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Photodynamic Therapy and TiO_2-Decorated Ag Nanoparticles: Implications for Skin Cancer Treatment

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ABSTRACT

This in vitro study was conducted to evaluate the effects of blue light (λ = 420–480nm), titanium dioxide-decorated silver (TiO_2/Ag) nanoparticles, and their combined application on the viability of A431 skin cancer cells. Cells cultured in a medium enriched with 10% fetal bovine serum were subjected to three distinct treatments: exposure to blue light, application of TiO_2/Ag nanoparticles, and a combination of both. The MTT assay was utilized to determine cell viability post 24-hour incubation, with the resultant color intensity measured via a plate reader. Findings indicated a significant reduction in cell viability: blue light exposure led to an approximately 82% decrease at the 45-minute mark; nanoparticle treatment resulted in a notable viability reduction across all concentrations ($p \leq 0.001$); and the combined treatment displayed an enhanced effect, reducing cell viability by 42.79% relative to the control group after 45 minutes. Notably, the highest nanoparticle concentration (400 μg/ml) demonstrated superior anticancer efficacy. The synergistic impact of the combined treatment was evident, surpassing the individual effects of either blue light or TiO_2/Ag nanoparticles in diminishing A431 cell numbers. This study underscores the potential of integrating photodynamic therapy (PDT) with nanoparticle technology in targeting skin cancer cells, highlighting a promising avenue for therapeutic intervention.

1. INTRODUCTION

Cancer nanomedicine, while traversing the complex landscape of Clinical Translational Science (CTS), has elicited a spectrum of responses, ranging from enthusiasm to skepticism [1, 2]. The interactions of nanoparticles with biological molecules within drug delivery systems remain an area of ongoing research, with many aspects yet to be fully elucidated [3]. Nanomedicine holds promise for enhancing the bioavailability of pharmaceuticals, mitigating drug degradation, improving cellular targeting, controlling site-specific drug release, and achieving therapeutic efficacy with reduced adverse effects. Specifically, the integration of nanomedicine with PDT is primarily aimed at reducing the side effects associated with cancer treatments. PDT is known to induce tumor cell death, either partially or completely, through mechanisms that are not attributable to a singular process [4]. Widely applied in various cancer treatments, PDT's advantages include minimal invasiveness, lack of cumulative damage, and resistance to drug resistance [5, 6]. The field of biomedical optics, emerging as a distinct discipline, has significantly influenced modern medicine through advanced lasers and optical devices. In the realm of cancer nanomedicine, innovative techniques such as photothermal therapy and PDT have been developed. However, the reliance on single-modality phototherapy often falls short in comprehensively addressing persistent cancers due to recurrence or metastasis risks, prompting the exploration of combinatory therapeutic strategies.

2. LITERATURE REVIEW

The application of PDT using laser light sources has been constrained by the limited irradiation area of these lasers, posing a challenge particularly in dermatological contexts where extensive skin areas require treatment [7]. This limitation has led to the adoption of alternative light sources, such as halogen, Xenon, and metal-halide lamps, in lieu of laser light. Such substitutions have yielded impressive clinical outcomes, significantly enhancing the feasibility and efficiency of PDT in dermatology [8, 9]. Concurrently, the integration of light therapy with chemical drugs has demonstrated heightened therapeutic efficacy [10, 11]. The synergy between chemotherapy and phototherapy is noteworthy, as the cytotoxicity of several chemotherapeutic agents is known to increase at elevated temperatures [12]. A key observation is that traditional phototherapeutic agents predominantly provide localized heat to the tumor area without incorporating drug delivery. This observation has steered research towards developing therapeutic strategies that concurrently deliver both chemotherapeutic agents and heat to

the tumor site. Such strategies are anticipated to significantly augment therapeutic effectiveness while minimizing adverse effects. In response to this need, novel devices have been engineered, capable of delivering both chemotherapeutic drugs and photothermal agents directly to targeted tumor locations, thereby optimizing the treatment efficacy and reducing systemic toxicity.

