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RESEARCH ARTICLE

Development of Promising Red-Light-Induced Chitosan-Based Photocatalysts for Cancer Therapy

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ABSTRACT: Cancer remains a global health challenge, with lung and skin cancers among the leading causes of mortality. Conventional treatments pose limitations, necessitating innovative approaches. This study explores the application of red-light-induced chitosan-based photocatalysts for photodynamic therapy (PDT). The unique properties of chitosan nanoparticles, derived from chitin, and the photosensitizer protoporphyrin IX (PPIX) are leveraged for targeted cancer treatment. Chitosan nanoparticles were synthesized through chitin deacetylation and ionotropic gelation, reducing the size from 1300 kDa to ~50 kDa. PPIX-chitosan complexes were formed at varying ratios (1:1, 4:1, 16:1, and 160:1) and evaluated for cytotoxicity. A laser device emitting 660 nm red light activated the complexes. A549 lung cancer cells and SK-MEL-2 skin cancer cells were tested, and cell viability was performed. The chitosan nanoparticles exhibited successful formation. Cytotoxicity results revealed optimal chitosan to PPIX ratios of 16:1 and 160:1, minimizing adverse effects. Laser-activated PPIX-chitosan complexes demonstrated significant cell death in A549 and SK-MEL-2 cells, validating their efficacy in cancer treatment. This study pioneers using chitosan-based photocatalysts for cancer therapy, showcasing their potential in PDT. The environmentally friendly nature and cost-effectiveness of chitosan further contribute to its viability for mass production and broad clinical applications.

KEYWORDS: Biomedical Engineering Medicine; Biomaterials and Regenerative; Cancer; Photodynamic Therapy; Chitosan-Protoporphyrin IX complex.

Introduction

Globally, a sizable proportion of cancer-related deaths are attributable to lung cancer. Smoking, exposure to carcinogens, a family history of lung cancer, or a genetic predisposition are all causes of lung cancer.[1] The carcinogens in tobacco smoke can damage lung cells over time, leading to cancer growth. Non-Small Cell Lung Cancer (NSCLC) and Small Cell Lung Cancer (SCLC) are the two main subtypes of lung cancer. Approximately 85% of all lung cancers are NSCLC. Adenocarcinoma, squamous cell carcinoma, and large cell carcinoma are subtypes of NSCLC. SCLC has a propensity to grow and spread rapidly and is frequently linked to excessive smoking.[2] About 15% of lung cancer cases are caused by it. The treatment options for lung cancer include surgery, chemotherapy, radiation therapy, targeted therapies, and immunotherapy. The choice of treatment depends on the cancer's stage and type.[3]

Skin cancers are mainly caused by sunlight's ultraviolet (UV) radiation. Sunburns, a family history of skin cancer, having many moles or atypical moles, and a weaker immune system are additional risk factors.[4] Basal Cell Carcinoma (BCC), Squamous Cell Carcinoma (SCC), and Melanoma are the three main kinds of skin cancer. The most common skin cancer is basal cell carcinoma, characterized by slow-growing, shiny, painless kinds that rarely spread but may result in local damage if neglected. Typically, squamous cell carcinoma appears thick, scaly, crusty bumps, or rapidly growing. The most fatal form of skin cancer, melanoma, often occurs as irregular, asymmetrical moles with a range of coloration and irregular bonds. Based on the stage and type, surgical excision, radiation therapy, Mohs micrographic surgery, electrodessication and curettage, or cryotherapy can be applied.[5]

Cancers are currently treated with various therapies which have been recently developed. In addition to using surgery, chemotherapy, and radiation therapy to target and kill cancer cells, immunotherapy uses the body's immune system. Depending on the patient's profile, targeted therapies target specific molecules crucial for tumor growth.[6] Current treatments are becoming more individualized and efficient. However, several limitations still exist in cancer therapy. For example, cancer cells can become resistant to therapeutic approaches, reducing their efficacy over time. Furthermore, many cancer treatments include serious side effects that might lower a patient's quality of life. Cancers diagnosed in late stages are also a problem due to the effective treatments. Patients may even suffer from the tremendous cost caused by the long-term treatment. To overcome these obstacles, continued research is needed on new treatments, cost-effective techniques, and approaches to lessen side effects.[7]

