In-vitro Study of Photothermal Anticancer Activity of Carboxylated Multiwalled Carbon Nanotubes

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ABSTRACT

Background and Objective: Multi-walled Carbon Nano Tubes (MWCNTs) as an important element of nanosciences have a remarkable absorption in the region of NIR window (650-900 nm) which can overcome the limitations of deep treatment in photothermal therapy. To disperse MWCNTs in water, it is proposed to attach carboxylated functional group (-COOH) to MWCNTs in order to increase dispersivity in water.

Materials and Methods: A stable suspension of MWCNTs-COOH with different concentrations (from 2.5 to 500 µg/ml) was prepared. Then, they were compared for their ability to increase temperature in the presence of 810 nm laser irradiation and through a wide range of radiation time (from 20 to 600 s) and three laser powers (1.5, 2 and 2.5 w). The temperature rise was recorded real time every 20 seconds by a precise thermometer.

Results: Absorption spectrum of MWCNTs-COOH suspension was remarkably higher than water in a wavelength range of 200 to 1100 nm. For example, using the concentrations of 2.5 and 80 µg/ml of MWCNTs-COOH suspension caused a temperature elevation 2.35 and 9.23 times compared to water, respectively, upon 10 min laser irradiation and 2.5 w. Moreover, this predominance can be observed for 1.5 and 2 w radiation powers, too. Our findings show that the maximum of temperature increase was obtained at 80 µg/ml concentration of MWCNT-COOH suspension for three powers and through all periods of exposure time. Our results show that the minimum required parameters for a 5°C temperature increase (a 5°C temperature increase causes cell death) were achieved through 2.5 w, 28 µg/ml concentration and 20 second irradiation time in which both concentration and radiation times were relatively low.

Conclusion: Our results showed that MWCNTs-COOH can be considered as a potent photothermal agent in targeted therapies. New strategies must be developed to minimize the concentration, irradiation time and radiation power used in experiments.

Keywords

Hyperthermia, Multiwalled Carbon Nanotubes, Nanoparticles, Near Infrared, Photothermal Therapy

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Introduction

Cancer as a major cause of death will be considered in today's world. For example, it caused 7.4 million people to die in 2007 and it is estimated that in 2030 this figure will reach 12 million. The important point is that the World Health Organization (WHO) emphasized that at least 30% of cancer deaths can be avoided [1]. Nowadays, the main treatment of malignant tumors includes radiotherapy,

chemotherapy and surgery in which their final goal is to increase treatment efficiency in tumor and decrease side effects in healthy tissues. However, most of these therapies are relatively effective and continue to recur which can be invasive in some cases [2-4].

The concept of using combination treatments, namely, heat and radiotherapy is a noticeable approach; however, it is more efficient if radiotherapy applies hyperthermia as an adjunctive therapy. Physicians have been using hyperthermia for cancer treatment since the 18th century [1, 5]. Cell death occurs at temperatures above 40 degrees centigrade [6,7]. If such cells have a temperature increase ranging from 41 to 47°C, the phenomenon of apoptotic death begins to emerge [5]. At temperatures above 40°C, cell death occurs due to protein denaturation or damage to cell membrane [7].

In hyperthermia, some heat generation sources including visible light, near infrared, microwaves, radiofrequency and ultrasonic waves are used [8]. Photo-Thermal Therapy (PTT) is considered to be a method of treating cancer so that the light-producing sources and related light-absorbing dyes are used to generate heat for killing cancerous cells [9-14]. The efficiency of PTT depends on various factors such as time, temperature and temperature increase rate; however, the main drawback of conventional PTT is nonspecific heating of healthy tissues causing to injure healthy tissues. In addition, temperature increase rate in conventional PTT is slow. Therefore, the healthy tissues are heated above tolerance threshold and then injured [15].

In other words, a new strategy must be developed. Consequently, solutions must be found to enhance accumulation of light-absorbing dyes with regard to PTT producing methods in tumor tissues, selectively. Secondly, the light-absorbing dyes must be able to increase the temperature rapidly and finally, these dyes prove the biocompatibility. To achieve these goals, targeted therapies based on nanotechnology are advantageous. Interesting and unique properties of nanoparticles make them effective agents for diagnosis and treatment procedures. For example, nanoparticles, nanorods and nanotubes are mentioned as agents with suitable absorption spectra in Near Infra-Red (NIR) region for Photothermal treatment [9-14, 16-20]. Optical absorption of these materials is much higher than that of existing natural dyes in texture (e.g. chromophores). This issue reduces the light doses required for treatment, as well as minimizes the damage to healthy tissues [11, 21-23].

Carbon Nano Tubes (CNTs) as an important element of nanotechnology have remarkable physical and chemical properties due to their atomic structure. The Introduction of CNTs have led to a new approach in the science of nanotechnology and are therefore used with considerable pace in various fields [24-27]. CNTs have a high thermal conductivity and strong absorption in the region of NIR optical window (650 - 900 nm) where the absorption coefficient of water, hemoglobin and other absorbers in tissues has a minimum value of absorption. Therefore, light at this wavelength range can easily pass through the tissues [5, 11, 14, 21-23, 28].

CNTs with unique absorption spectrum, as mentioned before, can overcome the limitations of deep treatment in PTT [7, 21-23, 28, 29]. The mechanism of heat generation about Single-Walled CNTs (SWCNTs) and Multi-walled CNTs (MWCNTs) is that the excitation of optical transitions with relaxation that leads to increase vibrational modes in CNT and finally, the heat is generated [7]. MWCNTs in NIR region have obviously more optical absorption than SWCNTs because of having more electrons per particle for absorption [22]. For example, in one experiment, it was observed that the temperature increase in 0.1 mg/ml concentration was 28°C and 4°C for MWCNTs and SWCNTs, respectively. In addition, the differences between MWCNTs and SWCNTs for temperature increase depend on concentration [21]. MWCNTs and SWCNTs

have high potential to conjugate to different agents so that they accumulate in tumor target selectively [7].

To disperse MWCNTs in a solvent, many different methods have been proposed. One of these methods is to attach carboxylated functional group (-COOH) to MWCNTs in order to increase dispersivity in water [30].

It is predicted that MWCNTs-COOH would increase the depth of treatment in PTT due to strong absorption in the region of NIR optical window. In addition, because of the remarkable efficiency of heat generating and the applicability of targeted therapy of MWCNTs-COOH, the treatment time is shortened. Moreover, increasing the dispersivity of MWCNTs-COOH compared to that of MWCNTs leads to an increase in absorption coefficient. For all the reasons stated, MWCNTs-COOH can be a good candidate for targeted PTT.

To the best of our knowledge, this study is the first comprehensive research on MWCNTs-COOH nanomaterial to be used as a potent PTT agent through many different concentrations (from 2.5 to 500 µg/ml, total: 11 different concentrations) as well as a wide range of radiation time (from 20 to 600 s) and three laser powers, none of which has ever been carried out comprehensively before.