Park et al. [13] have demonstrated the potential for reducing single drug doses in PDT, thereby minimizing adverse side effects while preserving treatment efficacy [14]. This aspect of dose reduction represents a significant advancement in the field. Further, research by Kah et al. [15] has established that PDT can induce DNA fragmentation and promote apoptosis in targeted cells. The extent of DNA fragmentation was found to be influenced by both time and dosage parameters. Additionally, these researchers observed damage to cytoplasmic structures and chromatin condensation towards the periphery of the nucleus. In terms of photocatalytic cytotoxicity, TiO_2/Ag nanoparticles have shown a remarkable efficiency, requiring only a quarter of the irradiation time needed by TiO_2 alone [16, 17]. The efficacy of TiO_2/Ag in cell destruction was reported to be over threefold greater than that of TiO_2 at equivalent concentrations. When examining the killing of HeLa cells, the comparative photoluminescence intensities of TiO_2 and TiO_2/Ag hybrids were analyzed. The findings indicated that the fluorescence intensity of TiO_2/Ag was significantly higher than that of TiO_2 alone. In this context, the current study employs TiO_2-decorated Ag nanoparticles and a Xenon lamp emitting a specific wavelength range (420-480 nm) to investigate wavelength-specific biological effects on cultured human skin cells.

3. MATERIALS AND METHODS

3.1 Cell line and culture condition

A cell line derived from a non-melanoma skin cancer called the A431 cell line was employed. used this line because it is commonly used in similar studies and has specific characteristics with a wavelength of 420–480 nm that make it suitable for PDT. This A431 cell line was obtained from the American Type Culture Collection (ATCC). ATCC offers scientists a range of high-quality cell authentication testing services backed by nearly a century of experience in biomaterial management and authentication standards. The cell lines were grown in a complete growth medium called RPMI-1640 (is a versatile and widely used cell culture medium that has many applications in the life sciences industry. Its ability to support the growth of a wide range of cell types makes it a valuable tool for studying disease), which was made according to the Gibco manual with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin antibiotics. The culture was carried out at a temperature of 37℃.

3.2 Nanoparticles of TiO_2 decorated Ag

Titanium Dioxide decorated silver Ag-TiO_2 Nanoparticles 20 wt.% Dispersion was obtained from "SIGMA-ALDRICH" fully dispersed in Water (Doped with 100 ppm 15 nm Ag light brown color, crystallite sizes for TiO_2/Ag, ranging from 5 to 25 nm).

The solution was prepared by dissolving an 800 µl of the TiO_2/Ag (1000 µg/ml) in 1200 µl of culture media to obtain 2000 µl at concentration 400 µg/ml done using the dilution relationship. then drag by micropipette 200 µl and placed into the plate.

This is done using the following relationship, which is called the dilution relationship:

$$C_1V_1 = C_2V_2 \quad (1)$$

where:

C_1: First concentration,

C_2: second concentration,

V_1: the necessary volume of the first concentration,

V_2: The volume needed to be added to the first concentration to obtain.

3.3 Exposure irradiation

Before the process of irradiation, cells were planted in 200 µl of media on sterile 96-well plates with a cover and the irradiation was carried out in a dark room after careful timing. Through the measurements, we found the response of the cells to each source is not constant. For each source, the response was according to the power of the source used, and therefore the change was made depending on the cell's response, and the best response was also searched for. The emitted light fully covered the irradiated field of each culture plate. The cells in the control group were not subjected to the light treatment, and the cells were allowed to remain at room temperature (RT). Xenon lamp was used in this paper. Emitting at around 420-480 nm. 40 W optical emission power for Xenon (12 V). The irradiate directly without distance between the source and plate.

3.4 MTT assay (methyl thiazolyl tetrazolium assay)

The absorbance of -3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma–Aldrich) in live cells was measured to evaluate the growth of the cells in accordance with the methodology outlined in the previous passage [18].

