ORIGINAL RESEARCH

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Effect of Light Emitting Diode and TiO_2-decorated Ag Nanoparticles on Skin Cancer A431 Cell Line

Duaa J. Dheaa, Samira A. Mahdi

Department of Physics, College of Science, University of Babylon, Babel, Iraq.

*Corresponding author:
duaa.jaber.Scihigh113@student.uobabylon.Edu.iq
sci.samira.adnan@uobabylon.edu.iq

Abstract

Background: Devices based on light-emitting diodes (LEDs) are the newest and safest method for treating a variety of ailments, and dermatologists are now routinely using them. To confirm the effectiveness of this type of treatment, more controlled research are still required.

Materials and Methods: A431 Cell line and treatment with blue LED at output light wave intensity 1200-1400 mw/cm. photodynamic therapy (PDT) with TiO_2/Ag using human skin cancer cells as experimental model were used. Cell death and a possible mechanism of action were studied.

Results: Blue light (wavelength 420–480 nm) exposure caused a rapid and significant decline in viability, which was followed by the death of over half of the cells. The current paper reviews the state of the art in skin LED-based treatment methods. The newest and safest treatment is LED therapy with nanoparticle, the treatment of radioactively irradiated cells with LED causes an increase in cell death. Blue light exposure might open up new possibilities for treating superficial skin cancers in people.

Conclusion: The blue light (420–480 nm) of the light-emitting diode had a better anticancer effect on the SCC cell line after 24 hours of incubation. The best results were obtained with a 240-second exposure time in light with a TiO_2/Ag concentration of 400 µg/ml.

Keyword

Skin cancer, Photodynamic therapy (PDT), Nanoparticle, Light emitting diode (LED).

Imprint

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1. Introduction

The term "cancer" denotes to a disease in which living cells grow and proliferate outside of the body's control due to changes in Deoxyribo-Nucleic Acid (DNA), with some types resulting in tumor mass formation[1]. Skin cancers are cancers that develop as a result of abnormal cell division or mutation. Skin tumor is classified into three kinds: basal-cell skin cancer (BCC), squamous cell skin cancer (SCC) and melanoma. Basal Cell Carcinoma and Squamous Cell Carcinoma skin cancers are both types of non-melanoma skin tumor, whereas melanoma is an aggressive cancer that typically appears as a mole. Overexposure to UV radiation causes nearly 90% of skin cancer cases [2]. UV radiation overexposure is typically the result of excessive. Overexposure to UV light is frequently the result of too much. The effects of the various wavelengths included in sun irradiation are not well understood, though. Despite this ignorance, a number of phototherapy techniques have evolved recently that use a variety of light sources to treat hyperproliferative skin conditions such psoriasis, acne, keratosis, and skin cancer are treated with a broad range of wavelengths (380-440 nm),, frequently including a sizable fraction of ultraviolet light, are used to treat both skin cancer and other diseases. which, through DNA damage and subsequent mutations, has been connected to the development of cancer [3]. As a result, it is a dangerous technique for treating skin conditions linked to a higher risk of tumor development. Determining specific wavelengths and energy densities that have biological benefits while preventing negative side effects is therefore crucial. Research in this area has advanced thanks to the use of coherent light sources (such as low-power laser therapy) and noncoherent light sources (such as light-emitting diodes, xenon arc, metal halide, tungsten filament, and fluorescent lamps) with very small bandwidths. This has made it possible to link specific biological effects to specific wavelengths. The biological relevance of these devices is undeniable [4, 5] notwithstanding the controversy surrounding their impact on cellular functions. Early in the 20th century, photodynamic therapy (PDT) was developed as an experimental therapy that uses a photosensitizing medication, light, and oxygen to destroy cancer cells [4]. PDT was initially used to treat cutaneous, esophageal,

bladder, and bronchial cancers. PDT provided possible tumor elimination with good cosmetic results, patient tolerance, and a speedy recovery time when compared to traditional surgery [6].

