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Photothermal Therapy Using Ag/TiO2 Core-Shell Nanoparticles Against Breast Cancer Cell Line: an *In Vitro* and *In Vivo* Study

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Abstract

Introduction: Pulsed Laser Ablation in Liquid (PLAL) is considered a facile and green method for synthesizing nanoparticles (NPs) with no byproducts formed during the synthesis process. Compared to other traditional treatment strategies, photothermal therapy (PTT) is considered a powerful alternative anti-tumor treatment methods. Ag/TiO_2 core-shell NPs were determined as a photothermal agent in the NIR-II window in the biomedical field. The anti-tumor effect (*in vitro*) and safety study (*in vivo*) of Ag/TiO_2 core-shell NPs were conducted in the current study.

Methods: TiO_2 nanoparticle colloid was prepared by PLAL using a fiber laser (1060 nm), In order to achieve a nanoparticle core-shell, Ag NPs were added to the as-prepared nanoparticle colloid. The anti-tumor effect of the produced Ag/TiO_2 core-shell NPs in D.W. and PBS as solvents, was investigated against the AMJ13 cell line both with and without using the laser at 1060 nm as the ablation source for the PTT *in vitro*. Also, histopathological changes following a single injection of the Ag/TiO_2 in mice were evaluated.

Results: *In vitro* results showed that the Ag/TiO_2 in PBS had anti-breast cancer cell line compared with REF normal cell line, while Ag/TiO_2 that was prepared in D.W. was biocompatible. Therefore, the anti-tumor potential impact of Ag/TiO_2 on PBS was selected to be tested as a photothermal agent *in vitro*. Interestingly, the results of the *in vivo* study showed Ag/TiO_2 no significant histopathological changes on mouse tissue. Moreover, the PTT study demonstrated that the best working laser parameters were 5W within 5 minutes, which achieved more than 57% inhibition percentage of tumor cell growth compared to the non-irradiated cancer cells.

Conclusion: Ag/TiO_2 core-shell nanoparticle is concluded to be a promising PTT agent in the NIR-II window against the AMJ13 breast cancer cell line, and it was safe on normal tissue *in vivo*.

Keywords: AMJ13, Cytotoxicity, Fiber laser, Histopathology, *In vitro*, *In vivo*, NIRII- window, PLAL, PTT

Introduction

The impact of cancer is increasing significantly, and breast cancer remains one of the most prevalent cancers worldwide among women, followed by prostate, lung, gastric, and colorectal tumors, along with non-melanoma skin malignancies, according to the International Agency for Research on Cancer in, Globocan, 2022. This pattern also holds true for Iraqi women.[1] Moreover, according to the Iraqi cancer registry, breast cancer is one of the top cancers spreading in Iraq, along with colorectal and cervical cancer.

Compared to other traditional treatment strategies, such as surgery, chemotherapy and radiotherapy, photothermal therapy (PTT) is considered a powerful replacement to the above-mentioned treatment methods.[1]

Extensive attention has been given to PTT for tumor ablation using NIR radiation.[2] PTT has the ability to achieve high temporal and spatial control of local heating under minimum adverse side effects. Hyperthermia generated by photothermal agents (PTAs) upon exposure to NIR irradiation can be applied to kill cancer cells while causing no damage to the nontargeted tissue.[3]

Two important factors of NIR irradiation, namely the minimal tissue scattering and absorption, must be considered in order to permit effective tissue penetration while a minimal self-heating must be maintained, with high photothermal conversion efficiency of the used light-absorbing material. The light in the second biological

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window (NIR-II) extends between 1000 and 1350 nm and is considered more suitable for biological applications compared to that in the first biological window (NIR-I) that extends from 700 to 1000 nm. Using the NIR-II has two superior benefits compared to NIR-I, which include an increased depth of penetration and a higher maximum permissible exposure (MPE) of the skin to the light. The maximum penetration depth is expected to be located at wavelengths of 1000-1100 nm, whereas ~1 W cm^{-2} is the MPE for the skin at the NIR-II window. While the MPE is ~0.7 W cm^{-2} for the 976 nm laser, it is ~ 0.54 W cm^{-2} for the 915 nm laser, and for the 808 nm laser, it is ~ 0.33 W cm^{-2} (according to the American National Standard for Safe Use of Lasers, ANSI Z136.1-2007). That is, higher NIR-II light tolerance is exhibited by skin cells when compared to shorter-wavelength light.[4]