Material and Methods

Multi-walled Carbon Nano Tubes (MWCNTs), -COOH functionalized (MWCNTs-COOH) were purchased from US Nano Company (purity > 99 %, OD: 20 - 30 nm, ID: 5 - 10 nm, length: 10 – 30 µm). The content of carboxylated functional group (–COOH) into the MWCNTs-COOH agent is 2.73 wt% and the method of manufacturing of CNTs was Carbon-vapor Deposition (CVD method).

To produce different concentrations of MWCNTs-COOH suspension, a certain amount of which is added to 15 ml deionized water. The final concentrations were prepared ranging 2.5 to 5oo µg/ml (11 different concentrations). To obtain a stable and homogeneous suspension, MWCNTs-COOH particles were dispersed in water through ultrasonic bath for 1.5 hour [30].

The temperature of water in ultrasonic bath was kept low (e.g. 15°C) because of preventing the sample temperature increase; otherwise it can reduce the intensity of absorption [30]. Each sample was centrifuged after ultrasonication at 5000 rpm for 30 min, in order to eliminate large aggregates and bundles, and then 80% above the solutions for the experiments to be collected [23, 30].

A double-beam UV/VIS/NIR spectrophotometer (UV-1700 Pharmaspect, SHIMADZU, Japan) was used for measuring optical absorption of samples with different concentrations in a wavelength range of 200 to 1100 nm (range of recorded absorbance: from 0.00 A to 3.99 A). In all experiments, the absorbance of deionized water is considered as a zero baseline.

MWCNTs-COOH solution and water samples were placed in special cuvettes made of quartz (SpectroCell Company, For 190 - 2500 nm, 45 × 12.5 × 12.5 mm3, Pathlenght: 10 mm, 3.5 ml). Thousand microliters of each sample was placed in a special NIR-transparent glass cuvette made of quartz into the experiment setup and then each sample irradiated with a 810 nm continuous-wave laser (MDL-2.5 w, CHANGCHUN New Industries OPTOElectronics TECH CO, Spot size = 6 × 8 mm2) in three different fixed powers (1.5, 2 and 2.5 w) for a ten-minute irradiation.

For measuring real-time temperatures during the 10-minute laser radiation, a thermometer (Testo-735) and its accessories including USB data transmission cable and special pc software (Testo ComSoft) were used to record the temperature every 20 seconds. In addition, the initial temperature of samples had been recorded before the laser irradiation started. The thermocouple probe was placed in the solution at fixed and known distance from the laser center to avoid direct radiation to the thermocouple and then temperatures were measured.

In this study, the relationship between temperature elevation related to different concentrations of MWCNTs-COOH suspension, three laser power and irradiation time were studied. All experiments were performed three times each, at the room temperature.

For statistical analyses, firstly we used student, t-test for surveying normality of data and then as the second step, we calculated P-values with multiway Analysis of Variance (ANOVA) for comparing the temperature increase among groups with and without MWCNTs-COOH agent. It is mentioned that P-value less than 0.05 was as a significant level. All statistical analyses were performed with SPSS software.

Results

Firstly, a stable and homogeneous suspension of MWCNTs-COOH with different concentrations was prepared. Then, they were compared for their capability to enhance heat generation and temperature in the presence of 810 nm laser irradiation.

Absorption spectra of MWCNTs-COOH suspension was measured and the data showed remarkably enhanced absorbance compared with water alone in a wavelength range of 200 to 1100 nm (Figure 1). This topic gives us the promise that samples with MWCNTs-COOH compared to water alone noticeably increased heat generation and temperature.

The temperature increase in different concentrations of MWCNTs-COOH suspension (11 different concentrations) and water were obtained in the presence of laser radiation. For example, the temperature increase as a function of time is shown for concentrations of 0 (water), 35 and 80 µg/ml at 2.5 w upon 10 min laser irradiation (Figure 2). Temperatures shown in Figure 2 were recorded real-time every 20 seconds.

According to Figure 2, the temperature rise for concentrations of 35 and 80 µg/ml of MWCNTs-COOH suspension is considerably higher than water. For example, the temperature increase (ΔT) for 2.5 w and 10 min irradiation was 6.3°C (from 32.2 to 38.5°C), 42°C (from 29.2 to 71.2°C) and 58.2°C (from 29.2 to 87.4°C) for water and concentrations of 35 and 80 µg/ml of MWCNTs-COOH suspension, respectively. In other words, concentrations of 35 and 80 µg/ml of MWCNTs-COOH

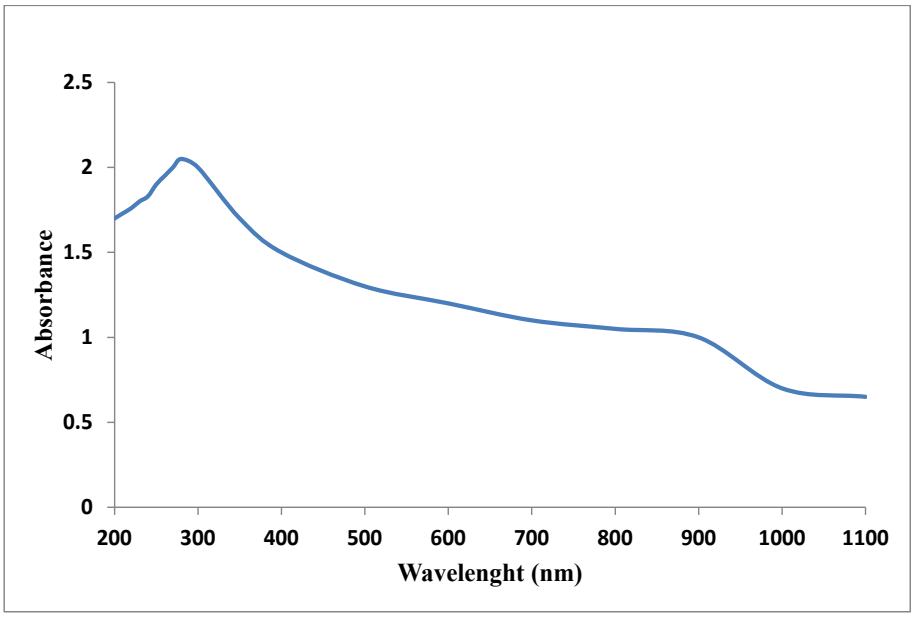


Figure 1: Absorption spectra of MWCNTs-COOH in water. It is mentioned water in quartz cuvette was used as baseline in spectrophotometer

