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Influence of carbon nanotubes and graphene nanosheets on photothermal effect of hydroxyapatite

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Abstract

Herein we present a successful strategy for efficient enhancement of photothermal efficiency of hydroxyapatite (HAP) by its conjugation with carbon nanotubes (CNTs) and graphene nanosheets (GR). Owing to excellent biocompatibility with human body and its non-toxicity, implementation of HAP based nanomaterials in photothermal therapy (PTT) provides non-replaceable benefits over currently using PTE agents. Therefore, in this report, it has been experimentally exploited that the photothermal effect (PTE) of HAP has significantly improved by its assembly with CNTs and GR. It is found that the type of carbon nanomaterial used to conjugate with HAP has influenced on its PTE in such a way that the photothermal efficiency of GR-HAP was higher than CNTs-COOH-HAP under exposure to 980 nm near-infrared (NIR) laser. The temperature attained by aqueous dispersions of both CNTs-COOH-HAP and GR-HAP after illuminating to NIR radiations for 7 min was found to be above 50 °C, which is beyond the temperature tolerance of cancer cells. So that the rise in temperature shown by both CNTs-COOH-HAP and GR-HAP is enough to induce the death of tumoral or cancerous cells. Overall, this approach in modality of HAP with CNTs and GR provide a great potential for development of future nontoxic PTE agents.

Graphical Abstract

Keywords

Hydroxyapatite; Graphene; Carbon nanotube; Photothermal effect; Near-infrared radiation

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Introduction

The employment of near-infrared (NIR) radiations in photothermal therapy (PTT) to treat cancer is current high pitched interest aside from its classical applications such as telecommunication, sensing ablation etc [1,2]. The reason is that NIR region (700–1100 nm) is ideal clinical phototherapeutic window for PTT as attenuation of NIR radiations by skin, blood and tissues is low and it allow for the treatment of deep-seated tumors. [3–5]. The application of PTT in the treatment of tumors has been identified as a minimally invasive alternative to conventional hyperthermia treatment owing to its remote controllability, low systemic toxicity, and minor side effects [6,7]. The conventional hyperthermia treatment, currently in use increases the rate of biochemical reactions, which induces the generation of reactive oxygen species. This oxidative stress gradually leads to destruction of plasma membranes, proteins, and nucleic acid [8–10]. For direct cell necrosis in conventional hyperthermia treatment, long-term exposures (>60 min) to temperatures is required [10,11]. On the other hand, temperature above 48 ° C instantaneously cause irreversible protein coagulation and DNA damage that lead to death of cells even for a short time exposure (4–6 min) [10]. Under these circumstance, PTT is a suitable method to destroy tumor tissues as it possess supercritical benefits such as spatiotemporal controllability, minimal invasiveness, and independence of tumor type [12]. While, the implementation of PTT relies on the development of suitable photothermal coupling agents. Among the rest of PTT agents, typical sp² carbon nanomaterials, such as carbon nanotubes (CNTs), graphene (GR) and fullerene, have been intensely investigated for PTT applications owing to their exceptional structural and functional properties [10,13]. On a per-mass basis, both CNTs and GR possess a larger extinction coefficient of NIR light absorption than gold nanomaterials, and consequently leads to higher photothermal conversion efficiency [14].

Meanwhile, one-dimensional (1-D) CNTs possess strong absorption in NIR region between 700–1100 nm, which is particularly attractive as the living tissues do not strongly absorb in this region and tissue penetration is optimal during hyperthermia treatment, so that CNTs are promising agents of PTT [3,14,15]. It is revealed that execution of CNTs is a superlative platform for NIR triggered destruction of cancer cells through photothermal ablation [16–19]. In addition, selective destruction of cancerous cells is possible through proper functionalization of CNTs without harming to healthy cells [20,21]. In particular, after functionalization, CNTs may easily come across the membrane into the cell via endocytosis and diffusion [22,23]. It means CNTs can serve as drug carriers that deliver drug molecules for chemical therapy to the targeted cells. The large surface area of CNTs, together with their hollow structure, enables them to be loaded with a large quantity of drug molecules [24,25]. The attachment of drug molecules to CNTs can also effectively prolong the circulation time of drug molecules in blood and thus enhances cellular uptake of the drug by cancer cells [24,26]. Moreover, owing to characteristic of Raman scattering, CNTs can be used as tracer to monitor the distribution of drug molecules in human body as well [27,28]. Apparently, CNTs can take a multiple role in malignant tumor therapy, for instance, drug carrier, light-inducing heat treating agent, and drug molecule tracer. Additional critical aspect for the selective CNTs mediated thermal ablation of cells is the physiological stability of the linkage between the targeting moieties and the CNTs.

Another carbon nanomaterial, which has equal prominence like CNTs is graphene (GR) and recently it is attracted tremendous attention owing to its two-dimensional (2D) structure, high surface area, easy surface functionality [29], biocompatibility [30–32], high thermal stability, and enhanced NIR absorption capability in the first and second biological window (650–950 nm and 1000–1350 nm) made it as an ideal theranostic platform for future nanomedicine [14,33]. Due to its excellent photon-thermal transfer efficiency under NIR irradiation, GR has been used for PTT in vitro [14,34] and in vivo [35] for combined PTT and chemotherapy or photodynamic therapy (PDT) [36]. This type of strategy is advantageous by minimizing therapy time and avoiding the utilization of multiple laser systems.