2. Materials and Methods

2.1. Cell line and culture conditions

Cell line A431 non melanoma skin cancer was used for the in vitro experiments. A431 Cell line were provided from the American Type Culture Collection (ATCC). The cell line was cultured with a complete growth medium, RPMI-1640, which was prepared according to the Gibco manual with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin antibiotics and incubated at 37 C.

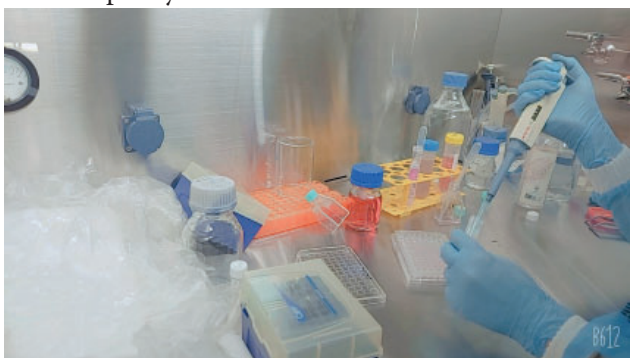

Figure 1. The 96-well plates for MTT assay test.

2.2. Light exposure

The LED (blue light) used in these experiments is based on a commercially available LED, emitting at around 420–480 nm with an output light wave intensity of 1200–1500 mW per cm. Blue light-emitting diodes were used to treat SCC cells at various irradiation times (10, 20, ….., 220, 230, and 240 seconds).

Figure 2.Setup of PhotodynamicTherapy (PDT) by blue LED in this work.

2.3. TiO_2/Ag Preparation

To begin creating the TiO_2/Ag composite colloids, 30 grams of industrial TiO_2 was ground and dissolved in 200 mL of deionized water. using a nanometer grinder for 30 minutes at 3000 rpm to produce nano-TiO_2.

Then, 100mL of PEG-600 were constantly mixed with 5 mL of TiO_2 slurry and 1g of $AgNO_3$ using a magnetic stirrer. Reactions were carried out for 4 hours at 60 °C. To create TiO2/Ag powder, the TiO_2/Ag colloids were centrifuged at 3000 rpm for one hour, washed with ethyl alcohol, and then dried in vacuum at 80 °C for twenty-four hours.

2.4. MTT assay (growth inhibition assay)

As previously mentioned [7], cell growth was measured by measuring the absorbance of 3-(4, 5-dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich). In 96 wall plates, 50000 cells per wall were seeded, and they were exposed to blue LED radiation for the specified amount of time. The solution of MTT (10 µl: 5 mg/ml in PBS) was added to each of the 96 wall plates after blue LED exposure. The plates were then incubated for an additional 3–4 hours at 37°C. The medium was then pipetted out, 100 of DMSO was added to each well to dissolve the formazan crystals. A micro plate reader was used to assess optical density at 570 nm (Molecular Devices, USA). The viability percentage was estimated using this equation:

3. Statistical Analysis

Sigma Plot version 13 and Microsoft Office Excel 2010 were used to collect and analyses all the data. The significance of differences between the data means was evaluated using the ANOVA test, where a p-value of between 0.001 and 0.05 was deemed statistically significant.

4. Result

4.1. Blue light inhibited the growth of A431 non-melanoma cells

The CCK-8 determines viability. The LED blue light at 420–480 nm had a significant cytotoxic effect, and the effect of cell death got bigger with increased exposure time. After exposure treatment for 240 seconds, I killed nearly half of the cells (Figure 3). Therefore, blue light 420–480 nm was used to treat SCC cells