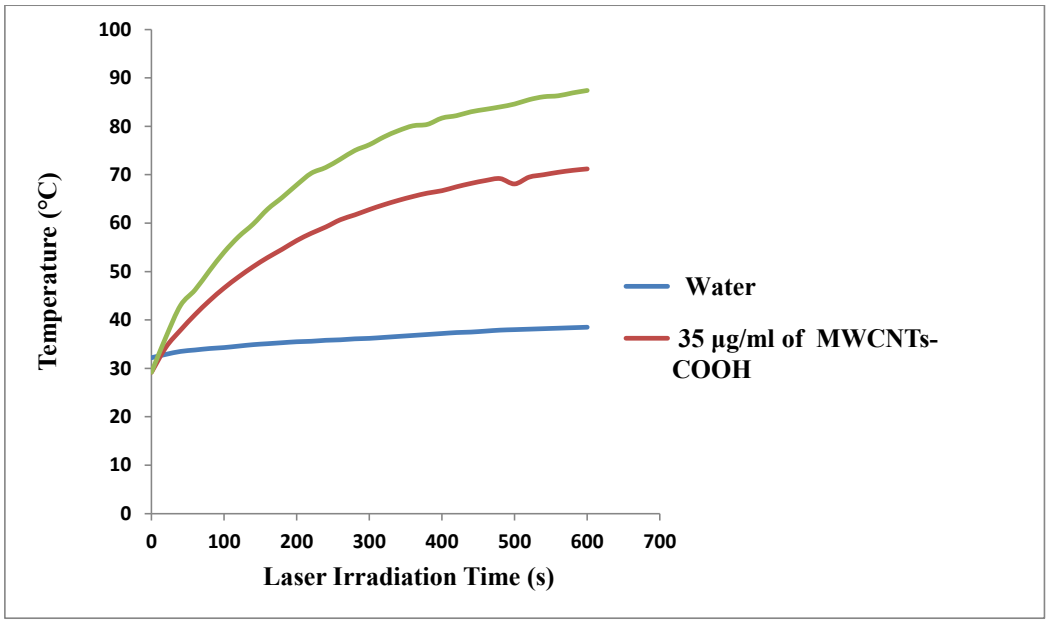


Figure 2: Increasing of temperature for water and 35 and 80 µg/ml concentrations of MWCNTs-COOH suspension at 2.5 w upon 10 min laser irradiation

suspension caused temperature elevation by 6.66 and 9.23 times compared to water, respectively.

To demonstrate the ability of sample containing with and without MWCNTs-COOH for temperature increasing, the data are shown at three power levels (1.5, 2 and 2.5 w) for concentrations of 0 to 500 µg/ml of MWCNTs-COOH suspension upon 10 min laser irradiation in Figure 3.

Temperature increase for all different concentrations of MWCNTs-COOH suspension

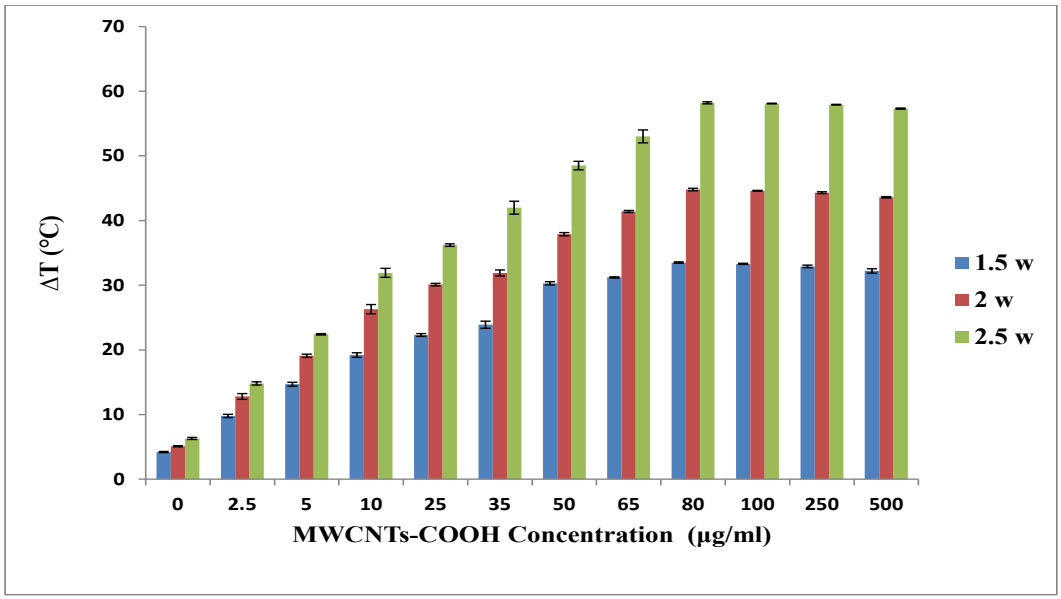


Figure 3: The temperature increase as the function of different concentrations of MWCNTs-COOH suspension compared to water at three power levels upon 10 min laser irradiation

(from 2.5 to 500 µg/ml) was noticeable and significant compared to water sample at three power levels of 1.5, 2 and 2.5 w (shown in Figure 3, P value < 0.001).

A more detailed explanation is that the temperature increase for the lowest concentration of MWCNTs-COOH suspension (2.5 µg/ml) was by 2.33, 2.50 and 2.35 times compared to water sample upon 1.5, 2 and 2.5 w, respectively. In addition, this superiority for 80 µg/ml MWCNTs-COOH suspension was 8, 8.8 and 9.2 times higher than that of water for 1.5, 2 and 2.5 w, respectively.

According to Figure 3, another result is that the maximum of temperature increase occurred for 80 µg/ml MWCNTs-COOH suspension which is 33.5, 44.8 and 58.2°C for 1.5, 2 and 2.5 w, respectively.

It can be observed that the temperature rise enhances by the increase in the concentration of MWCNTs-COOH suspension and irradiation power, up to 80 µg/ml MWCNTs-COOH suspension and above 80 µg/ml concentration, the temperature increase starts to decrease slightly. For example, the temperature increase for concentrations of 80, 100, 250 and 500 µg/ml at 2.5 w laser power is 58.2, 58.1, 57.9 and 57.3°C, respectively. It is mentioned that the level of temperature increase is reduced approximately 1°C from 80 to 500 µg/ml of MWCNTs-COOH suspension (P value for temperature rise between 80 and 500 µg/ml concentration was 0.483).

Moreover, the temperature increase for concentrations of 80, 100, 250 and 500 µg/ml at 2 w laser power was 44.8, 44.6, 44.3 and 43.6°C, respectively (P-value for temperature rise between 80 and 500 µg/ml was 0.004).

In addition, for the lowest laser power (1.5 w), the temperature increase for concentrations of 80, 100, 250 and 500 µg/ml was 33.5, 33.3, 32.9 and 32.2°C, respectively (P-value for temperature rise between 80 and 500 µg/ml was less than 0.001). Furthermore, the temperature increase had linear relationship with laser powers (1.5, 2 and 2.5 w) for all different concentrations of MWCNTs-COOH suspension ($R2 > 0.99$).