Therefore, the execution of CNTs and GR could help in development of innovative multimodal therapies that combine PTT and PDT. The PDT is a noninvasive phototherapy, which is currently using in clinical practice, [37,38], so that combination of PTT with PDT is highly feasible and it bring great clinical value. However, the poor dispersibility of CNTs and GR in water has restricted their application in PTT. To overcome this problem, functionalization of CNTs and GR with hydrophilic materials is needed. Among a series of hydrophilic conjugatives used in functionalization of CNTs and GR, hydroxyapatite (HAP) is frontline one as it possess excellent biocompatibility and bioactivity with human tissues owing to its identical chemical composition and crystal structure to mineral component present in human hard tissue such as bone and tooth [38,39]. Due to its high binding activity to DNA and proteins, HAP-based biomaterials are currently used in bone and tooth repair [40,41]. In addition, since more than a decade, HAP has been used as a drug carrier [42,43]. The HAP can safely deliver the drug by protecting gene, drug and protein present in cells because of its good adsorption capacity and increasing solubility in the lower pH or acidic environment of cells [44–46]. Since the 1990s, the inhibitory effect of HAP on the proliferation of cancer cells was investigated and reported [47,48]. It is revealed that HAP inhibit the proliferation of cancer cell and it demonstrated higher inhibitory effect on cancer cell than normal cells by preventing the synthesis of protein [49]. Therefore, execution of HAP in PTT is a smart movement to overcome from toxic effects caused over human tissue by use of non-biocompatible materials in the treatment.

In consideration of its importance and biocompatibility, herein we prepared the NIR active, HAP based nanocomposites comprised of CNTs and HAP (CNTs-COOH-HAP) and GR and HAP (GR-HAP). Both, CNTs-COOH-HAP and GR-HAP have exhibited excellent photothermal conversion ability under exposure to 808 and 980 nm NIR laser systems. While the biological systems are transparent to 700–1100 nm NIR radiations, the strong absorbance of CNTs-COOH-HAP and GR-HAP nanocomposites can be used for optical stimulated thermal generation inside body cells to afford various useful functions such as destruction of cancer cells. The CNTs-COOH-HAP and GR-HAP have demonstrated a significant photothermal conversion efficiency of 22.2 and 25.9 %, respectively at 980 nm, and it is comparable with the efficiency of reported photothermal agents. The CNTs-COOH-HAP and GR-HAP hybrid assemblies have possessed an rise in temperature above 50 ° C by irradiating to 980 nm laser, which is beyond the temperature tolerance of cancer cells. Owing to its difficult in the accomplishment of perfectly homogeneous composition between

CNTs and HAP, and GR and HAP by traditional mixing technology, herein we developed the facile *in-situ* method for uniform deposition of HAP over CNTs and GR nanosheets.

2. Experimental section

2.1. Materials

All the reagents were purchased from Aldrich and used without further purification unless otherwise noted. All the aqueous solutions were prepared with ultrapure water obtained from Milli-Q Plus system (Millipore).

2.2. Preparation of CNTs-COOH-HAP nanocomposite

Prior to grafting of HAP over CNTs, the CNTs were converted to CNTs-COOH to achieve an effective grafting of HAP over their surface. The carboxylation of CNTs was performed by refluxing the CNTs in a mixture of 1:3 (v/v) nitric acid and sulfuric acid under stirring at 70 ° C for 24 h, followed by centrifugation, repeated washings with DI water and drying under vacuum [50,51]. Thus produced CNTs-COOH was used for the deposition of HAP and it was carried out by the procedure reported by our group [52]. In brief, 100 mg of CNTs-COOH were dispersed in 25 mL DI water, mixed with 0.01 mol/L aqueous solution of calcium hydroxide and stirred for 1 h under ambient conditions. Subsequently, pH of the mixture was adjusted to 9 by addition of phosphoric acid under constant stirring and the mixture was allowed to stir at room temperature for 30 min. Thus resulted CNTs-COOH-HAP was centrifuged, washed with DI water and dried under vacuum.

2.3. Preparation of GR-HAP nanocomposite

The GR nanosheets utilized in the synthesis of GR-HAP nanocomposite were produced by the reduction of GO and the GO was prepared by graphite powder using Hummers and Offeman method with slight modifications [53,54]. Further, GO was reduced to GR through dispersion of 50 mg of GO in 50 mL ethanol by sonication for 5 mins and the dispersion was subjected to centrifugation and ethanol was removed. Subsequently, the GO was re-dispersed in ethylenediamine to yield a yellow-brown suspension, which was refluxed at 80 ° C for 1 hr. Then the resultant dark suspension of GR nanosheets was centrifuged, washed with ethanol and DI water and dried under vacuum. Then the GR nanosheets thus obtained were used in the grafting of HAP in aid to the method reported by us [55]. In detail, 40 mg of GR nanosheets were dispersed in 40 mL DI water by sonication for 5 mins. To this suspension, an aqueous solution of 0.01 mol L⁻¹ of calcium hydroxide was slowly added and the mixture was stirred under ambient conditions for 1 h and its pH was adjusted to 9 using phosphoric acid under constant stirring. Thus resulted HAP deposited GR nanosheets (GR-HAP) was separated by centrifugation, washed with DI water and dried under vacuum.