Several studies have incorporated the anti-cancerous effect of metal and metal oxide NPs, which cause cell death and DNA damage to breast cancer cell lines. Ag nanoparticles (NPs) synthesized using different methods have been extensively studied for different biomedical applications, including the anti-cancer agent.[5] So far, for PTT, the restricted list for the nanomaterials that considered to be active in the NIR-II window are, CuS-based NPs , 2-D nanosheets, gold nanostructures, MXenes and large conjugated polymer particles.[6-9] The studies of NIR-II photothermal agents are still in their early stages, with limited examples mentioned above. The negligible cytotoxic effect of NIR-II photothermal agents has rarely been reported. Therefore, there is a need to develop safe and efficient NIR-II photothermal agents.[10]

Pulsed laser ablation in liquid (PLAL) is considered a facile and green method for synthesizing NP with no byproducts formed during the synthesis process compared to chemical methods.[11,12] Therefore, the main objective of the current study is to investigate the ability of the Ag/TiO_2 core-shell nanoparticle to be used as a photothermal agent in the NIR-II window against the human breast cancer cell line AMJ 13. This is the first study to incorporate Ag/TiO_2 in the NIR-II window to destruct the AMJ-13 human breast cancer cell line.

Two solvents were used in this study (PBS, D.W.) due to their safety on normal cells. poly vinyl alcohol (PVA) was used as a stabilizer since the Ag NPs have the tendency to agglomerate and are considered biocompatible and chemically stable.

Materials and Methods

Materials

Ti plate with purity 99.9%, PBS, Ag NPs with the dimension of 20-30 nm, Polyvinyl alcohol (PVA) $(C_2H_4O)_n$ from Barcelona, Espana (Didactic), D.W., 96-microtiter well plate, φ 0.22 μm millipore filter, tissue culture flasks were used in this study. See Figure S1 in Supplementary file 1.

Method

Production Ag/TiO_2

Ag Np (particle size 20-30 nm) with a concentration of 2 mg/mL was added to the as-prepared solution and was stirred for 30 minutes with a magnetic stirrer to achieve Ag/TiO_2 as a nanoparticle core-shell. More information about the synthesis of TiO_2 is provided in Supplementary file 1.

The anti-tumor activity of the produced Ag/TiO_2 core-shell nanoparticle was investigated against the AMJ 13 cell line both without and with the use of the 1060 nm fiber laser as the ablation source for the PTT *in vitro*. The as-prepared nanaocolloid of $PVA/Ag/TiO_2$ in D.W. and Ag/TiO_2 in PBS are referred to as $PVA/Ag/TiO_2$ and Ag/TiO_2 in the article, respectively. See Figure S2, S3 and S4 for the characteristics of the as-prepared Ag/TiO_2.

In Vitro Antitumor Therapy

To study the effect of both $PVA/Ag/TiO_2$ and Ag/TiO_2 core-shell NPs *in vitro,* the cytotoxicity assay was performed to investigate the cytotoxic impact of the Ag/TiO_2 on two cell lines, AMJ13 as a locally Iraqi established human breast cancer cell line,[13,14] and REF, a normal rat embryonic fibroblast cell line as the control that established and authenticated in experemantal therapy department/the Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR). Both cell lines were established at the Experimental Therapy Department, Iraqi Center for Cancer and Medical Genetics Research, Mustansiriyah University, and they were cultured in RPMI-1640 medium (US biological, USA), with 10% FBS (Capricorn- Scientific Germany), 100 units/mL penicillin, and 100 μg/mL streptomycin.[15]

Five different concentrations of Ag/TiO_2 NPs were used as follows: 266, 133, 66.5, 33.25, 16.625, and 0.00 (control) μg /mL, while the concentrations of $PVA/Ag/TiO_2$ NPs were 40, 20, 10, 5, 2.5, and 0.00 (control) μg /mL for two incubation times (24 and 72 hours) for both solvents (PBS and D.W.) with five replicates for each concentration.