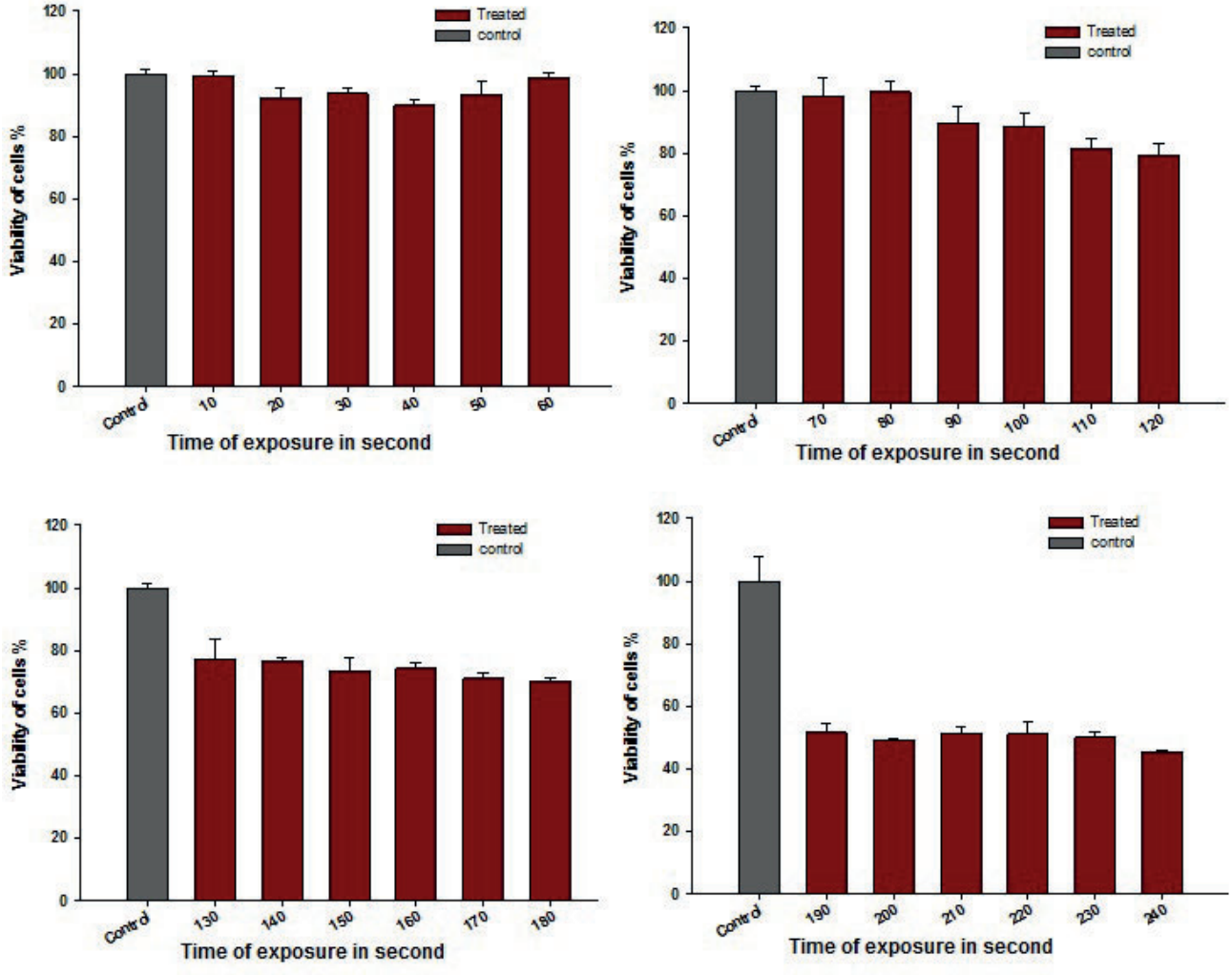

Figure 3.Effect of different irradiation times of Blue LED on A431 cell line after incubation for 24 hours.

in order to study cell reactions to PBM at various irradiances and doses.

4.2. Effect TiO_2/Ag nanoparticles on skin cancer cell line

Nanoparticles concentrate inside the tumor by direct injection. The cell viability in the presence of the TiO_2/Ag nanoparticles was investigated through the MTT assay, as depicted in Figure 4.Compared to the control group.

