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Polyphenols attached graphene nanosheets for high efficiency NIR mediated photodestruction of cancer cells

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ABSTRACT

Green tea-reduced graphene oxide (GT-rGO) sheets have been exploited for high efficiency near infrared (NIR) photothermal therapy of HT29 and SW48 colon cancer cells. The biocompatibility of GT-rGO sheets was investigated by means of MTT assays. The polyphenol constituents of GT-rGO act as effective targeting ligands for the attachment of rGO to the surface of cancer cells, as confirmed by the cell granularity test in flow cytometry assays and also by scanning electron microscopy. The photo-thermal destruction of higher metastatic cancer cells (SW48) is found to be more than 20% higher than that of the lower metastatic one (HT29). The photo-destruction efficiency factor of the GT-rGO is found to be at least two orders of magnitude higher than other carbon-based nano-materials. Such excellent cancer cell destruction efficiency provided application of a low concentration of rGO (3 mg/L) and NIR laser power density (0.25 W/cm^2) in our photo-thermal therapy of cancer cells.

1. Introduction

Graphene is a single-atom-thick sheet of sp^2-bonded carbon atoms in a hexagonal two-dimensional lattice, which promises unique potential applications in condensed-matter and high-energy physics [1–3], material science [4–7] and a broad range of technological fields [8] such as ultrasensitive gas sensors, gas storage media, energy production and storage and electronic devices [9–16]. Moreover, because of their unique physicochemical properties, graphene and its derivatives have attracted great attention in biological and biomedical areas [17–19]. Graphene nanosheets have also been used in bio-electro-catalysis [20]. In addition, a high bio-recognition capability has been observed for graphene paper [21]. More recently some graphene based bio-devices such as DNA transistors [22] and electrochemical biosensors [18,23–27] have been developed. These nanolayers have been thoroughly investigated in the field of cell biology. In addition they have been used for controlling the differentiation of human stem cells [28] and offer an ideal platform for cell culture experiments owing to their easy handling [29]. In particular, graphene is a potential candidate for biological applications, such as drug delivery and bio-analysis [17,30]. For instance, it was found that graphene oxide (GO) could deliver doxorubicin into cancer cells for the therapeutic purposes [17].

Recently, carbon nanotubes have been used as noninvasive, harmless and efficient vehicles for photo-thermal therapy of cancer disease. These nano-structures can be activated by a skin-penetrating NIR (near infrared) irradiation which promises to be a highly efficient therapeutic technique. Both single wall and multi-wall carbon nanotubes have been studied [31–35] for such cancer cell photo-thermal therapy due to their high absorbance in NIR wavelength and their capability of cancer cell destruction.

With regard to graphene-based materials new investigations have shown similar effect on cancer photo-thermal therapy using PEG-layered graphene oxide (nanoGO) with a high injected dose (20 mg/Kg) and NIR laser power (2 W/cm^2) [36]. The toxicity and bio-compatibility of graphene is also very crucial in their applications in cancer photothermal therapy. Many studies have been focused on the subject of biocompatibility of graphene in both live cells and bacteria media [37,38]. However there are some controversial reports about the cytotoxicity of graphene based materials [39–43]. These reports suggest that graphene owns a dose dependent toxicity. So, the lower volume and concentration of graphene with higher optical absorbance efficiency is more suitable for photo-thermal therapy. Recently, nano-sized PEG layered rGO was used by Robinson et al. [37] for cancer photothermal therapy with an increased cancer cell destruction using a lower graphene concentration as compared to the previous reports [36,44]. They used rGO with a lower concentration of 6.6 mg/L for each 4000 cancer cells and a diode laser with a power density of 15 W/cm^2 to obtain destruction efficiency more than 80%. Furthermore, in their study a branched amphiphilic surfactant was used to noncovalently coat nano-rGO structures to achieve stability and biocompatibility in biological buffer solutions.

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Usually hydrazine is used to realized rGO, although there have been reports of using certain anti-oxidant bio-materials with aromatic structures such as vitamin C [45], melatonin [46,47] and green tea [48] which can be applied for the reduction of graphene oxide. Compared to hydrazine, these bio-materials enjoy certain advantages in cancer photothermal therapy as mentioned in Ref [36,44]. Apart from their biocompatibility, their aromatic rings could make a π–π binding structure with rGO sheets and attach to it [47]. In addition, the aromatic rings of GT (green tea) including the polyphenol groups can be bound to cancer cell's surface receptors [49]. One of the important agents for this binding process is epigallocatechin gallate (EGCG), the main polyphenol in GT, which binds to the cancer cell surfaces [50].

In this work, we applied GT for bio compatible reduction of GO and used GT-rGOs for cancer cell photothermal therapy. EGCG groups which exist in GT-rGO sheets were employed as the binding agent of rGO layers to the cancer cell surfaces. Using this approach, we have been able to lower the concentration and volume of the injected rGO as well as the laser power in the photothermal therapy of colon cancer cells by a great amount and achieve high cell destruction efficiencies.