Finally, under aseptic conditions, the supernatant of each well was discarded, and 100 μl of crystal violet stain (C.V) was added to each well, and the plates were covered and incubated for 20 minutes at 37 °C. After that, each well was washed with tap water and left to dry for a while. At a wavelength of 492 nm, the optical density (OD) was determined with a Flourometer (BMG LABTECH, Germany).[14,15] The microscope images of both treated and control cells were captured using the Leica inverted light microscope (Germany).

Photothermal Therapy Study

After allocating the best exposure time (24, 72) and the best as-prepared nanocolloid, the Ag/TiO_2 core-shell nanoparticle was used in the PTT study for a 24-hour exposure time. The same concentrations mentioned

earlier, without laser exposure, were used for the PTT study. The laser power and the exposure time were tested with and without the presence of the Ag/TiO_2. Two controls were used in the PTT experiment: The first control was conducted without a laser with zero concentration of the Ag/TiO_2 core-shell nanoparticle, while the second one was done with a laser with zero concentration of the Ag/TiO_2 core-shell nanoparticle. The temperature of the cells was monitored using a thermal imager NF-521. Figure S4 illustrates a schematic diagram of the laser source used. Table S1 and S2 illustrates the laser parameters used in the study.

In Vivo Study

An *in vivo* study was performed to investigate whether the Ag/TiO_2 core-shell nanoparticle has toxicopathological effects on mice.

Animal resource and housing. Six-eight-week-old male Albino mice (n=40) weighing 20-22 gm were obtained, housed and authenticated in the animal house unit of the ICCMGR at Mustansiriyah University. The mice were acclimated to the animal house unit of the ICCMGR for 2 weeks prior to the experiment. They were monitored daily and maintained on a light-dark cycle (12-h and 12-h) with ad libitum access to pelleted food and water. All experiments and animal care were conducted in compliance with the institutional animal care guidelines.

Experimental design and histopathological study. Male albino mice (n=40) were randomly divided into two groups. The mice of the first group (n=10) were injected with 500 µL/I.P/mouse of PBS and considered a negative control, but the mice of the second group (n=30) were injected with a single dose of Ag/TiO_2 at 266 mg /mL (500 L/I.P/mouse) see Figure 1. Both groups were daily monitored, and body weight and survival rate were recorded. At 14- and 28-day post-injection, half of the mice of each group were sacrificed by CO_2 asphyxiation followed by cervical dislocation, and none of the mice were excluded from the current study. To determine histopathological changes post-Ag/TiO_2 injection, liver, spleen, kidney, and heart were collected and fixed in neutral buffer formalin (10%). Tissue specimens were processed in gradually dehydrated ethyl alcohol, cleared and embedded in xylene and paraffin, respectively. Then, tissues were sectioned (5 µm) and stained with hematoxylin and eosin.[16,17]

Statistical Analysis

The experimental data were analyzed via two-way analysis of variance (ANOVA) and one-sample t-test for statistical comparisons between the studied groups (control and samples) using five replicates for each experiment. A P value ≤ 0.05 and 0.01 was considered statistically significant. For this analysis, we used the GraphPad Prism 8 software (GraphPad Software, Inc., San Diego, California).

Results

Temperature

The heating produced due to the exposure of 1060 nm laser wavelength at an average power of 5 W at a concentration of 266 ppm for Ag/TiO_2 in PBS raised the temperature from 25 °C to nearly 60 °C after 5 minutes (of laser exposure). For 1/10 of the concentration of 400 ppm of $PVA/Ag/TiO_2$, the temperature reached 35 °C (upon laser exposure) (Figure S5a). The saturation in heat changes reached after four minutes of irradiation with a value of nearly 30 °C for the Ag/TiO_2 core-shell nanoparticle in PBS. Thus, by comparing the Ag/TiO_2 in PBS with $PVA/Ag/TiO_2$, the PBS showed a temperature change of only 10 °C and the $PVA/Ag/TiO_2$ reached 13.5 °C during the same laser exposure time (Figure S4). These high values of the temperature at the concentration of 266 nm made the Ag/TiO_2 in PBS a promising candidate as a photothermal agent operating at a laser wavelength of 1060 nm (Figure S5a and b), while the concentration of $PVA/Ag/TiO_2$ was too low to achieve the specified ablation temperature.