Figure (4) that PDT treated A431 cells show major cell death, almost 80%, at 50 µg/ml and it becomes 50% up till TiO_2/Ag concentration of 400 µg/ml which has been considered as optimal concentration in current study.

the results of photodynamic treatment of human skin cancer cell line with optimum parameter 400 µg/ml, there was obvious cytotoxicity in A431 cells in the concentration 400 (P>0.05) (figure 5) viability of cell rate was all approximately 20% with increasing time of

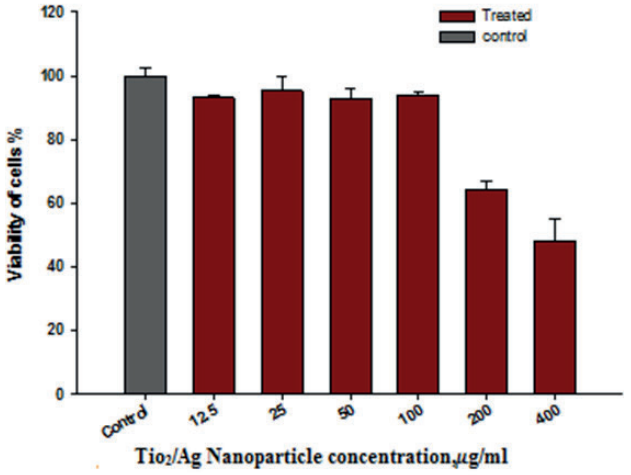

Figure (4): Effect TiO_2 decorated Ag nanoparticles on skin cancer cell line

exposure by applying the equation (1) on skin cancer without and with using nanoparticles with Photodynamic therapy, which indicated the Ag@TiO_2 NPs exhibited good biocompatibility. The cell could still show approximately 20% of vitality in time period 240 sec of exposure. we get a decrease in the number of surviving

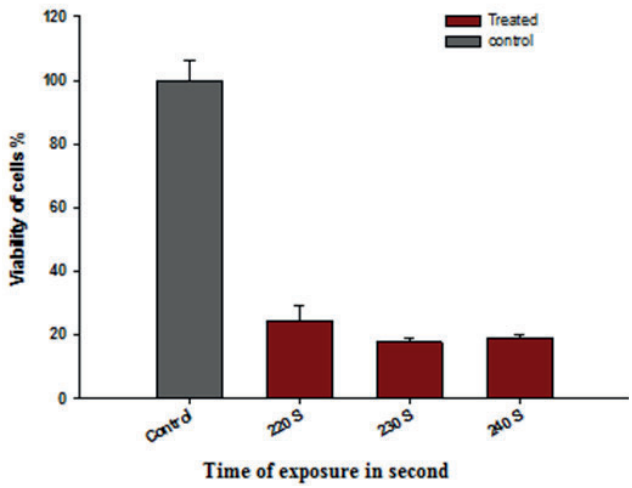

Figure (5): Effect TiO2 decorated Ag nanoparticles (400µg/ml) after exposed to different exposure.

cancer cells due to presence of Tio_2/Ag nanoparticles with increasing time of exposure. The number of surviving malignant cells is decreasing exponentially with increased time of exposure. as shown in figure (5) The evidence at hand points to multiple processes that may be involved in cell death as the mechanism underlying the anticancer characteristics.

According to a theory put up by De Matteis et al., 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 [8]. According to Gurunathan et alreport,. 's the MDA-MB-231 breast cancer cell line was susceptible to the cytotoxicity of silver nanoparticles by a conventional p53-dependent apoptotic mechanism [9]. Autophagy, however, has also lately been put out as a potential mechanism. According to Lin et alresearch,. 's administering an autophagy inhibitor to patients increases the anticancer effect of nanoparticles [10]. It is well known that the plasma and mitochondrial membranes may be harmed by oxidative stress brought on by a high quantity of ROS [8]. This ROS production, specifically for silver nanoparticles, has repeatedly been noted as a key mechanism underlying their cytotoxic activity [8, 11].

4.3 Tio_2/Ag Nanoparticle characterization

Under specific conditions, the X-ray diffraction (XRD) technique was used to determine the type of crystal structure, the major crystalline stages, and the orientation of the films that were generated as well as to identify some structural elements including the crystal size.. Figure (6) shows the (XRD) pattern of theTiO_2/Ag produced films. XRD pattern showed peaks at 2θ = 25.4° correspond to planes (101) this agreement with [12]. Furthermore, weak peaks corresponding to metal silver can be found at "2 θ = 44.3° (2 0 0), 64.4° (2 2 0) and 77.4° (3 3 0) ". It showed that Ag had attached itself to the TiO_2 supports successfully. Unluckily, this is because there are few metals present and the TiO_2 supports have high diffraction peaks [5, 8]. The strength of the primary signal in PEG following Ag photo reduction on the surface of TiO_2 declines, showing that the anchoring procedure did not alter TiO2's distinctive crystal surface structure [12]. My samples had rather tiny crystallite sizes for TiO_2/Ag, ranging from 5 to 25 nm.