2. Experimental section

2.1. Cell culture

HT29 and SW48 tumor cell lines were obtained from the National Cell Bank of Iran, Pasteur Institute. HT-29 and SW48 cell lines were isolated from a primary and Grade IV colon tumor respectively. 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 and SW48 cells were harvested with 0.25% trypsin_EDTA solution (Invitrogen).

2.2. rGO preparation

Natural graphite powder (45 μm, Sigma-Aldrich) was used for synthesis of GO through a modified Hummers method. More details on the synthesis of GO powder by this method were previously reported elsewhere [39,51]. Then, a GO suspension was prepared by dispersing the prepared GO powder in distilled water (0.1 mg/mL). Green tea has been used for the reduction of the GO suspension. First, 10 g of GT were infused in 100 mL water at a temperature of 80–86 °C for 10 min followed by the filteration of the extracted suspension. In the next subsequence, the GT solution and GO suspention were mixed by equal volumes at room temperature. Using a magneto-stirrer heater, the prepared GO-GT suspension was heated up to 80 °C while constantly stirred at 400 rpm for 10 min.

2.3. Heat increment measurement of NIR ablated rGO

To measure the heat increment of rGO by NIR irradiation, a biocompatible synthesized rGO solution (rGO concentration: 100 mg/L) was irradiated by the 780-nm diode laser at 0.25 W/cm^2. The temperature was measured at each 20 s intervals with a thermocouple placed inside the solution for a total of 2 min. Special care must be taken to avoid direct exposure of the thermocouple to the laser beam to minimize any undesired heating of the thermocouple by the laser.

2.4. Flow cytometry for biocompatibility testing of rGO

To prepare the sample for flow cytometery (Protech Co.), the cell culture media were first removed and cells were resuspended using trypsin. Then, trypsin was inactivated using the old cell culture media of each well. Resuspended cells were washed two times with PBS. They were then precipitated in a centrifuge at 2000 rpm. The supernatant, which is cell free, was discarded and the cell pellet was resuspended in 500 μl of PI along with 1 μg of RNase.

2.5. rGO based cancer photothermal treatment

For cancer photothermal investigations, the incubations of rGO nano sheets solutions with HT29 and SW48 cells which were grades 1 and 4 of colon cancer, respectively, were placed into 96-well plates, while the cells have been seeded for 24 h before incubation (3×10^4 cells per well). rGO solution was then added to each well at a final concentration of 3 mg/L. The incubations were carried out at 37 °C and in 5% CO_2 atmosphere for 6 h. Some of the wells which contained rGO added cell solution and cell solution without rGO were exposed to NIR diode laser for different time durations. The exposure times ranged from 10 to 20 min.

2.6. MTT assays for testing cancer cell's viability after photothermal process

After the exposure process, the wells were placed in an incubator for 12 h. In the next step the cell medium was removed from the wells and the cells were washed and detached from the surface by the addition of trypsin-EDTA solution (Invitrogen) for the subsequent characterization like MTT. The cell proliferation was assessed by MTT assay in accordance with the Sigma-Aldrich (M2128) guideline. The colorimetric 3-(4,5-dim ethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) test evaluates viability and proliferation of cells on the basis of reduction of yellow tetrazole to purple formazan in living cells. The metabolic activity of cells is proportional to the color density development. SW48 and HT29 cells were cultured in different wells at a concentration of 5×10^5 cells/ml in RPMI medium and supplemented with 10% FBS for 24 h. The negative controls and rGO and laser treated samples are all incubated on the same 96-well culture plate. For viability analysis the media were removed and replaced with 90 μL of serum-free media. 10 μL of the MTT solution with the concentration of 5 mg/mL was added to each well. Then the plate was incubated for 4 h at 37 °C and 5% CO_2. The supernatant was then removed, and 100 μL of dimethylsulfoxide was added to each well. After 20 min shaking to dissolve the MTT formazan, the light absorbance was recorded at 493 nm using a D.E.E READ (Dia Med Eurogen Co.) microplate reader. The reference data was taken from wells without cell which have been incubated with the MTT solution. The cell viability was calculated by the normalization of optical densities (OD) to the negative control.

2.7. Cell sample preparation for SEM imaging

After rGO's incubation process, the cells sample was fixed with 5% glutardhyde solution and dried in air. The samples have been thoroughly investigated using field-emission SEM apparatus (Hitachi 4160 unit).

3. Results and discussion

In this section, we have examined the physical properties of graphene-oxide prior and after the reduction process has been accomplished. In the subsequent sections, the bio-compatibility properties of such layers and their effectiveness in thermal therapy of cancerous cells will be discussed.

3.1. Physical properties

In an attempt to obtain a microscopic view of the graphene sheets, we have used an AFM apparatus. AFM images of the rGO platelets have been shown in Fig. 1. It is seen that the films consist of overlapping GO and rGO platelets so that any given region might comprise of one layer or overlapped layers with typically two or three overlapped