The temperature changed during the On-Off cycle of the as-prepared Ag/TiO_2 core-shell nanoparticle in PBS (Figure S6-a). From this result, it was obvious that the heating time was 300 seconds while the cooling time was nearly 350 seconds.

Figure S6-b shows the thermal image of the Ag/TiO_2 core-shell nanoparticle in PBS, where the temperature has reached its maximum value of 57.7 °C at a concentration of 266 ppm for the as-prepared colloid.

In Vitro Study

The cytotoxicity of the Ag/TiO_2 core-shell nanopatricles in D.W and PBS on AMJ13 breast cancer cells demonstrated concentration dependence at both exposure times (24 and 72 hours), and the cell viability decreased significantly (at P value ≤ 0.05) with the highest effect at a concentration of 40 µg /mL in DW as shown in Figure 2b and 266 µ /mL in PBS as shown in Figures 2a and S7 compared with the control.[12]

Interestingly, the results showed the non-significant effect

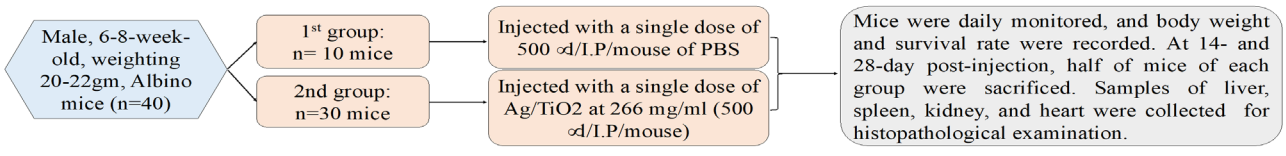

Figure 1. Study Plan and Experimental Design

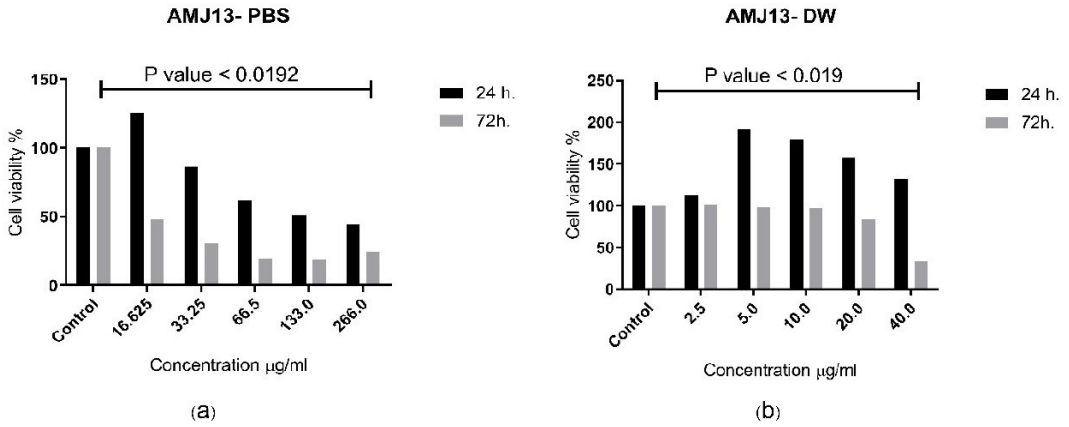

Figure 2. The percentage of cell viability of breast cancer cells line AMJ13 after (24 and 72 h) incubation time after being exposed to Ag/TiO2 nanoparticle core-shell showed a significant effect with highly effect on AMJ13- PBS at P value ≤ 0.05 (a) PBS and (b) D.W.

of Ag/TiO_2 core-shell on normal cell line REF on both DW and PBS at a *P* value ≤ 0.05 (Figure 3 and Figure S8), which showed its safety on normal cells.

According to the above results, 24 hours was the best exposure time, which showed a cytotoxic effect on cancer cells and safety on normal cells, so it was selected for the PTT experiments against AMJ13 in this study.