2.4. Photothermal effect

The photothermal effect of CNTs-COOH-HAP and GR-HAP was evaluated using 808 and 980 nm NIR diode laser systems (Armlaser Inc. USA) with an output power of 2 W/cm². In each experiment, 1 mL aqueous dispersion of the sample was transferred into 1 × 1 × 4 cm³ quartz cuvette and illuminated with NIR laser. Then the temperature variation mediated by the exposure to laser radiations was measured using Hanna precision digital thermometer

(Model: HI93510) carrying a thermocouple, which was immersed in the aqueous dispersions during experiment.

2.5. Characterization

ATR-IR spectra were recorded using Smiths ChemID diamond attenuated total reflection (DATR) spectrometer and the powder XRD patterns were recorded on Scintag X-ray diffractometer (PAD X), equipped with Cu K α photon source (45 kV, 40 mA) at scanning rate of 3°/min. SEM measurements were carried out on a JEOL JXA-8900 microscope and the Raman spectra were recorded with Renishaw R-3000QE system in the backscattering configuration using an Argon ion laser with wavelength 785 nm. The UV-vis absorption spectra were recorded using Varian Carry 50 Bio UV-vis spectrophotometer.

3. Results and discussion

The ATR-FTIR spectra of CNTs-COOH, CNTs-HAP, HAP, GO, GR and GR-HAP are depicted in Fig. 1. The spectrum of CNTs-COOH (Fig. 1a) displayed the characteristic peak corresponding to vibrations of -COOH groups, bonded to the surface of CNTs at 1755 cm⁻¹, which reveals the successful oxidation of CNTs. The bands observed at 559 and 600 cm⁻¹ in the spectrum of HAP (Fig. 1b) were assigned to P—O bending of phosphate group and the band at 1021 cm⁻¹ was attributed to O—P—O carbonate ions of hydroxyl sites [52]. The bands at 3366 and 1648 cm⁻¹ were attributed to stretching and bending vibrations of hydroxyl (—OH) group. The band at 1419 cm⁻¹ was owing to the vibrational mode of carbonate ion [52]. Overall, the spectrum of HAP displayed well resolved characteristic bands of natural HAP. The spectrum of CNTs-HAP (Fig. 1c) exhibited all the characteristic bands of HAP, while the bands related to CNTs were not distinctly observed, which might be due to low concentration ratio of CNTs compared to HAP. The spectrum of GO (Fig. 1d) shows a broad band around 3395 cm⁻¹ due to O—H stretching and an intense band at 1619 cm⁻¹ owing to aromatic C=C vibrations. In addition, the bands related to C=O, carboxy C—O, epoxy C—O and alkoxy C—O groups exist on the surface of GO nanosheets were observed at 1719, 1377 and 1161 and 1039 cm⁻¹, respectively. After reduction of GO, the characteristic bands of oxygen containing functional groups such as O—H, C=O and C—O disappeared in the spectrum of GR (Fig. 1e). Moreover, a new band was observed at 1509 cm⁻¹, which is ascribed to skeletal vibration of GR nanosheets [55]. The conformational changes displayed in the spectrum of GR facilitates the successful conversion of GO to GR. Further, the spectrum of GR-HAP (Fig. 1f) demonstrated the characteristic bands of HAP. The bands corresponding to P—O bending of phosphate group were observed at 563 and 599 cm⁻¹ and the vibrations of O—P—O carbonate ions of hydroxyl sites was displayed at 1021 cm⁻¹. While, the band appeared at 1422 cm⁻¹ was attributed to vibrations of carbonate ions. Overall, the spectra of CNTs-HAP (Fig. 1c) and GR-HAP (Fig. 1e) are identical to the spectrum of HAP (Fig. 1b), which reveals that HAP has retained the essential feature of its native structure in both CNTs-HAP and GR-HAP.

In addition, formation of CNTs-COOH-HAP and GR-HAP and the structural transformations were monitored using XRD patterns depicted in Fig. 2. The pristine CNTs (Fig. 2a) revealed the presence of two diffraction peaks at 26.4° and 42.6° corresponding to