Another important point about Figure 2 is that the temperature increase rate decreases by the increase in the exposure time. Therefore, the temperature increase for 5 min compared to 10 min laser irradiation was compared for different concentrations of MWCNTs-COOH suspension. Results showed (Table 1) that major increase in temperature achieved in the first 5 min laser radiation for different samples (for example 73% to 86% of total temperature

Table 1: The temperature increase for 5 min compared to 10 min laser radiation was calculated for different concentrations of MWCNTs-COOH suspension in this study

Power	Concentration(µg/ml)	2.5	5	10	25	35	50	65	80	100	250	500
1.5 w	ΔT after 5 min	7.8	11.2	14.6	16.8	17.8	23.5	24.4	25.8	25.6	25.2	24.9
	ΔT after 10 min	9.9	14.7	19.2	22.3	23.9	30.3	31.2	33.5	33.3	32.9	32.2
	(ΔT (5min)) / (ΔT (10min))*100	0.79	0.76	0.76	0.75	0.74	0.78	0.78	0.77	0.77	0.77	0.77
2 w	ΔT after 5 min	11	14.5	22.7	24.3	24.6	27.8	33	35.2	35	34.7	34
	ΔT after 10 min	12.8	19.1	26.3	30.1	31.9	37.9	41.4	44.8	44.6	44.3	43.6
	(ΔT (5min)) / (ΔT (10min))*100	0.86	0.76	0.86	0.81	0.77	0.73	0.80	0.79	0.78	0.78	0.78
2.5 w	ΔT after 5 min	11.2	17.1	24.6	28	33.6	39.4	44.4	47	46.9	46.7	46.1
	ΔT after 10 min	14.8	22.4	31.9	36.2	42	48.5	53	58.2	58.1	57.9	57.3
	(ΔT (5min)) / (ΔT (10min))*100	0.76	0.76	0.77	0.77	0.80	0.81	0.84	0.81	0.81	0.81	0.80

ΔT: Temperature increase

increase after 10 min laser irradiation was related to initial 5 min laser irradiation). Therefore, we focused on the temperature increase in the initial 5 min laser radiation.

Temperature increase after 20 s laser irradiation for different concentrations of MWCNTs-COOH suspension (11 concentrations) and for three power levels are shown in Figure 4.

It is quite clear that at concentrations higher than 80 µg/ml (e.g. 100, 250 and 500 µg/ml), the temperature increase started to decrease slightly. Furthermore, this behavior was observed at 1.5, 2 and 2.5 w power levels (Figure 5).

It should be emphasized that the temperature increase for concentrations higher than 80 µg/ml (e.g. 100, 250 and 500 µg/ml) in all periods of exposure time (20, 40, 60, 120, 180, 240 and 300 s) and for different power levels (1.5, 2 and 2.5 w) has the same behavior to that shown in Figure 4 (i.e. the temperature increase above 80 µg/ml concentration started to decrease slightly). Therefore, to better illustrate the temperature increase at low concentrations, concentrations higher than 100 µg/ml (e.g. 250 and 500 µg/ml) are not shown in next diagrams. Moreover, high concentrations (e.g. 250 and 500 µg/ml) may not be suitable for cell and animal studies.

At this stage, temperature increase as the function of concentration for different concentrations of MWCNTs-COOH suspension (from 2.5 to 100 µg/ml) and for different radiation power upon various radiation time of laser (20, 40, 60, 120, 180, 240 and 300 s) is shown in Figures 5a, b, c, d, e, f and g.

It can be observed that the temperature elevation enhanced by increasing the concentration of MWCNTs-COOH suspension and irradiation power in all figures (Figures 5a, b, c, d, e, f and g). For a typical example, temperature increase for concentrations of 2.5, 10, 35, 50 and 80 µg/ml was 4, 9, 11.9, 14 and 17°C, respectively upon 60 s laser irradiation at 2.5 w. Moreover, the temperature increase for 80 µg/ml was 4.25 times compared to 2.5 µg/ml concentration. The temperature rise continues to 80 µg/ml concentration, and after 80 µg/ml concentration, the temperature increase starts to decline slightly. To be more precise, the maximum temperature rise occurs at 80 µg/ml concentration of MWCNTs-COOH suspen-