Red-Light-Induced Photocatalyst is a promising approach to photodynamic therapy (PDT). A photosensitizer (a light-sensitive substance) is given to the patient during PDT and accumulates in the cancerous tissue.[8] Then, upon red light activation, the PPIX photosensitizer generates ROS, primarily singlet oxygen. These highly reactive species cause oxidative stress within cancer cells, damaging cellular components such as lipids, proteins, and DNA. Red light suits PDT because it can reach deeper-located cancers and penetrate tissues more deeply than shorter wavelengths. By precisely applying red

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light to the tumor, adjusting the photosensitizer's placement there, and considering how cancer cells react differently to ROS compared to healthy cells, it is possible to conduct therapy selectivity.[9] Despite the potential of red-light-induced photocatalysts, there are obstacles, such as making sure the photosensitizer only accumulates in cancer cells, not healthy tissue. Therefore, nanotechnology advancements are being developed to enhance cancer cell targeting and improve light absorption by designing photocatalysts based on nanomaterials.[10]

A sugar called chitosan is derived from chitin, which is commonly found in the exoskeleton of shellfish like crab, lobster, and shrimp.[11] Glucosamine and chitobiose are the two main building blocks of chitosan, which has a double helix structure comprising six sugar residues forming a spiral plane. Small chitosan particles known as chitosan nanoparticles (ChNP) exhibit unique characteristics, including surface and interface effects and small size.[12] ChNPs are used in nanomedicine to deliver medications to specific cells or tissues. Chitosan is known to be well-tolerated by the body, which reduces the likelihood of adverse reactions. It is also biodegradable, minimizing long-term adverse effects as it gradually breaks into non-toxic metabolites. As a result, researchers are actively exploring the environmentally, economically, and biologically friendly characteristics of chitosan nanoparticles as a promising material for medicine.[13]

Protoporphyrin IX (PPIX) is a type of porphyrin, an organic chemical that makes up the central portions of molecules such as hemoglobin and chlorophyll. Four pyrrole rings bridged by four sp2 hybridized carbon atoms create porphyrin. PPIX, a heme metabolite, is a hydrophobic photosensitizer that accumulates the lipid structures of cancer cells.[14] Even though it remains inactive until exposed to specific wavelengths of light, PPIX produces reactive oxygen species (ROS) through a photochemical reaction when exposed to light. The generated ROS are highly reactive and toxic to cellular components, which helps destroy tumor tissue while sparing surrounding healthy tissue. Therefore, PPIX is often utilized as a photosensitizer in photodynamic treatment (PDT), and it has gained significance in medical studies.[15]

This study focuses on investigating and creating cutting-edge nanotechnology-based methods to improve the effectiveness and safety of PDT for cancer treatment. It explores the efficacy and non-invasiveness of killing cancer cells using chitosan nanoparticles as a carrier, PPIX as a photosensitizer, and 660 nm red light as a trigger. Therefore, the findings of this study may aid in developing safer, more effective photodynamic therapy techniques for cancer treatment, enhancing therapeutic results and minimizing side effects.

Methods and Materials

Converting chitin to chitosan:

Chitin was fragmented by dissolving 5 grams of chitin in 150 mL of HCl at 50 ˚C for 18 hours. Through filtering, 3 grams of chitin powder were collected. Fragmented chitin was then dissolved in 100 mL of 30% NaOH at 50 ˚C for 18 hours, and chitosan powder was collected and dried after washing with distilled water.