PTT: According to the *in vitro* study, the results showed no effect of the $PVA/Ag/TiO_2$ on both the normal and cancer cell lines, which indicated the biosafety of this material. Therefore, the PTT study was applied only for the Ag/TiO_2 core-shell nanoparticle.

Power and Time

The laser power and the exposure time were varied with and without the presence of the Ag/TiO_2. From Figure 4, it was obvious that the best working laser parameters were at 5W for 5 minutes at an incubation time of 24 hours. This showed a significant effect (at a *P* value > 0.01) because other working parameters might cause burns if they were tested on cancerous mice (Figure S8).

Figure 5 showed the cell viability of the Ag/TiO_2 core-shell nanoparticle in PBS against the cancer cell line (AMJ13). The low cell viability with increasing Ag/TiO_2 core-shell nanoparticle concentration might be due to the release of Ag ions in PBS since it has a low particle size of 10 nm.[6] The Ag/TiO_2 core-shell nanoparticle against REF has shown a low cytotoxic effect with high cell viability with significant effects (at a *P* value > 0.01), especially at low concentrations, indicating the biocompatibility of the as-prepared material (Figure 3 & Figure S9). Comparing the cell viability with the AMJ13 after 24 hours of incubation, we can easily see that as the concentration of the Ag/TiO2 core-shell increases, the cell viability decreases, indicating that more NPs could enter the pores of the cell membrane, inducing damage to the cells. The concentration of the Ag/TiO_2 in PBS combined with a laser has achieved the maximum reduction in cell viability at 266 ppm compared with non-radiated cells, with a percentage reduction in cell viability of 40%.

Figure 6 shows the temperature of the AMJ13 cells incubated with the as-prepared Ag/TiO_2. The temperature was monitored and recorded by a thermal camera after the cells had been irradiated for 5 minutes with a 5 W laser. It was obvious that the highest temperature was obtained when the concentration of the as-prepared Ag/TiO_2 core-shell NPs was the lowest (16.625 ppm), and due to this, the highest cell death compared to the non-irradiated cells was achieved at this concentration (Figure S7). The photothermal conversion efficiency reached η=16.1% and the time constant of the heat transfer system was 163.08 see (Figure S10). Increasing the concentration of the Ag/TiO_2 core-shell nanoparticle has not increased the photothermal effect, and this might be attributed to the increased agglomeration of the as-prepared Ag/TiO_2 core-shell nanoparticle, so there will be an obstacle in entering the pores of the cells' membrane. A comparison between non-irradiated and laser-irradiated cells revealed that the 16.625 ppm treatment had the highest reduction percentage, with more than 57% of the cells being killed by the laser due to the highest ablation temperature achieved at this concentration.

In Vivo Study

Only the Ag/TiO_2 core-shell nanoparticle demonstrated anti-tumor activity in vitro, so it has been selected for the *in vivo* study.

The mice inoculated with either a single dose of PBS (control) or an Ag/TiO_2 core-shell nanoparticle at 266 mg /mL were daily monitored; their body weight and survival rate were recorded for any side effect development associated with Ag/TiO_2 core-shell nanoparticle injection. Interestingly, none of the mice injected with the Ag/TiO_2 nanoparticle core-shell developed any clinical signs, and they showed a 100% survival rate (Figure S11) with no significant differences in body weight compared with the control group (Figure S12).