In brief, cells were seeded at a density of 50,000 cells per wall on 96-wall plates, and then they were irradiated with blue light for the amount of time that was specified. After exposing all 96 of the wall plates to blue light, the MTT solution, consisting of 5 mg/ml in PBS and 10 microliters, was added to each plate. After a further three to four hours of incubation at 37℃, The formazan crystals will be formed, the plates were pipetted to remove the medium from the wells, and then one hundred microliters of Dimethyl Sulfoxide (DMSO) were added to each well in order to dissolve the formazan crystals. The optical density was measured at 570 nm with a micro plate reader made by (Molecular Devices in the United States) as shown in Figure 1. The findings were presented as a percentage compared to the values of the control group. Every measurement was taken three times to improve the precision. Absorbance readings from test samples must then be divided by those of the control and multiplied by 100 to give percentage cell viability or proliferation. Absorbance values greater than the control indicate cell proliferation, while lower values suggest cell death or inhibition of proliferation. Percent of cell viability was calculated by the following formula:

Viability of cells % = 100- (optical density of control wells - optical density of test well) / optical density of control wells (X 100). (2)

Figure 1. The 96-well plates for the MTT assay test

4. RESULTS

4.1 Toxicity of Xenon lamp exposure on A431 cell line

The skin cells were subjected to irradiation at wavelengths ranging 420-480 nm. power of 40 W at 15, 30 and 45 min. When we exposed the cells to direct light for different periods of time, through the measurements, we found the response of the cells to the power density of the source used, and therefore the change was made depending on the cell's response, and the best response was also searched for. the Xenon lamp had an effect of 82% in time 45 minutes, as shown in Figure 2. In times 15 and 30 minutes, we observed enhancement in cells rather than death, which its trust is due to the high direction and non-monochromatic nature of the lamp.

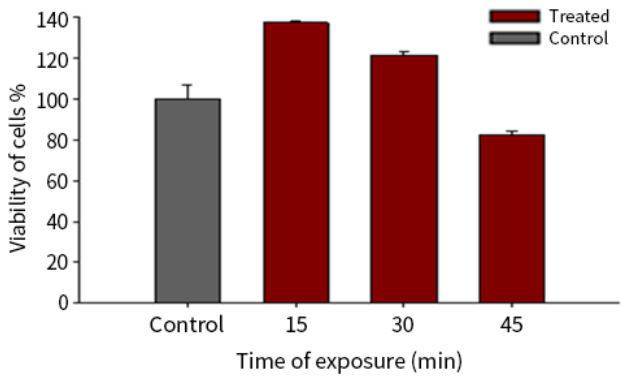

Figure 2. Effect of different irradiation times of Xenon lamp (420-480 nm) on A431 cell line after incubation for 24 hours

4.2 Cytotoxicity of TiO_2/Ag nanoparticles on A431 cells

The MTT test was used to assess the viability of human skin cells exposed to serial doubling increases in TiO_2/Ag nanoparticle concentrations ranging from 12.5 to 400 µg/ml. Nanoparticles' considerable suppression of cell viability was clearly detected in a dose-dependent manner. The cytotoxicity of TiO_2/Ag NPs against cancer cells was dependent on size because nanoparticles are taken up by cells by endocytosis. The released cytosolic silver ions may then produce large amounts of intracellular ROS, which may eventually lead to DNA damage and mitochondria-related apoptosis. Figure 3 shows the cell viability of TiO_2/Ag NPs with the concentration of nanoparticles.

The viability of cells decreases as the concentration of TiO_2/Ag nanoparticles was increased.

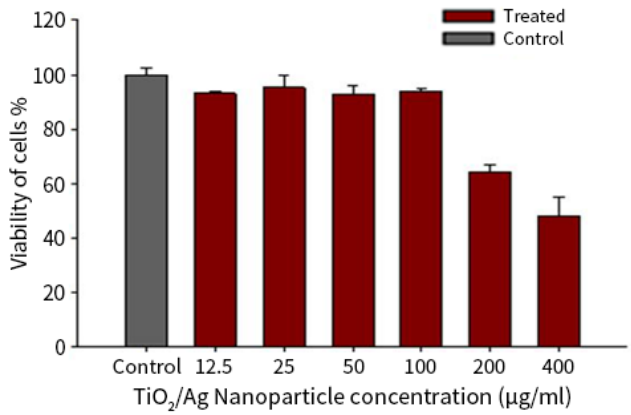

Figure 3. Effect of different concentrations of TiO_2/Ag on the A431 cell line after incubation for 24 hours

4.3 Combined effect of TiO_2/Ag nanoparticles and Xenon lamp on A431 cells

Figure 4 shows that the combination results showed that there was a significant decrease in cell viability percent ($p \leq 0.001$) at exposure times (15, 30, and 45 min) for 24 hours of incubation and that PDT by Xenon lamp-treated A431 cells showed major cell death, almost 42.79% in comparison to the control group, as shown in Table 1, at 400 µg/ml of TiO_2/Ag, which has been considered the optimal concentration in this study.