Discussion

Cancer of the squamous cell is a form of malignant skin neoplasm with a high risk of morbidity [13], making it crucial for the creation of innovative anti-malignant tumor therapies. LED-based complementary treatments (phototherapy). These intriguing findings suggested that blue light therapy might be an effective method for treating skin tumors. In comparison to the cell cycle blockade observed by Ohara et al., blue light in the current investigation displayed stronger and faster cytotoxic effects. In line with Godley et al., we pro-

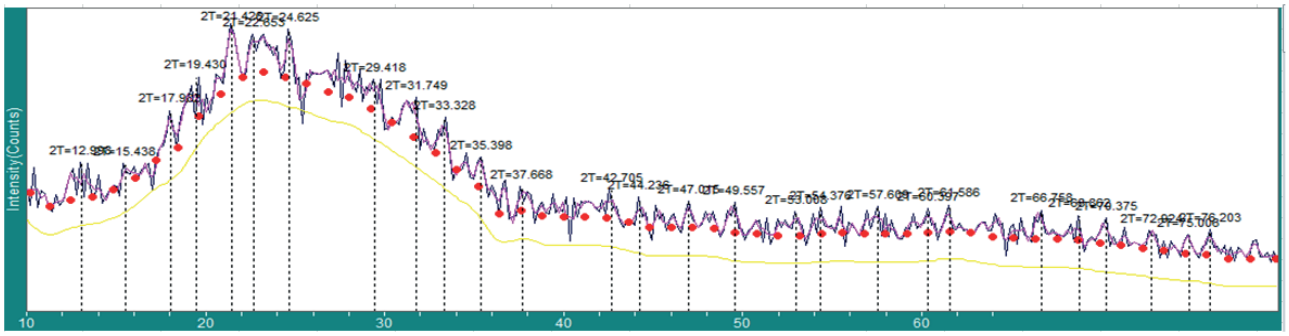

Figure (6): The XRD analysis of sample of Tio_2/Ag nanoparticles

pose that the cytotoxic effects of blue light on non-pigmented epithelial cells are brought on by various types of damage. [14] In v-Ha-ras transgenic mice, exposure to blue LEDs also greatly decreased the incidence and amount of papilloma's brought on by TPA (12-O tetradecanoylphorbol-13-acetate) administration[15]. 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. [16]. These findings are intriguing and imply that adding blue light to a photosensitizer could boost PDT's effectiveness even further. Porphyrins or any photosensitizer are more able to endure blue light and strongly absorb it. Despite having limited skin penetration, blue light during PDT might have a synergistic effect with porphyrins. Our findings contradict previous research that claimed blue light exposure increased cell proliferation in human uveal melanoma cells compared to controls [17]. There is a mismatch between visible light (450–490 nm) and ultraviolet (UV–400 nm), and the spectrum of radiation (100–400 nm) was not covered in many articles. most likely due to a small amount of UV [18]. As a result, the best absorption wavelength of A431 cell photoreceptors at (1200–1400 mW/cm) was suggested to be nearly 450 nanometers. We hypothesized that this was due to the best-wavelength photoreceptor to treated of skin cancer cell.

Conclusion

The blue-light (420-480 nm) of light-emitting-diode had a better anticancer effect on SCC cell line after 24 hours. had a stimulatory effect at lower exposure time (170 second) it had an anticancer effect at 24 hours. While it had an anticancer effect at all other exposure time (190, 200, 210, 220, 230, 240 seconds) for 24 hours. The effect was dose-time dependent, The best results were obtained with a 240-second exposure time in light with a TiO_2/Ag concentration of 400 µg/ml.

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