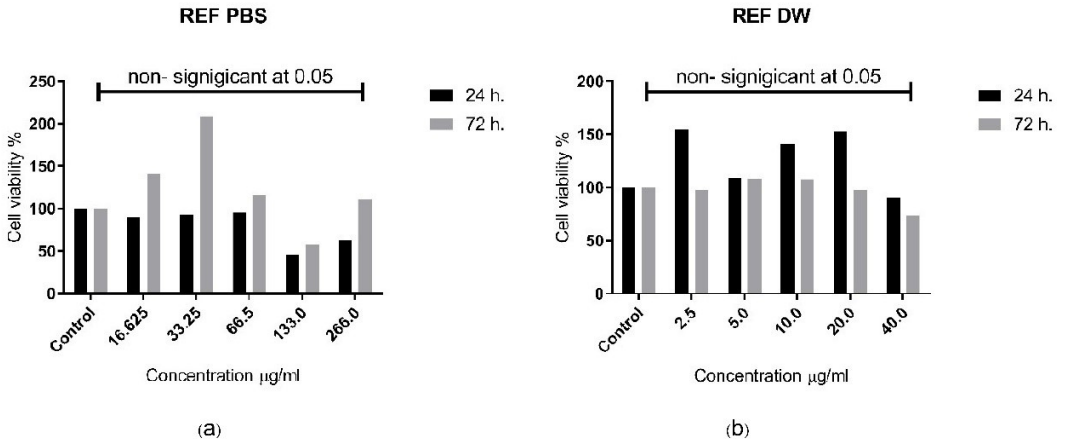

Figure 3. The effect of Ag/TiO2 nanoparticle core-shell on normal REF cells for both 24 and 72 h. incubation time in that showed non-significant effect between all concentrations in both PBS and DW (a) PBS and (b) D.W.

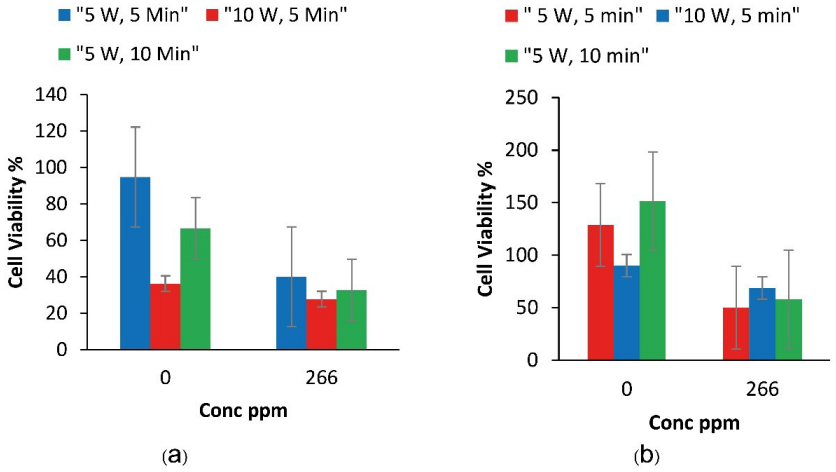

Figure 4. Cell viability of AMJ13 without and with Ag/TiO2 using laser power of 5, 10 W and irradiation time 5, 10 min that showed a significant effect (a) incubation time 24 h, (b) incubation time 12 h

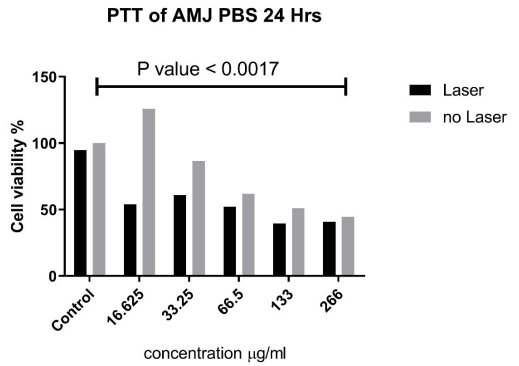

Figure 5. Investigating the cell viability% of AMJ13 with different concentrations of Ag/TiO_2 nanoparticle core-shell with and without laser exposure of 5W, 5 min incubation time 24 h. that showed a significant effects

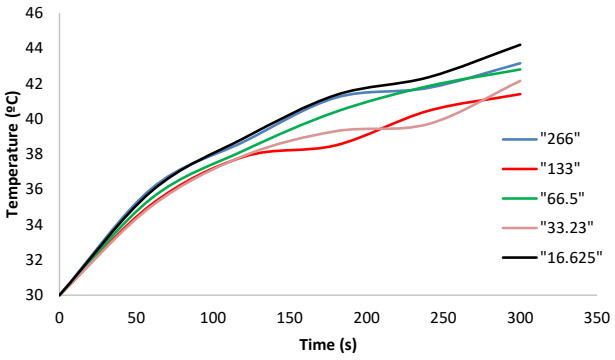

Figure 6. The temperature of the incubated AMJ13 cell with different concentrations of Ag/TiO_2 nanoparticle core-shell in PBS at an irradiation of 5W, 5 min