Table 1. Parameters of TiO_2/Ag in combination with Xenon lamp on A431 cell line

Time	Optical Density of Test Well	Ave. Optical Density of Control Wells	Viability of Cells %	Ave. Viability of Cells %
15 min	0.221	0.562	39.300	41.790
	0.258		45.880	
	0.226		40.189	
30 min	0.257	0.562	45.702	39.419
	0.206		36.633	
	0.202		35.921	
45 min	0.234	0.562	41.612	42.797
	0.218		38.767	
	0.27		48.014	

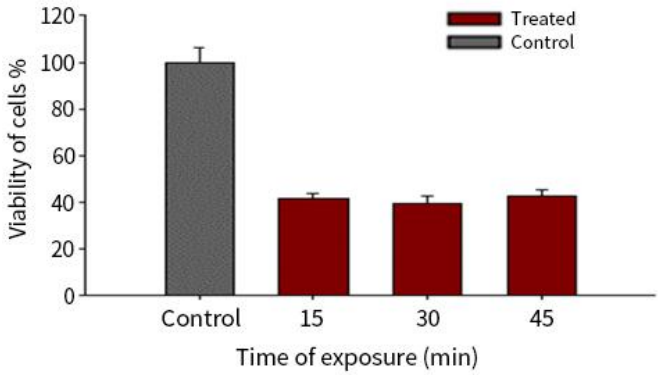

Figure 4. Effect of TiO_2/Ag in combination with Xenon lamp on A431 cell line after incubation for 24 hours

4.4 The morphology of skin cancer cell

Figure 5 shows that morphology of A431 cells at irradiance

of 40 W at irradiance time at 15 min, 30 min and 45 min significantly viability of cell migration compared with the control group, Figure 6 shows that morphology.

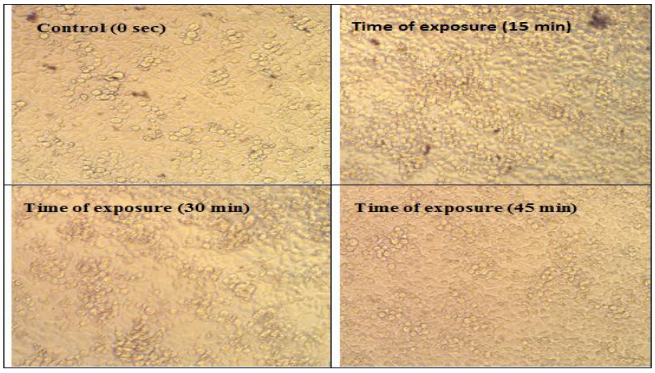

Figure 5. Observations of A431 cell morphological changes after exposure by Xenon lamp, the cells were observed by optic microscope directly

At optimal concentrations of NPs (400 µg/ml) there were obvious changes in cell survival, and as the exposure time increased, there was a modest change in cell survival, but as the time reached 45 min, cell survival dropped dramatically. Cells grown for 24 hours in media containing NPs exhibit a modest decrease in viability. There is a consistent change in cell viability with irradiation and NPs, and this change is distinct from that of cancer cells only (control).

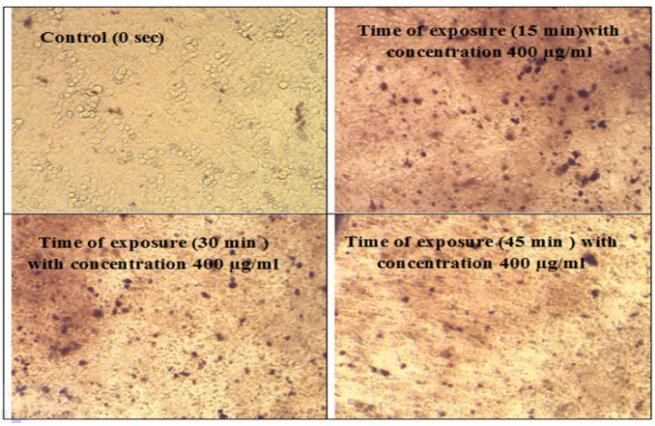

Figure 6. Observations of A431 cell morphological changes after exposure Xenon lamp with TiO2/Ag nanoparticles (400 µg/ml), the cells were observed by optic microscope directly