Chitosan Nanoparticle Preparation:

0.5 grams of chitosan were dissolved in 500 mL of 0.5% (v/v) acetic acid solution (2.5 mL acetic acid mixed with 497.6 mL water). Chitosan concentration could depend on the desired nanoparticle size and concentration. The chitosan solution was stirred to ensure it was well-mixed and homogeneous with five minutes incubation time. To form chitosan nanoparticles from chitosan solution, in the dropwise addition method, 3.95 mL of 0.10 wt % (2.7 mM) tripolyphosphate (TPP) solution was slowly titrated (at a rate of roughly 1 drop/s) into 10 mL of 0.10 wt% (5.2 mM in its cationic glucosamine units) chitosan solution, yielding a final chitosan concentration of 0.072 wt% (3.7 mM). Stirring of the mixture was continued for 10 minutes to allow nanoparticle formation. Then, chitosan nanoparticles were purified through centrifugation to separate them from the solution. Collected nanoparticles were dried using the refrigerator.

Protoporphyrin IX (PPIX) Preparation:

Protoporphyrin IX (PPIX) (CAT# P8293-1G) was purchased from Sigma Aldrich. The purchased PPIX was dissolved in dimethyl sulfoxide (DMSO) to create a 10 mg/ml PPIX solution. The solution was thoroughly stirred to ensure the PPIX was well-dissolved and evenly dispersed.

PPIX-Chitosan Complex Formation:

The PPIX solution was added to the 0.072 wt% (3.7 mM) chitosan nanoparticle solution. The mixture was gently stirred for 10 minutes to allow for a stable complex formation between the PPIX and the chitosan nanoparticles. The complex formation occurs due to the interaction between the chitosan's amino groups and the PPIX. During the procedure, four samples with different Chitosan to PPIX molar ratios were made: 1:1 (478 µL:100 µL), 4:1 (478 µL:25 µL), 16:1 (478 µL:6.25 µL), and 160:1 (478 µL:0.625 µL). The complex was purified through centrifugation to separate it from the solution. The complex was washed with 0.5% (v/v) acetic acid solution to remove any unbound PPIX or impurities. The complex was dried and stored in the refrigerator.

Human Cancer Cell Culture:

A549 lung cancer cells and SK-MEL-2 skin cancer cells were purchased from Korea Cell Line Bank. RPMI1640 cell culture media with 10% fetal bovine serum and 1% penicillin and streptomycin antibiotics. The cells were incubated in a CO2 incubator at 37 ˚C.

PPIX-Chitosan Complex treatment:

A549 lung cancer cells and SK-MEL-2 skin cancer cells were treated with 100 µL of 1:1, 4:1, 16:1, and 160:1 chitosan to PPIX complex solutions for 24 hours. Then, the laser wavelength of 660 nm was applied to the cancer cells for 24 hours. Lastly, the cell viability was performed, and a cell image was taken to check the cancer cell integrity.

Cell viability assay:

Cell viability was analyzed using a LUNA-FL automatic cell counter device. The Acridine Orange and Propidium Iodide, cell staining solution, was used to stain the live and dead

cells. Live cells were stained green fluorescent, and dead cells were stained red fluorescent.

Laser device setup:

A laser wavelength of 660 nm was developed and set up with the plastic adjuster. The adjuster allows the height of the laser to be arranged. The laser on-off switch was also set up in the device. The cells were treated with the laser for indicated hours throughout the experiments.

Cell image analysis:

The ImageJ program was used to analyze the dead cell area in pixels. The area of the cell pixel was quantified using the Image J program.

Essential notes for reproducibility of PPIX-Chitosan complex:

Several critical factors must be meticulously controlled to ensure the reproducibility of the PPIX-Chitosan complex synthesis. First, filtering the chitosan solution is essential to remove undissolved particles that could interfere with uniform nanoparticle formation. The tripolyphosphate (TPP) addition rate must also be carefully managed, ideally with a syringe pump, to maintain a consistent drop rate, as fluctuations can result in non-uniform particles. Maintaining a constant stirring speed during TPP addition is crucial to ensure uniform nanoparticle size. Washing steps should be performed carefully to prevent the loss of nanoparticles, and centrifugation conditions such as speed, time, and temperature must remain consistent across batches to achieve reliable particle separation. By adhering to these steps, the reproducibility and consistency of the PPIX-Chitosan complex synthesis can be ensured, which is vital for obtaining reliable experimental results and for further applications of the nanoparticles.