(0 0 2) and (1 0 0) reflections of carbon atoms of CNTs, respectively [25]. The well pronounced peak at 42.6° in CNTs is clearly visible in Fig. S1. The d-spacing value calculated for CNTs was found to be 0.346 nm. The high intensity of reflection peaks in CNTs demonstrates the high graphitic structure of pristine CNTs. After carboxylation, the (0 0 2) and (1 0 0) reflections of CNTs have slightly shifted to 24.0° and 43.4° in CNTs-COOH (Fig. 2b), which could be resulted from the defects generated over the surface of CNTs by the process of oxidation. Moreover, the characteristic peaks in CNTs-COOH (Fig. S1) have significantly broadened compared to CNTs, which is accounted for shortening of CNTs by the treatment of acid mixture. By the process of oxidation the d-spacing value in CNTs-COOH has raised to 0.380 nm and this increment is ascribed to introduction of -COOH groups and the defects over the surface of CNTs. Then the principle reflections appeared in Fig. 2(c) at 2θ value of 25.7° , 31.9° , 32.7° , 39.6° , 46.7° , 49.4° , 53.1° and 64.3° are assigned to (0 0 2), (2 1 1), (1 1 2), (3 1 0), (2 2 2), (2 1 3), (0 0 4) and (3 0 4) planes of HAP, respectively (JCPDS File No. 09-0432). All the characteristic peaks of HAP were displayed in the pattern of CNTs-COOH-HAP (Fig. 2d) in addition to the bands associated to CNTs. While, the principle peaks of CNTs and HAP corresponding to their (0 0 2) have overlapped and appeared as a single peak at 25.9° in the pattern of CNTs-COOH-HAP. Overall, no radical shift in the position of characteristic peaks of CNTs-COOH (Fig. 2b) was observed for CNTs-COOH-HAP (Fig. 2d), which suggests, retainment of original structure of CNTs-COOH after grafting of HAP also. The pattern of graphite (Fig. 2e) displayed a high intense peak at 26.4° owing to (0 0 2) plane of graphite and it correspond to an interlayer d-spacing value of 0.346 nm. In Fig. 2(f), the characteristic peak of GO narrated to its (0 0 2) reflection was displayed at 13.2° . The substantial shift in the characteristic peak of GO compared to native material, graphite indicates complete oxidation of graphite to GO and disruption in ordering of graphene layers of GO. After oxidation, the interlayer d-spacing in GO was increased to 0.676 nm and it is ascribed to presence of oxygen containing functional groups such as carboxyl (-COOH), hydroxyl (-OH), epoxy groups and inserted H_2O molecules and the structural defects exists in GO [56]. Further, the GR (Fig. 2g) exhibited two distinctive peaks at 2θ value of 26.5° and 44.5° , and these peaks were assigned to (0 0 2) and (1 0 0) reflections of hexagonal structural GR, respectively [55]. After reduction of GO to GR, the d-spacing value in GR was reduced to 0.35 nm, which facilitates the successful conversion of GO to GR and effective removal of oxygen containing functional groups situated over the surface of GO. In addition, the widening of the principle peak at 26.5° in GR indicates its existence in nanosize. In GR-HAP (Fig. 2h) all the characteristic peaks related to HAP were observed in addition to two prominent peaks at 25.3° and 44.9° corresponding to (0 0 2) and (1 0 0) reflections of GR, respectively, it reveals the effective grafting of HAP over GR nanosheets. The slight modifications of peaks displayed for GR-HAP (Fig. 2h) compared to HAP (Fig. 2c) and GR (Fig. 2g) implicates that the native structure of both HAP and GR was well retained after their conjugation also. The variation in the value of d-spacing for all the samples were estimated and presented in Table 1. The interplanar d-spacing value calculated for CNTs-COOH-HAP (0.354 nm) and GR-HAP (0.360 nm) indicates the existence of high ordered graphitic structure in them.

The Raman spectrum of pristine CNTs shown in Fig 3(a) displayed its G-band at 1587 cm^{-1} and D-band at 1306 cm^{-1} , while the position of these G- and D-bands was shifted to 1598

and 1325 cm^{-1} , respectively in the spectrum of CNTs-COOH (Fig 3b). The shift occurred in frequency of G- and D-bands for CNTs-COOH towards high frequency compared to CNTs is attributed to generation of defects in the graphite structure of CNTs due to oxidation. Subsequently, owing to anchoring of HAP over CNTs-COOH, the location of G- and D-bands was displaced to 1594 and 1313 cm^{-1} , respectively, in CNTs-COOH-HAP (Fig 3c). The repositioning of G- and D-bands in CNTs-COOH-HAP towards low wavenumbers side compared to CNTs-COOH shows the defect density of CNTs-COOH has reduced by effective deposition of HAP in CNTs-COOH-HAP. It is known that the G-band is a characteristic feature of graphitic carbon layers corresponding to tangential vibration of carbon atoms and the D-band is a typical signature of presence of defective graphitic carbon. Therefore, the intensity ratio of D to G-band (ID/IG) suggests the order of surface modification in the measured samples. The estimated value of ID/IG for all the samples is given in Table 2. The value of ID/IG calculated for CNTs was 0.40 and this value for CNTs-COOH was 1.07. The lower value of ID/IG for CNTs signifies that the CNTs utilized here were consist of well-crystallized graphite. However the higher ID/IG ratio estimated for CNTs-COOH indicates that disordering of intrinsic nanotube structure. Further, the reduction in the value of ID/IG assessed for CNTs-COOH-HAP can be narrated to internal disordering graphite structure in CNTs-COOH has affected by the grafting of HAP. In addition, the spectrum of GO (Fig. 4a) exhibits its G- and D-bands at 1589 and 1308 cm^{-1} , respectively. After reduction of GO, the G- and D-bands in GR (Fig. 4b) were relocated to 1591 and 1315 cm^{-1} , respectively. The consequent functionalization of GR with deposition of HAP resulted to repositioning of G- and D-bands in GR-HAP (Fig. 4c) at their frequency of 1581 and 1296 cm^{-1} , respectively. The downward shifting of G- and D-bands in GR-HAP compared to GR show the stacking of GR nanosheets in GR-HAP by the integration of HAP. It is revealed that the position of G-band in the single layered GR sheets shifts to lower wavenumber after stacking into number of GR layers [57,58]. So that the position of G-band at 1591 cm^{-1} in GR could be attributed to existence of single layered GR nanosheets. Moreover, the appearance G-band with high intensity in the spectrum of GR suggests the high graphitization of GR nanosheets prepared in this process. Then the value of ID/IG calculated for GO was 1.61, which is significantly higher than the value estimated for GR (0.84), this assist the successful conversion of GO to GR by effective removal oxygen containing functional groups persist over the surface of GO. Furthermore, the ratio of ID/IG in GR-HA has reduced to 0.74 from its original value of 0.84 in GR after grafting of HAP. It signifies that the graphitic planes in GR were influenced by the conjugation of HAP.