To investigate the microscopic changes following the single injection of Ag/TiO_2 core-shell nanoparticle, their liver, spleen, kidney, and heart were collected for histopathological examination. No significant histological changes were detected in all evaluated organs at both time periods, 14- and 28-days post-injection (Figure S13) (The results of 28 days were not shown because of the similarity between the results of 14 and 28 days). Tissue sections demonstrated normal architecture of hepatic cords with no histological changes in the white and red pulp of the spleen. Also, the microscopic examination of the kidney and heart revealed no marked lesions in

these organs, indicating the safety of Ag/TiO_2 with no toxicopathological effect on animals.

Discussion

The AMJ13 breast cancer cell line was in its invasive breast carcinoma cell lines,[13] AMJ13 breast cancer cell line was shown to be a good breast cancer model to be studied with interesting results and it has been shown to be a good breast cancer model to be studied with interesting results.[13,14,17-19] Therefore, it was selected in our study. According to the above results, 24 hours was the best exposure time, which showed a cytotoxic effect on cancer cells and safety on normal cells, so it was selected for the PTT experiments against AMJ13 in this study.

In the PTT results, the low cell viability with increasing Ag/TiO_2 core-shell nanoparticle concentration might be due to the release of Ag ions in PBS since it has a low particle size of 10 nm.[6]

Also, due to the agglomeration of the Ag/TiO_2 NPs shown previously in the TEM (Figure S1), the interaction between the AMJ13 cells and the Ag/TiO_2 NPs was reduced, leading to a reduction in photothermal effect.[7] Ag/TiO_2 produce free radicals (i.e. reactive oxygen species) which result in: (1) the death of cancer cells, (2) alter in the cell morphology, (3) decrease in the metabolic activity of cells, (4) increase in the oxidative stress, (5) mitochondria damage and damaging DNA to a large extent.[8] Increasing the concentration of the Ag/TiO2 core-shell nanoparticle has not increased the photothermal effect, and this might be attributed to the increased agglomeration of the as-prepared Ag/TiO2 core-shell nanoparticle, so there will be an obstacle in entering the pores of the cells' membrane Table 1 shows the information of some selected previous studies regarding using NPs (Ag or TiO_2) separately or as a mixture solution. These published studies have investigated the *in vitro* and *in vivo* effects of acute and chronic exposure to Ag or TiO_2. Different methods were used to prepare the NPs, and different doses and exposure times were utilized in their methodology. Some of these studies showed the cytotoxic effect of these NPs and others did not.

However, in the current study, the Ag/TiO_2 core shell prepared using the green method was used. This could be one of the reasons behind the lack of toxic effect of Ag/TiO_2 on the normal cells *in vitro*, while the antitumor effect was obvious and it is considered an interesting observation that needs more investigation. Moreover, no pathological changes were seen in the mice that were injected with a single dose of Ag/TiO2. We believe a single injection of 266 mg /mL of Ag/TiO2 (500 µL/mouse/IP) was safe. More studies are required to investigate

Table 1. Summary of Some Selected Previous Studies of Using Ag and TiO_2 Nanoparticles *In Vitro* and *In Vivo*