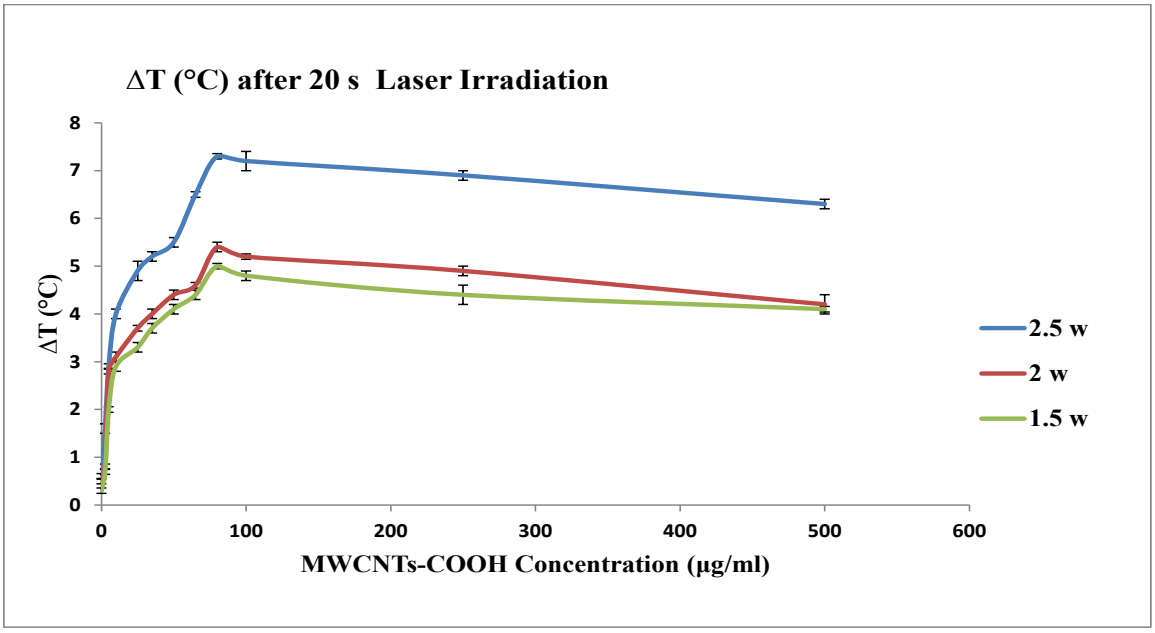
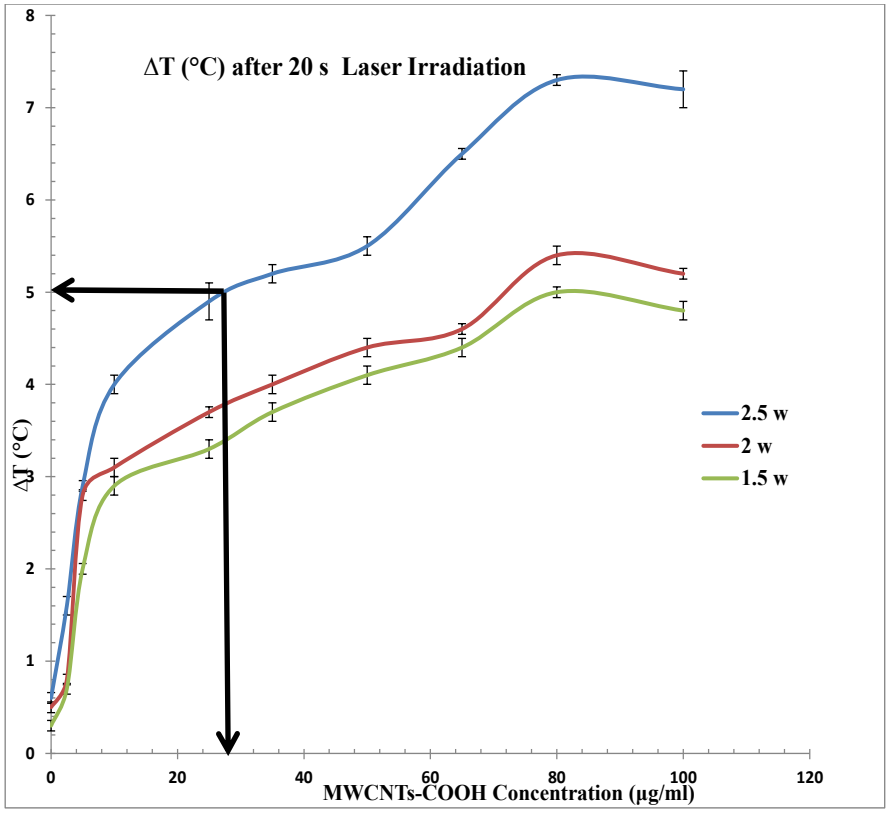
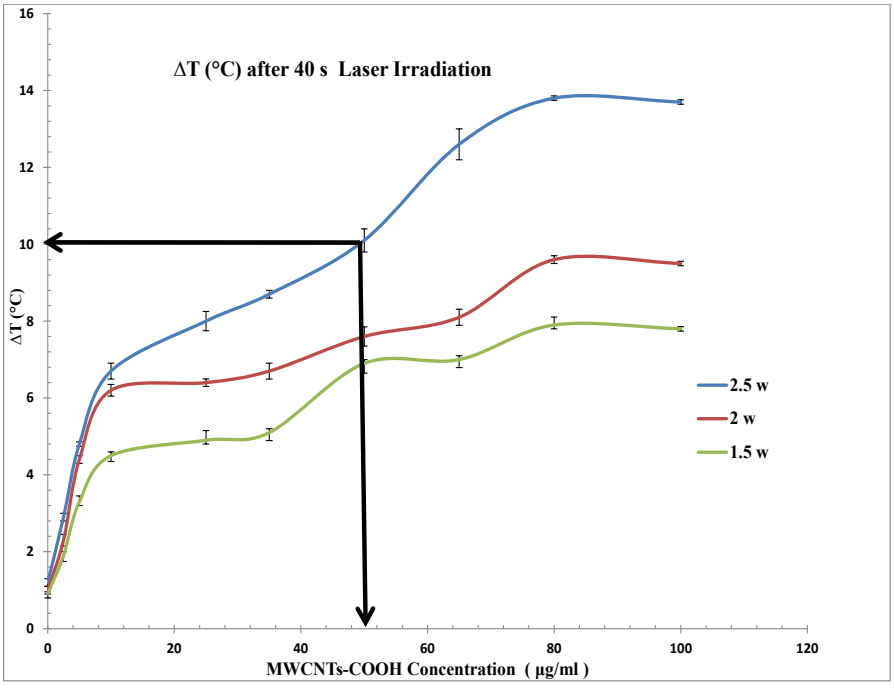


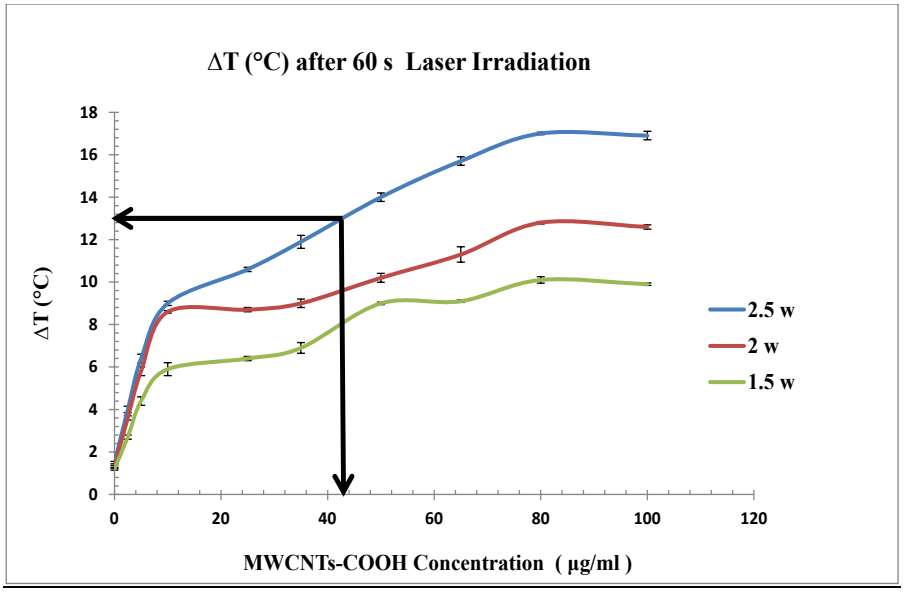
Figure 4: The temperature increase as the function of different concentrations of MWCNTs-COOH suspension (from 2.5 to 500 µg/ml) at three power levels upon 20 s irradiation


ΔT (°C) after 20 s Laser Irradiation
ΔT (°C)
MWCNTs-COOH Concentration (µg/ml)
2.5 w
2 w
1.5 w
0
1
2
3
4
5
6
7
8
0
20
40
60
80
100
120