The SEM image of CNTs-COOH (Fig. 5a) shows the successful oxidation of CNTs and effective separation of each CNT from other due to occurrence of repulsion between carboxylic groups persists on the surface of oxidized CNTs. The images of CNTs-HAP (Figs. 5b and c) reveals efficient deposition of HAP over individual CNT without formation of agglomeration. It can be seen that the porous structure has generated by complete covering of CNTs with HAP. It is visible that the shape of CNTs in Fig. 5(a) resembles with CNTs found in Figs. 5(b) and (c), it clarifies that the integral shape of CNTs has not altered by grafting of HAP. Further, the image of GO shown in Fig. 5(d) exhibits the effectual loosening of GO nanosheets and their porous structure due to opening of planer carbon networks wedged at the edge surface of crystallite by the process of oxidation. It is also

noticeable that GO is effectively exfoliated into thin nanosheets and possess wrinkled-paper-like morphology. From the images of GR-HAP (Figs. 5e and f) it is accessible that both surface of GR nanosheets and also their inter layers are densely packed with HAP, which resulted in the formation of sandwich-like layered structure between GR nanosheets and HAP. Moreover, the GR nanosheets persist in GR-HAP are of single-layered, curled and entangled. Overall, the SEM images of CNTs-HAP and GR-HAP exhibits the formation of homogeneous nanocomposites and the intimate contact occurs between CNTs and GR with HAP. During the process of purification of CNTs-HAP and GR-HAP, series of washing of with DI water did not detach the HAP from CNTs and GR nanosheets, which demonstrates the strong adherence of HAP over the surface of CNTs and GR nanosheets.

The optical property of the aqueous dispersion HAP, CNTs-COOH-HAP and GR-HAP was examined using UV-vis-NIR spectroscopy. The spectrum of HAP, shown in Fig. 6(a) does not show any typical absorption band in UV-vis region, while it exhibited a strong absorption band in the NIR region between 900–1100 nm. The spectrum of CNTs-COOH-HAP (Fig. 6b) displayed the characteristic absorption band of C=C bonds in CNTs at 244 nm [50,51,59]. Moreover, the spectrum of GR-HAP (Fig. 6b) demonstrated an absorption band at 242 nm corresponding to π $\rightarrow$ π^* transitions of the aromatic C-C bonds in GR [54,56]. In addition to their characteristic absorption bands, both CNTs-COOH-HAP and GR-HAP shown the strong absorption band in the NIR region. The strong absorption revealed for CNTs-COOH-HAP and GR-HAP in the NIR region could be originated from the electronic transitions between the first or second van Hove singularities of the CNTs and GR nanosheets [60,61].

The strong SPR absorption exhibited by CNTs-COOH-HAP and GR-HAP in the NIR region inspired us to analyze the PTE of these nanocomposites since such a strong absorption in the NIR region implies a potential for their photothermal conversion. The photothermal conversion occur in NIR region is very fruitful because the NIR region is considered as a biological window owing to living cells and tissues have low light scattering and adsorption in this region. [21]. Herein, the conjugation of CNTs and GR nanosheets with HAP does not only improve its absorption ability to NIR radiations but also enhances its photothermal conversion efficiency as it is revealed that both CNTs and GR are promising PTE candidates owing to their excellency in conversion of photo-excitation energy into thermal energy [62]. To access the PTE of CNTs-COOH-HAP and GR-HAP and provide a clarification to use them as potential PTE agents, the elevation in temperature of aqueous dispersions of CNTs-HAP and GR-HAP was measured under exposure to 980 nm NIR laser at their concentration level of 4, 2 and 1 mg/mL. Further to evaluate the PTE in detail, the photothermal conversion ability of CNTs- COOH-HAP was compared with those of water, CNTs, CNTs-COOH and HAP at their concentration level of 4 mg/mL. Fig. 7 exhibits the time-dependent temperature rise in response to 980 nm NIR irradiation. After 7 min of irradiation, water shows a slight raise in temperature of 8.3 ° C (Table 1), which indicates that the absorption ability of water to NIR radiations is very low. Under identical conditions, the CNTs explored an elevation in temperature of 37.0 ° C and after oxidation of CNTs, the photothermal conversion capability of CNTs-COOH has lowered to 35.5° C. The observed lowering in PTE of CNTs-COOH compared to CNTs could be due to defects generated in the hexagonal framework of graphite of CNTs-COOH by the harsh treatment of mixture of strong acids