5. DISCUSSION

5.1 Light exposure therapy

Skin tumors are therefore naturally one of the objectives for blue light exposure therapy, particularly when there are many of them and they are still in the early stages of development when surgical excision is difficult but external light exposure is easily accessible. Due to the utilization of a narrow, high-intensity band of blue light, that may match the pea absorption wavelength at which an undiscovered chemical is transformed, phototherapy in the current study was likely more successful than using daylight. These findings are intriguing and imply that adding blue light to a photosensitizer or nanoparticles could boost PDT's effectiveness even further. Researchers led by Liebmann et al. [19] conducted an experiment in which they irradiated human keratinocytes and skin-derived endothelial cells with light-emitting diode devices of several wavelengths in order to investigate the effect that this had on the physiology of the cells. It was shown that light with wavelengths of (632 – 940 nm) has no impact; however, blue light with wavelengths of (412 - 426 nm) has hazardous effects when it is exposed to high influences [19]. In recent years, there has been a rise in the use of blue-light therapy, which often encompasses a diverse spectrum of wavelengths and incorporates a sizeable quantity of ultraviolet-absorption spectrum light (380–400 nm). In addition, several blue-light-generating lamps with emission maxima ranging from 400 - 440 nm are readily accessible and are touted as having positive effects on one's health. PBM is developing as a significant strategy in most health-care applications, and blue light has been utilized effectively in dermatological and aesthetic domains [20, 21].

5.2 Silver nanoparticles

According to a theory put forth by De Matteis et al. [22] silver nanoparticles are taken up by cells by endocytosis. The released cytosolic silver ions may then produce large amounts of intracellular ROS, which may eventually lead to DNA damage and mitochondria-related apoptosis [22]. According to Gurunathan et al. [23], the MDA-MB-231 breast cancer cell line was susceptible to the cytotoxicity of silver nanoparticles by a conventional p53-dependent apoptotic mechanism. Autophagy, however, has also recently been put forward as a potential mechanism. According to Lin et al.'s research [24], administering an autophagy inhibitor to patients increases the anticancer effect of nanoparticles. It is well known that the plasma and mitochondrial membranes may be harmed by oxidative stress brought on by a high quantity of ROS [23]. This ROS production, specifically for silver NPs, has repeatedly been noted as a key mechanism underlying their cytotoxic activity [23, 25]. As a result, the best absorption concentration of A431 cell nanoparticles was 400 µg/ml at 45 min of exposure.

5.3 Future recommendations

Use different laser sources with different wavelengths, as well as other cancer cells such as melanoma and colon cancer. Apply the PDT to the skin human cancer cells.

6. CONCLUSION

The main presumption used in the production of this paper is the use of PDT in skin tumor treatment at various exposure times. After putting the cells through an exposure treatment for intervals of 15, 30, and 45 minutes for Xenon lamps, it was found that the irradiation dose with a nanoparticle of 400 µg/cm^2 killed over half of the cells at 45 minutes. The results of adding TiO_2/Ag NP have demonstrated that the size, shape, and concentration of the nanoparticles, as well as the type and energy of the irradiation, play an important role in the treatment enhancement. The best results were obtained with a 45min exposure time in light with a TiO_2/Ag concentration of 400 µg/ml. The combination of the nanoparticle with light had a synergistic effect by reducing the cell numbers more than that noticed during using the nanoparticle or photodynamic

therapy separately. According to the findings of this study, an increase in the number of cancer cells that were eliminated indicates that the work that is being done here improves photodynamic therapy as a treatment for cancer. The quantity of data that can be used to facilitate the planning of subsequent operations in vivo investigations and is sufficient to motivate investigation into the use of titanium oxide-decorated silver nanoparticles with irradiation and adding nanoparticles into cell lines and exposure to phototherapy showed a 40% reduction in viability of cells, and this is a good result.

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