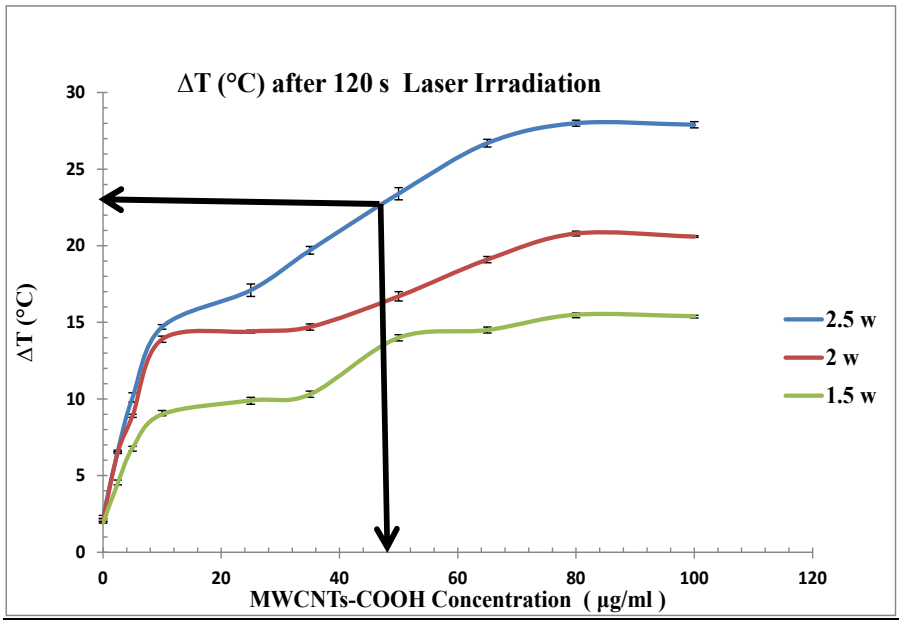
a


ΔT (°C) after 40 s Laser Irradiation
ΔT (°C)
MWCNTs-COOH Concentration (µg/ml)
2.5 w
2 w
1.5 w
0
2
4
6
8
10
12
14
16
0
20
40
60
80
100
120

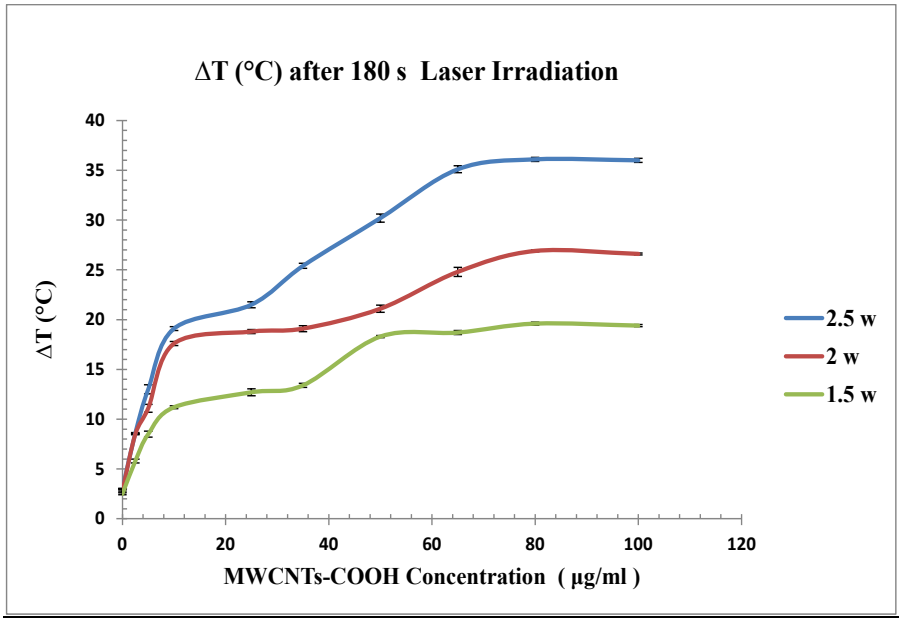
b


ΔT (°C) after 60 s Laser Irradiation
ΔT (°C)
MWCNTs-COOH Concentration (µg/ml)
2.5 w
2 w
1.5 w

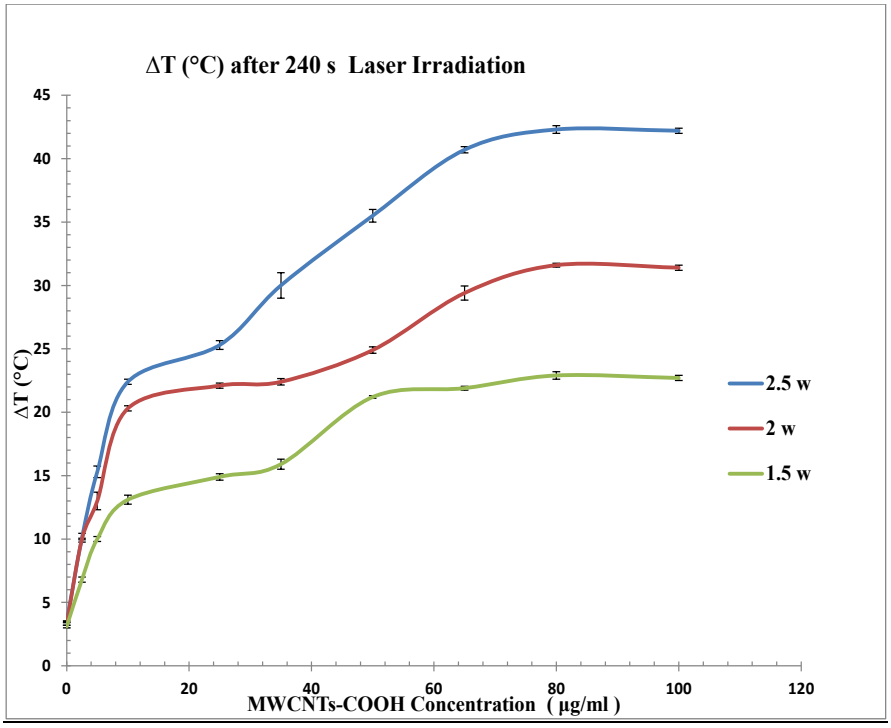
c


ΔT (°C) after 120 s Laser Irradiation
ΔT (°C)
MWCNTs-COOH Concentration (µg/ml)
2.5 w
2 w
1.5 w