during the process of oxidation. Thus resulted defects over the graphite layer of CNTs-COOH could be lowered the absorption rate of NIR radiations, which deteriorated its PTE. The subsequent functionalization of CNTs-COOH by the grafting of HAP caused to significant promotion in PTE of CNTs-COOH-HAP. The considerable increment in PTE of CNTs-COOH-HAP (45.6 ° C) compared to its individual components, *viz.*, CNTs-COOH (35.5 ° C) and HAP (21.9 ° C) could be attributed to enhancement in the absorption power to NIR radiations by the effective deposition of HAP over graphite surface of CNTs-COOH and existence of strong interaction and intimate contact between them. As the CNTs exhibited higher PTE than CNTs-COOH, so that we measured the PTE of CNTs-HAP (37.7 ° C), which is considerably lower than the rise in temperature found for CNTs-COOH-HAP (45.6 ° C). The higher PTE possessed by CNTs-COOH-HAP could be recited to defects formed over the graphite layer in CNTs-COOH provides an ideal platform for effectual deposition of HAP over the surface of CNTs-COOH. Conversely, absence of defects in pristine CNTs does not support the efficient grafting of HAP over their surface, which hinders the PTE.

Further, the PTE of GR-HAP was elucidated by its quantitative comparison with those of water, graphite, GO, GR and HAP under exposure to 980 nm laser at their concentration level of 4 mg/mL by maintaining the identical irradiation conditions (Fig. 8). The PTE of water was about 8.3 ° C, which was significantly lower than the PTE of graphite (36.0 ° C). The light to heat conversion ability of graphite was additionally improved through its oxidation to GO (40.5 ° C). This observation is contradictory to above mentioned result that is the PTE of CNTs (37.0 ° C) was lowered by their oxidation to CNTs-COOH (35.5 ° C). On basis of this, the enhancement observed in the PTE of GO, compared to graphite could be related to breaking down of the micrometer (μm) sized graphite sheets to nanometer (nm) sized GO sheets by the process of oxidation. As a supportive to this statement, the existence of GO in its nanometric size was revealed by SEM image (Fig. 5d). However, the rise in temperature observed for GO was found to be substantially lower than its reduced form, GR (46.8 ° C). This improvement in the PTE of GR might be due to effective removal of various functional groups such as O-H, C=O and C-O persists over the surface of GO nanosheets, which amplified the absorption of NIR radiations by GR nanosheets. Ultimately, the high absorption rate of NIR radiations over GR nanosheets led to its high light-to-heat conversion ability. Consequently, in a marked contrast to GR and HAP, the PTE of GR-HAP was turned to substantially higher to 48.7 ° C. The additional rise in temperature of 26.8 ° C (=48.7-21.9 ° C) in GR-HAP compared to PTE of HAP (21.9 ° C) is accounted to introduction of GR nanosheets in HAP.

Furthermore, it was noticed that the PTE of aqueous suspensions of CNTs-COOH-HAP and GR-HAP was linearly depends on their concentration level. The PTE of CNTs-COOH-HAP and GR-HAP was relatively increased in accordance to their concentration level in the order of 1, 2 and 4 mg/mL, respectively (Figs. S2 and S3). Both CNTs-COOH-HAP and GR-HAP did not show any photo bleaching or structural rupture by retaining the almost constant PTE for the same aqueous dispersion used in three consecutive cycles (Figs. S4 and S5). In addition, the PTE of CNTs-COOH-HAP and GR-HAP was evaluated using 808nm laser also and it was perceived that the PTE of both the samples at 808 nm is lower than its value at 980 nm (Fig. S6, Table 4). The reduction in the PTE of CNTs-COOH-HAP and GR-HAP by

switching to 808 nm could be explained on the basis of their UV-vis-NIR spectra (Figs. 6b and c) since the absorption of these samples at 808 nm is lower than 980 nm. To recognize any possibility of PTE of CNTs-HAP and GR-HAP under exposure to UV and visible light, the irradiation experiments were performed by replacing the NIR laser with UV and visible light, which did not show any PTE.

Moreover, the photothermal conversion efficiency of CNTs-COOH-HAP and GR-HAP was measured according to Roper's report with slight modifications [1,63–65]. The variation in temperature of the aqueous dispersion of CNTs-COOH-HAP and GR-HAP (4 mg/mL) was monitored as a function of time under exposure to 980 nm laser for 7 min, where the variation in temperature was reached to steady condition. In case of CNTs-COOH-HAP, 7 min of irradiation raised its temperature to 69.0 °C and for GR-HAP the temperature was elevated to 72.2 °C under identical experimental conditions. After 7 min of irradiation, the shut off of laser source implied to drop in temperature of aqueous dispersion of CNTs-COOH-HAP and GR-HAP (Fig. 9). The reduction in temperature of aqueous dispersions was systemically monitored to evaluate the rate of heat transfer from aqueous dispersion to surrounding environment using the following equation (1).

$$\eta = \frac{hS(T_{max} - T_{surr}) - Q_{dis}}{I(1 - 10^{-A_{980}})} \quad (1)$$

