the single-layer graphene sheets (1585 cm^{-1}) shifts about 6 cm^{-1} into lower frequencies after stacking more graphene layers (for 2–6 layers G band shifts to 1579 cm^{-1}) [6].

3.1 Biological tests results:

Figure 4 presents the ratio of remaining live cancer cells after the MTT process of each laser ablated and non ablated cell solution with and without the graphene nano-sheets. As seen from this figure, the exposure of the graphene-included cell solutions with a little concentration of 3%wt of the whole solution to NIR laser ablation would lead to the total destruction of the colon cancer cells. As an important event, the MTT results show that more than 85% of the cells which held with autoclaved graphene solution remained alive after 24hr. Moreover, NIR laser exposure with a 250 mw diode laser (780 nm) to colon cancer cells (without graphene inclusion) for 15 min kills less than 9% of colon cancer cells but exposing the NIR beam to the graphene-added cells for 25 min kills more than 66% of the cells. The NIR absorption of the graphene nano-layers results in the heat increment of the graphene sheets which in turn destructs the colon cells. These results suggest that bio-compatible graphene may potentially serve as an effective photo-thermal agent and pave the way to future cancer therapeutics.

Group1. Control : cells without any treatment
Group2. cancer cells + NIR 15 min continuous irradiation.
Group 3. cancer cells + graphene (without any NIR ablation).
Group 4. cancer cells + graphene + NIR 15 min pulse irradiation.
Group 5. cancer cells + graphene + NIR 15 min continuous irradiation.
Group 6. cancer cells + graphene + NIR 25 min continuous irradiation.

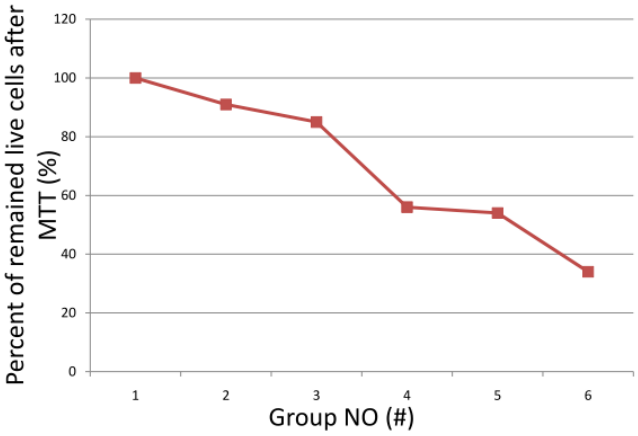

Figure 4. The effect of graphene and NIR irradiation on the destruction of colon cancer cells. Group 1 represents the original cells whereas groups 4 to 6 have been treated. Not a significant difference is observed between groups 4 and 5 where countious or pulsed mode irradiations have been practiced for a total time of 15 mins.

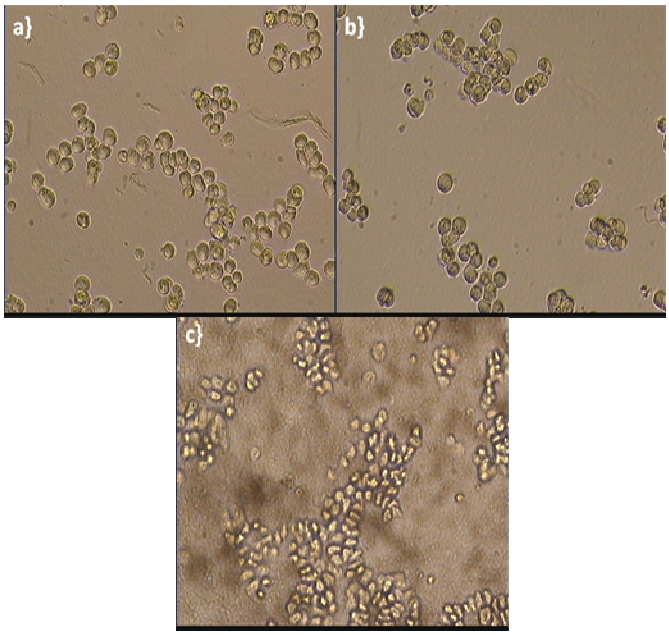

Figure 5. optical microscopy images of a) control colon cancer cells. b) after 15 min NIR ablation c) after 15 min NIR ablation to graphene added colon cancer solution. The rather darker background in the last image is due to the presence of graphene layer. In addition, the smaller size of the observed species corroborates the effectiveness of this technqiue in killing the cancerous cells.

Finally, in Figure 5 a collection of optical microscopy images of colon cancer cells and the effect of graphene layers and NIR ablation are observed. In this figure, part (a) corresponds to the control samples of cell solution without laser ablation whereas part (b) shows the results after laser ablated cell solution for 15 min. The size and clearness of the soultion remain the same, evidencing the little effect of the mere irradiation. However, figure 5-c represents the graphene added cell solution which has been exposed to NIR irradiation for 15 min. It is observed that the combination of graphene nano sheets and laser ablation results in an efficient destruction of cancer cells. Smaller size and deformability of the cells in figure 5-c confirm these effect. All images have been obtained with similar magnifications.

4 CONCLUSION

We have studied the favorable effect of near-IR irradiation on the properly prepared cancer cells. The NIR absorption of the graphene nano-layers results in the heat increment of the graphene sheets which in turn destructs the colon cells. These results suggest that bio-compatible graphene may potentially serve as an effective photo-thermal agent and pave the way to future cancer therapeutics. Further work on the application of this technique for future cancer therapy is being pursued. Authors wish to acknowledge the assistance of Dr. Peirovi for his technical discussions.

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