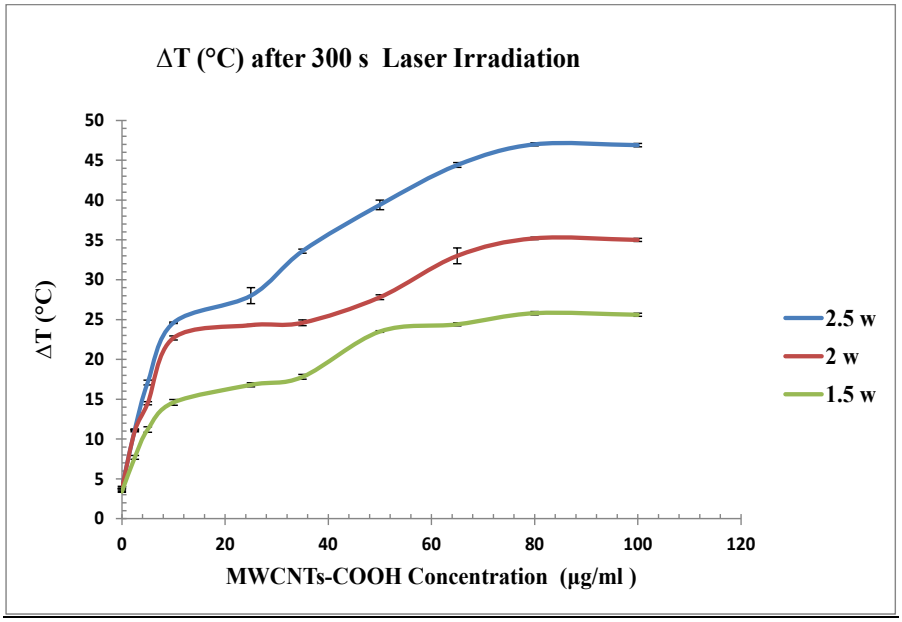
d


ΔT (°C) after 180 s Laser Irradiation
ΔT (°C)
MWCNTs-COOH Concentration (µg/ml)
2.5 w
2 w
1.5 w

e


ΔT (°C) after 240 s Laser Irradiation
ΔT (°C)
MWCNTs-COOH Concentration (µg/ml)
2.5 w
2 w
1.5 w

f



g

Figure 5: The temperature increase as the function of different concentrations of MWCNTs-COOH suspension (from 2.5 to 100 µg/ml) and for three power levels on various laser irradiation times : 20 s (a), 40 s (b), 60 s (c), 120 s (d), 180 s (e), 240 s (f) and 300 s (g)

sion for three power levels (1.5, 2 and 2.5 w) and for all periods of exposure time (Figures 5a, b, c, d, e, f and g). For example, the temperature elevation for 60 s laser radiation (2.5 w) for concentrations of 80, 100, 250 and 500 µg/ml was 17, 16.9, 16.7 and 16°C, respectively, and with these same radiation parameters, except exposure time changing from 60 second to 120 second, the temperature rise is 28, 27.9, 27.5 and 27.1°C, respectively.

Another point is that the temperature increase is enhanced with the increasing of radiation power. For a typical example, the temperature rise for 180 s irradiation time (80 µg/ml concentration) and at 1.5, 2 and 2.5 w was respectively 19.6, 26.9 and 36.1°C, and with these same radiation parameters, except that the concentration changes from 80 to 5 µg/ml concentration, the temperature rise is respectively 13, 11.1 and 8.5°C in which the temperature rise for 2.5 w was 53% more than 1.5 w (Figure 5e).

It is mentioned that a 5°C temperature increase causes cell death due to the protein denaturation or damage to the cell membrane [7]. A 5°C temperature increase is achieved at 2.5 w for at least 28 µg/ml concentration upon 20 s irradiation (Figure 5a). Moreover, the same temperature rise is obtained upon 20 s irradiation for at least 70 µg/ml concentration and only 80 µg/ml concentration for 2 and 1.5 w, respectively (Figure 5a).

At temperature increase range of 41 to 47°C, the phenomenon of apoptotic death begins to emerge [5]. Therefore, a 10°C temperature increase (i.e. 47°C -37°C =10°C) is considered, too. In Figure 5b, a 10°C temperature increase is obtained for at least 50 µg/ml concentration at 2.5 w upon 40 s irradiation and the same temperature increase does not occur at other power levels (1.5 and 2 w) and temperature increase is elevated to 9.6°C only at 80 µg/ml concentration for 2 w. In Figure 5c, a 10°C temperature increase is obtained for at least 50

μg/ml concentration and only 80 μg/ml concentration for 2 and 1.5 w, respectively (for more details, the temperature rise for 2 w and 50 μg/ml concentration is equal to 10.2°C).

In addition, necrosis and coagulation process are revealed above 50°C in which a 13°C temperature increase is studied [31]. For 2.5 w, the temperature elevation for 60 second radiation time is achieved 14°C for 50 μg/ml concentration (Figure 5c). The same temperature increase is obtained upon 120 s irradiation time for at least 10 μg/ml and 50 μg/ml concentrations for 2 w and 1.5 w, respectively (Figure 5d). Importantly, a 23°C temperature increase is obtained at 2.5 w for at least 50 μg/ml concentration upon 120 second radiation time in which at these conditions the thermal ablation begins (Figure 5d). It is mentioned that the thermal ablation begins at threshold of 60°C in which a 23°C temperature increase is in desirable [31].

In Figure 5e, a 23°C temperature increase does not occur at 1.5 w for 180 second irradiation time in which the maximum temperature rise for this power reaches 19.6°C. Moreover, the temperature increase is elevated up to 24.8°C for 65 μg/ml concentration, 2 w and 180 s radiation time. Furthermore, the temperature increase with these radiation conditions (e.g. 180 s, 2.5 w and 65 μg/ml concentration) is elevated up to 35.1°C. The temperature increase for 240 s radiation time and 80 μg/ml concentration is 22.9, 31.6 and 42.3°C for 1.5, 2 and 2.5 w, respectively (Figure 5f).

The temperature elevation for 300 second radiation time and a low concentration of MWCNTs-COOH suspension (10 μg/ml) is achieved 22.7 and 24.6°C For 2 w and 2.5 w, respectively (Figure 5g). The MWNTs-COOH concentrations required for 5, 10, 13 and 23°C temperature increases are indicated by horizontal and vertical arrows at 2.5 w in Figures 5a, b, c and d, respectively.

In summary, a certain temperature elevation (e.g. 5°C) is achieved at a higher power and a lower concentration, vice versa. In addition, using lower concentrations is invaluable for in vivo experiments especially when lower concentrations are less likely toxic.

In summary, the maximum of temperature increase is related to 2.5 w and 80 μg/ml concentration of MWCNTs-COOH suspension for different laser radiation times including of 20, 40, 60, 120, 180, 240 ,300 and 600 s that is 7.3, 13.8, 17, 28, 36.1, 42.3, 47 and 58.2°C, respectively (Figure 3 and Figures 5a, b, c, d, e, f and g).

Temperature increase for all different concentrations of MWCNTs-COOH suspension (from 2.5 μg/ml to 500 μg/ml) was noticeable and significant compared to water sample at three power levels (1.5, 2 and 2.5 w) and in different periods of exposure time (20, 40, 60, 120, 180, 240 ,300 and 600 s, P value < 0.001).

In summary, the minimum concentration required for a certain amount of temperature increase for specified values of radiation power and time are shown in Table 2.

According to Table 2, for temperature enhancing above the specified value (e.g. 5°C) in each row, it is possible to increase radiation parameters namely, increasing radiation power, concentration and radiation times.