Results

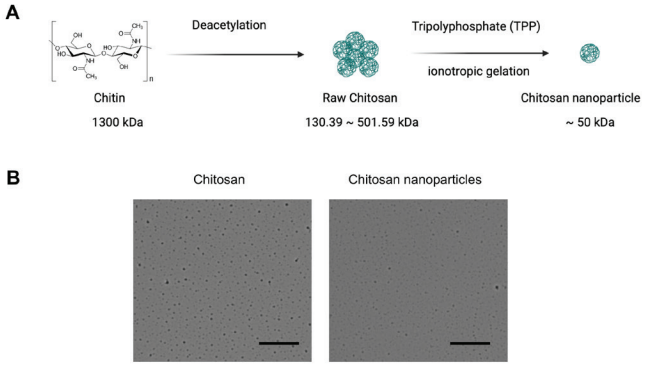

Figure 1: Chitosan nanoparticle formation through deacetylation and ionotropic gelation. (A) Schematic representation of the experimental process aimed at converting chitin to chitosan. Chitin was subjected to 30% NaOH solution for 18 hours, inducing deacetylation by breaking acetyl groups from the chitin polymer chain. This resulted in the formation of chitosan. To create nanoparticles, the cross-linking agent tripolyphosphate (TPP) was employed to stabilize the chitosan nanoparticles and prevent aggregation. The positively charged amino groups of the chitosan solution engaged with negatively charged TPP molecules through ionic interactions during dropwise addition to the TPP solution, leading to ionotropic gelation and the creation of chitosan nanoparticles. (B) Microscopic images illustrating the particle sizes before and after ionotropic gelation. The left picture displays pure chitosan particles, while the right picture exhibits chitosan nanoparticles. The smaller size of the chitosan nanoparticles is evident, showcasing the success of the ionotropic gelation process in reducing particle size from 1300 kDa to 130~500 kDa. Scale bar = 1 μm

We put chitin into a 30% NaOH solution for 18 hours to collect chitosan. Sodium hydroxide breaks the acetyl groups from the chitin polymer chain through deacetylation, forming chitosan (Figure 1A). To create nanoparticles, we used the cross-linking agent tripolyphosphate (TPP), which helps to stabilize the chitosan nanoparticles and prevent them from aggregating.[16] The positively charged amino groups of the chitosan solution engage with the negatively charged TPP molecules through ionic interactions when it is added dropwise to the TPP solution while being stirred. Chitosan nanoparticles are created due to this reaction called ionotropic gelation (Figure 1A). This experimental process aimed to convert chitin to chitosan through deacetylation and chitosan to chitosan nanoparticles using TPP solution through ionotropic gelation. Chitosan nanoparticles are widely used in medication delivery because of their efficient delivery and binding ability from the small size with high surface area.[17] Therefore, in this experiment, chitosan nanoparticles were used as a carrier of PPIX since relatively positively charged chitosan will be attracted to relatively negatively charged cancer cells, which we are targeting. We could reduce the particle size from 1300 kDa to 130~500 kDa by conducting Chitin deacetylation. Following the process, through ionotropic gelation, we could produce ~50 kDa chitosan nanoparticles from 130~500 kDa sized collected chitosan (Figure 1A). The black dots on the figure are the particles of chitosan (Figure 1B). The right picture shows pure chitosan particles, and the left picture shows the chitosan nanoparticles after the ionotropic gelation. In the figure, the chitosan nanoparticles(right) have much smaller sizes than the pure chitosan particles(left) (Figure 1B). We could successfully develop chitosan nanoparticles from chitin through deacetylation and ionotropic gelation of chitosan.