Where η is the photothermal efficiency, h is heat transfer coefficient, S is the surface area of the sample container. T_{max} is the maximum temperature attained by system of aqueous dispersions and it is 69.0 and 72.2 °C for CNTs-COOH-HAP and GR-HAP, respectively. T_{surr} is the surrounding temperature, which was 23.4 °C for CNTs-COOH-HAP and 23.5 °C for GR-HAP. Subsequently, the value of $(T_{max} - T_{surr})$ was 45.6 °C for CNTs-COOH-HAP and it was 48.7 °C for GR-HAP. I is the power of laser source, which is 2000 mW. A_{980} is the absorbance of aqueous dispersions of CNTs-COOH-HAP (1.215) and GR-HAP (1.258) at an excitation wavelength of 980 nm. The Q_{dis} is the rate of heat dissipated due to absorption of light by solvent and container.

To calculate the value of hS , a dimensionless driving force of temperature, θ is introduced and scaled using the maximum system temperature, T_{max} and the surrounding temperature, T_{surr}

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \quad (2)$$

and the sample system time constant, τ_s was evaluated using the following equation (3)

$$\ln \theta \quad (3)$$

Then the value of τ_s was calculated by Figs. S7 and S8, and using its value the unknown parameter, hS was evaluated with the help of following equation (4).

$$m_D C_D \tau_s \quad (4)$$

where m_D (1.01g) mass of DI water and C_D is its heat capacity. The value of Q_{dis} was measured separately using quartz cuvette containing only DI water without any sample and it was found to be 25.9 mW.

Hence the 980nm photothermal conversion efficiency (η) calculated for CNTs-COOH-HAP was 22.2 % and for GR-HAP it was 25.9%. The photothermal conversion efficiency estimated for CNTs-COOH-HAP and GR-HAP is better than the value reported for Au nanoshells (18%) [66], Au nanorods (22%) [66] and Cu_{2-x}Se nanoparticles (22%) [67], and close to the value obtained for Au nanoshells (25%) [68] and Cu_9S_5 nanoparticles (25.7%) [1]. The outstanding PTE observed in CNTs-COOH-HAP and GR-HAP could be related to their high absorption ability to NIR radiations, which was confirmed by UV-vis absorption spectra, causes rapid photoexcitation of electrons in CNTs-COOH-HAP and GR-HAP under illuminating to NIR laser. Hence the process of electron excitation leads to generation of high amount of thermal energy in both CNTs-COOH-HAP and GR-HAP. On the basis of this elucidation, mechanism persist with excellent PTE of CNTs-COOH-HAP and GR-HAP can be illustrated as shown in Fig. 10.

It is established that the PTE of CNTs-COOH-HAP was lower than the GR-HAP under illumination to 980 nm laser radiations (Fig. 11). Accordingly, the PTE of GR was found to be higher than CNTs (Table 3). The superior PTE of GR-HAP compared to CNTs-COOH-HAP could be ascribed to giant two-dimensional planar structure of GR, which extends the optical absorption ability of GR-HAP and it facilitates the charge transportation. In addition, the defective sites persists over the surface of GR nanosheets provide an ideal platform for HAP to deposit effectively over their surface and it bring an intimate contact between GR nanosheets and HAP. It results in the structural difference between one dimensional cylindrical CNTs and the two dimensional flat GR. It is touted that the GR possess excellent electrical conductivity since its discovery [69,70]. In real, GR is essentially a CNT cut along its axis and unrolled to lay flat, and it can provide conduction pathways to a greater area per unit mass than CNTs, which should translate into improved conductivity at lower optical densities [71]. Another likely reason for the diminished PTE of CNTs-COOH-HAP could be the cylindrical shape of CNTs may reduce the absorption of NIR photo radiations over their surface compared to the flat surfaced GR nanosheets. The enhanced PTE observed here for GR-HAP compared to CNTs-COOH-HAP is analogous to previous reports, in which a superior photocatalytic activity was found for GR based nanocomposites compared to CNTs based nanocomposites [72,73].

Herein, the temperature attained by aqueous dispersions of CNTs-COOH-HAP and GR-HAP after irradiating to 980 nm laser for 7 min is beyond 50 ° C at their all measured concentration levels (Table 1), which is beyond the temperature tolerance of cancer cells [74,75]. So that the rise in temperature shown by both CNTs-COOH-HAP and GR-HAP is

sufficient to induce the death of tumoral or cancerous cells. These results suggest that both CNTs-COOH-HAP and GR-HAP could be effective photothermal agents and pave the way to future cancer therapeutics. Additional benefit of CNTs-COOH-HAP and GR-HAP is the presence of HAP, as it is known that the HAP is a unique bioceramic that has significant level of current clinical applications. The generous medical applications found for HAP are owing to its mineralogical and chemical properties are identical to the apatite exists in human bone and tooth. Moreover, HAP do not possess any toxic effects with human body tissue [76]. In general, employment of HAP has tremendous bioactivity and excellent biocompatibility with human body compared to rest of the PTE agents used so far. In addition, both CNTs [77,78] and GR [79,80] also have good biocompatibility with human body. Overall, this study reveals that CNTs-COOH-HAP and GR-HAP have possessed significant PTE, so that both these nanocomposites could serve as synergistic agents for efficient implementation of hyperthermia therapy.