For a typical example, with regard to 2 w row (related to $\Delta T=10°C$ in Table 2), three cases (a, b and c) are surveyed (Table 2).

a: If the concentration increases from 48 μg/ml to 65 μg/ml (other parameters are invariable), the temperature rise is enhanced from 10 to 11.3°C (Figure 5c).

b: In addition, if the radiation time increases from 60 to 80 s (other parameters are invariable), the temperature rise increases from 10 to 12.4°C (the related Figure is not shown in this paper).

c: And then, if the irradiation power enhances from 2 to 2.5 w (other parameters are invariable), the temperature rise increases from 10 to 13.8°C (Figure 5c).

Discussion

In this paper, we studied a unique nanomate-

Table 2: The minimum concentration required for a certain amount of temperature increase for specified values of radiation power and time in this study

ΔT	Radiation Power (w)	The minimum concentration required (µg/ml)	irradiation time (s)
5°C	2.5 w	28	20
	2 w	72	20
	1.5 w	80	20
10°C	2.5 w	49	40
	2 w	48	60
	1.5 w	80	60
13°C	2.5 w	43	60
	2 w	9	120
	1.5 w	44	120
23°C	2.5 w	48	120
	2 w	58	180
	1.5 w	80	240

ΔT: Temperature increase

rial called carboxylated Multi-Walled Carbon Nano Tubes (MWCNTs-COOH) that exhibits noticeable optical absorption in NIR region (650-900 nm) where the absorption coefficient of water and other natural chromophores in tissues have a minimum absorption and scattering. Therefore, light at this wavelength range can penetrate into the tissues [5, 11, 21-23, 28].

Absorbance is an acceptable criterion for predicting heat generation and consequently a temperature increase is considered to be [22]. In our study, MWCNTs-COOH showed outstandingly enhanced absorbance compared to water alone in a wavelength range of 200 to 1100 nm (Figure 1). Moreover, attaching carboxylated functional group (-COOH) to MWCNTs leads to increasing the dispersivity of MWCNTs-COOH.

For these reasons, we used MWCNTs-COOH as a potent photothermal agent for therapy. We obtained useful results in which the superiority of MWCNTs-COOH compared to water for increasing the temperature is proven under many different conditions. Data was obtained through many different concentrations (from 2.5 to 500 µg/ml) as well as a wide range of radiation times (from 20 to 600s) and three laser powers, none of which has ever been performed comprehensively in the past. Moreover, in this study, the temperature increase was recorded real time every 20 seconds by a precise thermometer (thermocouple) which was connected to a special software program.

Compared to water, MWCNTs-COOH was able to enhance the temperature increase drastically (Figures 2 and 3). For example, using concentrations of 80 and 2.5 µg/ml of MWCNTs-COOH suspension caused a temperature elevation 9.23 and 2.35 times, respectively, upon 10 min laser irradiation and 2.5 w (Concentration of 2.5 µg/ml was as the lowest concentration). Moreover, this predominance can be observed for 1.5 and 2 w radiation powers, too (Figure 3). As reported in another study, the temperature elevation for 100 µg/ml of MWCNTs suspension upon 5 min laser irradiation (for 3 w and 1064 nm) was obtained 43°C which was lower than that in our study (i.e. 47°C for 80 µg/ml upon 5 min and 2.5 w) [22]. In particular, in our research the radiation power density (5.2 w/cm2 Vs 15.3 w/cm2) and the concentration (80 µg/ml Vs 100 µg/ml) were lower compared to the described

study. Our better results are due to better dispersivity of MWCNTs-COOH compared with only MWCNTs.

In our study, the temperature increase (ΔT) at 2.5 w upon 10 min irradiation was 58.2 and 6.3°C respectively, for 80 µg/ml concentration of MWCNTs-COOH suspension and water (Figure 2 and 3). In other words, it can be assumed that the temperature of healthy tissues surrounding the tumor can reach 44°C approximately which is harmful for non-targeted organs leading to thermal injuries. In fact, there is a major drawback that cannot justify the rational treatment. For this reason, it is an important requirement that the temperature rise must be controlled appropriately to minimize thermal injuries for non-targeted organs. For dissolving this problem, there must be a new strategy to be able to reduce the concentration, radiation time and irradiation power used in the experiments.

To achieve these goals, we must be able to enhance the accumulation of nanomaterial agents in target tissues selectively; this approach is obtained through attaching special molecules to nanomaterial agents. In addition, the efficiency of nanomaterial agents for heat generation must become better; this goal is achieved through functionalization of nanomaterial agents [9-14, 16-20]. Moreover, using lower concentrations is invaluable for in vivo experiments especially when lower concentrations are less likely toxic. In other words, lower concentrations are more probable to be present in human treatment [7].

A more detailed explanation is that the temperature increase for MWCNTs is more than SWCNTs and some other nanomaterials (e.g. carbon nanohorns, gold nanoshells and nanorods) [21].

Compared to MWCNTs suspension (15.3 w/cm2 and 5 min), samples without MWCNTs for having the same thermal dose and cell viability, the laser power and radiation time should be 50.9 w/cm2 and 10 min. These differences indicate the value of MWCNTs for heat generation [22].

Our findings show that the temperature rise continues to 80 µg/ml concentration, and then for concentration above 80 µg/ml, it starts to decrease slightly. In fact, the maximum temperature increase was obtained at 80 µg/ml concentration of MWCNTs-COOH suspension for three power levels (1.5, 2 and 2.5 w) and through all periods of exposure time (20 s, 40 s, 60 s, 2 min, 3 min, 4 min, 5 min and 10 min) (Figure 3 and Figures 5a, b, c, d, e, f and g). It is stated that the temperature decrease is due to enhanced scattering and bundling effects with increasing the concentration of MWCNTs-COOH suspension [21].

It is emphasized that a 5°C temperature increase causes cell death. Moreover, in our study, the minimum required parameter for a 5°C temperature increase was achieved through 2.5 w, 28 µg/ml concentration and 20 second irradiation time that both concentration and radiation times were relatively low (Figure 5a). Our findings show that necrosis and coagulation processes (approximately ΔT = 13°C) occurred at 2.5 w, at least 43 µg/ml concentration and upon 60 second laser irradiation time (Figure 5c). Furthermore, the minimum required parameters for thermal ablation (approximately ΔT = 23°C) was obtained at 2.5 w, 48 µg/ml concentration and upon 120 second radiation time (Figure 5d).

Targeted photothermal therapies based on nanotechnology can overcome the limitations of deep treatment in conventional photothermal therapy. In this paper, we studied MWCNTs-COOH as an important element of nanotechnology having remarkable optical absorption in the NIR region. Our results showed that MWCNTs-COOH can be considered as a potent photothermal agent in targeted therapies.

New strategies must be developed to minimize the concentration, radiation time and irradiation power used in experiments in which the thermal injuries for healthy organs are minimized. In addition, using lower concen-

trations are more likely to be present in the human treatment due to lower toxicity.

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Conflict of Interest

The authors have no conflicts of interest.

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