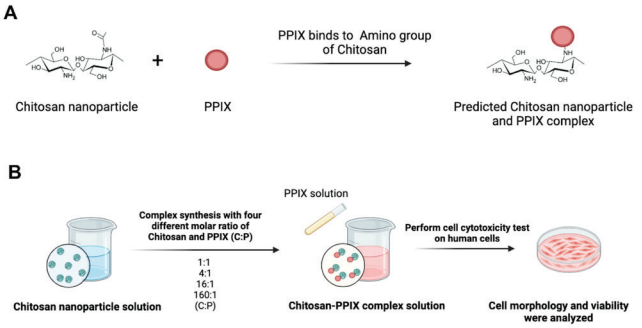

Figure 2: Formation and evaluation of PPIX-Chitosan nanoparticle complexes. (A) Schematic representation illustrating the stable bonding between chitosan, a biocompatible and biodegradable polysaccharide derived from chitin, and PPIX, a stable and non-toxic photosensitizer. The amino group of chitosan facilitates a stable bond with PPIX, emphasizing the compatibility and safety of this complex. (B) Experimental setup involving the combination of chitosan nanoparticles with a PPIX solution at distinct molar ratios (1:1, 4:1, 16:1, and 160:1) to create PPIX-chitosan nanoparticle complexes. This step aims to assess the most effective complex for cancer treatment by varying the concentrations of chitosan and PPIX. Subsequent evaluation of cell cytotoxicity provides insights into the optimal ratios for inducing cancer cell death.

Chitosan is a polysaccharide derived from chitin, a biocompatible and biodegradable source. Therefore, it does not cause measurable toxicity or immune responses in the body.

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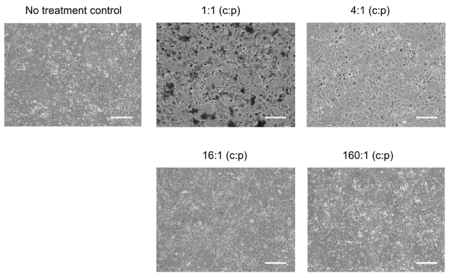
Similarly, even though it becomes excited and reacts with oxygen when exposed to a specific wavelength, PPIX is relatively stable and non-toxic without light activation. These two components, carrier and synthesizer, can be bonded stably through the chitosan's functional group, the amino group (Figure 2A). First, we combined chitosan nanoparticles with a PPIX solution at four distinct molar ratios to assess the most effective complex for cancer treatment: 1:1, 4:1, 16:1, and 160:1 (Figure 2B). Next, we applied each complex to cancer cells to check the cell cytotoxicity.



	Total cell concentration	Live cell concentration	Dead cell concentration	Viability
No treatment control	7.14 x 10^5 cells/mL	4.88 x 10^5 cells/mL	2.26 x 10^5 cells/mL	68.4 %
1:1 (c:p)	5.03 x 10^5 cells/mL	0 cells/mL	5.03 x 10^5 cells/mL	0%
4:1 (c:p)	6.96 x 10^5 cells/mL	1.64 x 10^4 cells/mL	6.79 x 10^5 cells/mL	2.4 %
16:1 (c:p)	7.18 x 10^5 cells/mL	3.99 x 10^5 cells/mL	3.19 x 10^5 cells/mL	55.5%
160:1 (c:p)	6.50 x 10^5 cells/mL	4.12 x 10^5 cells/mL	2.38 x 10^5 cells/mL	63.4 %

Figure 3: Comparative evaluation of cell viability under different Chitosan-to-PPIX ratios (1:1, 4:1, 16:1, and 160:1): After a 24-hour treatment period, cell viability was measured using a cell counter. The 16:1 and 160:1 Chitosan-to-PPIX ratios were the most favorable conditions due to their lower toxicity in human cells. Scale bar = 300 μm