4. Conclusions

In conclusion, the unique hybrid structure generates in HAP through conjugation of CNTs and GR enhances its absorption ability to NIR radiations, which significantly enhances the PTE of HAP. The PTE of CNTs-COOH-HAP and GR-HAP was found to be significantly higher than their individual components, *viz.*, CNTs, GR and HAP. The temperature attained by aqueous dispersions of CNTs-COOH-HAP and GR-HAP after irradiating to 980 nm laser for 7 min is above 50 ° C, which is beyond the temperature tolerance of cancer cells. So that the rise in temperature shown by both CNTs-COOH-HAP and GR-HAP is sufficient to induce the death of tumoral or cancerous cells. The hybridization of HAP with CNTs and GR not only improved the photothermal efficiency of CNTs-COOH-HAP and GR-HAP but also their photostability. This strategy provides a multipronged approach to overcome from the non-biocompatible PTE agents currently in use. Apart from PTT, the excellent NIR absorption ability of CNTs-COOH-HAP and GR-HAP is advantageous to other biologically relevant applications such as biosensing. We believe that both CNTs-COOH-HAP and GR-HAP could be promising multimodal platforms to improve current cancer therapeutics owing to their excellent biocompatibility and multifunctionality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. ATR-FTIR spectra of (a) CNTs-COOH, (b) CNTs-HAP, (c) HAP, (d) graphene oxide, (e) graphene, and (f) GR-HAP.

Fig. 2. XRD pattern of (a) pristine CNTs, (b) CNTs-COOH, (c) HAP, (d) CNTs-COOH-HAP, (e) graphite, (f) graphene oxide, (g) graphene, and (h) GR-HAP.

Fig. 3.
Raman spectra of (a) pristine CNTs, (b) CNTs-COOH, and (c) CNTs-COOH-HAP.

Fig. 4.
Raman spectra of (a) graphene oxide, (b) graphene, and (c) GR-HAP.

Fig. 5. SEM images of (a) CNTs-COOH, (b and c) CNTs-HAP, (d) graphene oxide, and (e and f) GR-HAP.

Fig. 6. UV-vis-NIR spectra of (a) HAP, (b) CNTs-COOH-HAP, and (c) GR-HAP.

Fig. 7.
Temperature variation of aqueous dispersions as a function of irradiation time with 980 nm NIR laser.

Fig. 8.
Temperature variation of aqueous dispersions as a function of irradiation time with 980 nm NIR laser.

Fig. 9. Temperature variation of aqueous dispersions of CNTs-COOH-HAP and GR-HAP by irradiation for 7 min with 980 nm laser and followed by laser shut off.

Fig. 10.
Mechanism underlying the photothermal effect of CNTs-COOH-HAP and GR-HAP.

Fig. 11.
Photothermal effect of aqueous dispersions of HAP, CNTs-COOH-HAP and GR-HAP under exposure to 980 nm laser.

Table 1

Sample	d-spacing (nm)
CNTs	0.346
CNTs-COOH	0.380
CNTs-COOH-HAP	0.354
Graphite	0.346
GO	0.676
GR	0.346
GR-HAP	0.360

Table 2

Sample	Position of D-band (cm ⁻¹)	Position of G-band (cm ⁻¹)	ID/IG
CNTs	1306	1587	0.40
CNTs-COOH	1325	1598	1.07
CNTs-COOH-HAP	1313	1594	0.88
GO	1319	1589	1.61
GR	1309	1591	0.84
GR-HAP	1312	1580	0.74

Table 3

Sample	Concentration of aqueous dispersion (mg/mL)	Initial temperature before irradiation with 980 nm laser (° C)	Final temperature after irradiation with 980 nm laser for 7 min (° C)	T _{980nm} (T _{final} -T _{initial})
CNTs	4	22.6	59.6	37.0
CNTs-COOH	4	23.2	58.7	35.5
HAP	4	23.5	45.4	21.9
CNTs-HAP	4	23.9	61.4	37.5
CNTs-COOH-HAP	4	23.4	69.0	45.6
Graphite	4	22.4	58.4	36.0
GO	4	23.4	63.9	40.5
GR	4	23.5	70.3	46.8
GR-HAP	4	23.5	72.2	48.7
HAP	2	23.5	40.6	17.1
CNTs-COOH-HAP	2	23.7	59.1	35.4
GR-HAP	2	23.7	68.9	45.2
HAP	1	23.7	32.5	8.8
CNTs-COOH-HAP	1	23.4	52.8	29.4
GR-HAP	1	23.6	57.8	34.2
Water	-	24.4	32.7	8.3

Table 4

Sample	Concentration of aqueous dispersion (mg/mL)	Initial temperature before irradiation with 808 nm laser (° C)	Final temperature after irradiation with 808 nm laser for 7 min (° C)	T _{808nm} (T _{final} -T _{initial})
HAP	4	23.3	38.1	14.8
CNTs-COOH-HAP	4	23.7	47.5	23.8
GR-HAP	4	23.9	61.4	37.5
Water	-	24.1	26.0	1.9