Nanoparticle	Particle size and morphology	*In vitro*	*In vivo*	Results	Ref.
Nano-sized TiO_2 TiO_2 was prepared by (chemical method) sol–gel method under acidic conditions	100 nm (Uniformly scattered nanocrystalline structure)	-	Adult mice were injected intraperitonially with nano-sized TiO_2 (0, 324, 648, 972, 1296, 1944 or 2592 mg/kg) for 7 and 14 days (acute study)	Different pathological lesions in the spleen, liver, lung, and kidney	17
Individual and combined toxicity effects of Ag- and TiO_2- NPs (the method of synthesis was not mentioned)	Ag (Spherical) diameter (7.29, 16.9, 1.06 nm) TiO_2 diameter (32.3 ± 26.9 nm)	-	Common carp as an aquatic animal model, Acute toxicity (96 hrs) of AgNPs and AgNPs þ TiO_2 NPs. Chronic toxicity (10 days + 10 day recovery) of AgNPs, TiO_2 NPs and AgNPs + TiO_2 NPs, different doses	Acute toxicity of Ag NPs and TiO_2 NPs to the juvenile common carp was increased by their co-exposure. Chronic toxicity tests included a 10-day exposure and a 10-day recovery period. In most cases, histological damages were more severe in co- exposure to Ag- and TiO_2- NPs compared with the individual Ag NPs	18
TiO_2 (Flame- Spray Pyrolysis) and Ag NPs have been used separately in this study.	Ag spherical agglomerates, 26.2 ± 10.7 nm TiO_2 (non-homogeneous) agglomerates, 40.2 ± 17.6	Rat primary cortical cultures, acute (0.5h), subacute (24 and 48 h), and chronic (14 days) toxicity at concentration 1–100 µg/mL	----	TiO_2 NPs a moderate dose- and time-dependent decrease in neuronal activity. Ag NPs clearly reduces neuronal- and network activity of rat primary cortical cultures in a dose- and time-dependent manner	19
Green plant- based synthesis Ag–TiO_2 nanocomposites	Core-shell nanoclusters, TiO_2 (20-26 nm) Ag (5-8 nm)	Fibroblast cells.	*In vivo* dermal toxicity, Acute (applied once) and subacute (applied once daily) dermal toxicity, in rats	*In vitro* study: showed no toxic effect. *In vivo:* on Acute and sub-dermal toxicity testing of Ag–TiO_2 NCs at up to 10 × (6250 mg mL)/ applied daily for 28 days MIC in the CF rats did not cause any significant edema and erythema in the treated skin area in acute and sub-acute dermal toxicity	20
Green synthesized using PLAL Ag/TiO_2 core shell	Core-shell (10.63 nm)	* Human breast cancer cell line AMJ13 * REF (Rat embryonic cell line) Concentrations (266, 133, 66.5, 33.25, 16.625, 0.00 µg /mL)	Mice were injected intraperitoneally with a single dose of Ag/TiO_2 at 266 mg / mL (500 µL/mouse).	*In vitro* study: Ag/TiO_2 showed significant antitumor effect against breast cancer cell line, while no effect on normal (REF) cell line. *In vivo* study: No significant histological changes were seen in mice following Ag/TiO_2 injection	This work

the toxicopathological effects of Ag/TiO2 *in vivo* using different doses and time points.

Conclusion

From the results above, we can conclude that the Ag/TiO_2 core-shell nanoparticle in PBS was proven to be safe in both *in vitro* and *in vivo* studies. The fiber laser of wavelength 1060 µm can be considered a promising tool to be used in the NIR-II window for PTT with the PT agent Ag/TiO_2 core-shell nanoparticle in PBS against the breast cancer cell line AMJ 13.

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Investigation: Huda Mahmood Al-attar, Maeda H. Mohammad, Aous Kahtan Almzaien.
Methodology: Huda Mahmood Al-attar, Adnan Khazaal Ajeel, Haydar Adnan Hassoon, Rasha Abd Al-Amir Hussein, Aysar A. Ahmed.
Project administration: Huda Mahmood Al-attar, Maeda H. Mohammad.
Supervision: Huda Mahmood Al-attar, Maeda H. Mohammad, Aous Kahtan Almzaien, Ali Hussain Alwan.
Validation: Huda Mahmood Al-attar, Maeda H. Mohammad, Aous Kahtan Almzaien.
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Writing–original draft: Huda Mahmood Al-attar, Maeda H. Mohammad, Aous Kahtan Almzaien, Ali Hussain Alwan.
Writing–review & editing: Huda Mahmood Al-attar, Maeda H. Mohammad, Omar Hussein Khalaf.

Competing Interests

The authors declare that there are no conflicts of interests in publishing this manuscript.

Consent for Publication

All authors have given consent for publication.

Ethical Approval

This study was conducted according to the rules of the Animal House Unit, Iraqi Center for Cancer and Medical Genetic Research, Mustasiriyah University.

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Supplementary Files

Supplementary file 1 contains Tables S1-S2 and Figures S1-S13 .

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