While PPIX is generally known to be non-toxic and remains inactive without light exposure, an excessive concentration of PPIX molecules in conjunction with Chitosan may induce cell cytotoxicity. Ensuring the non-toxicity of these particles in the absence of red light, which triggers the PPIX reaction, we conducted the experiments to assess cell viability by varying the molar ratio of chitosan and PPIK mixtures under conditions without red light stimulation. The viability of the cells when there was no treatment (nanoparticle and PPIX complex) was 68.4%, looking normal. In contrast, dark areas are shown in the images of a 1:1 ratio solution and a 4:1 ratio solution (Figure 3). The dark portions are speculated to be the aggregated nanoparticles with cells, increasing toxicity. Numerically, when there was treatment with a 1:1 ratio solution, the cells showed 0% viability, and the treatment with a 1:4 ratio solution also showed only 2.4% viability of the cells. However, when there was less PPIX concentration, the 16:1 solution showed 55.5% cell viability, and the treatment of the 160:1 solution resulted in 63.4% viability, almost like the one with no treatment. Looking at the result, we concluded that excessive PPIX molecule concentration adversely impacts cell viability, particularly with a 1:1 and 1:4 chitosan to PPIX ratio. As a result, we ended up using a 16:1 or 160:1 chitosan to PPIX ratio solution for the following experiments.

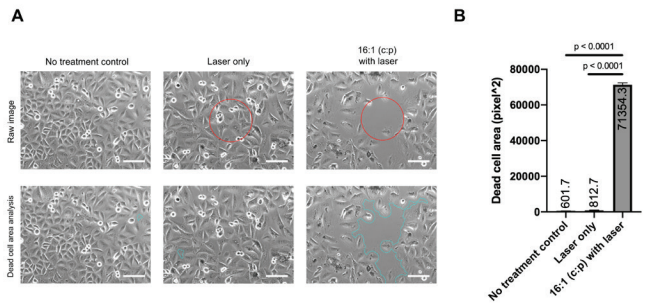


Figure 4: PPIX-Chitosan nanoparticle complex efficiently induced cell death on A549 Lung Cancer Cells with 660 nm Red Light Laser. (A) Cell image of the PPIX-chitosan nanoparticle complex's effectiveness on A549 lung cancer cells using a 660 nm red light laser. The treatment group with a 16:1 (chitosan: PPIX) complex, when exposed to the laser, demonstrated a substantial decrease in cell viability, with nearly all cells in the laser-treated area undergoing cell death. (B) Quantitative analysis of the dead cell area difference between the control sample and the laser-treated group with the 16:1 complex. Scale bar = 100 μm. A one-way ANOVA test with Tukey's posthoc test was used to calculate the p-value.

In this experiment, the effectiveness of PPIX-chitosan nanoparticle complexes on lung cancer cells is tested using a 660 nm red light laser. The cell line used in the experiment is A549, commonly used in lung cancer research. When comparing the no-treatment control with the laser-only group, almost no difference was observed. It resulted in only about 200 pixels of dead cell area of difference. However, when the 16:1 (chitosan: PPIX) complex was treated with a laser, almost all the cells in the laser-treated area died (Figure 4A). Approximately 70,500 pixels of dead cell area difference was observed compared to the control sample (Figure 4B). These results indicate that PPIX-chitosan nanoparticle complexes effectively kill cancer cells when exposed to a 660 nm laser.

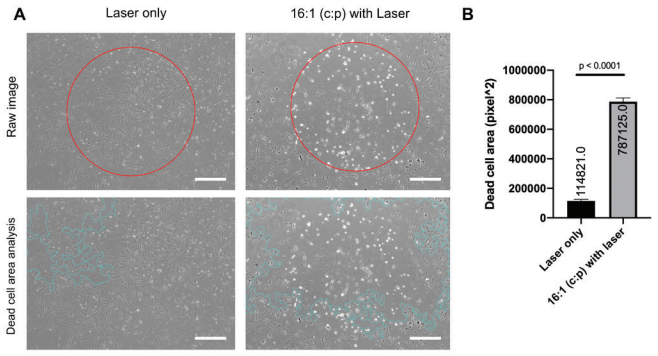


Figure 5: PPIX-Chitosan nanoparticle complex efficiently induced cell death on SK-MEL-2 skin cancer cells with a 660 nm red light laser. (A) Cell image of evaluating the effectiveness of PPIX-chitosan nanoparticle complexes on SK-MEL-2 skin cancer cells. The 16:1 (chitosan: PPIX) complex, when subjected to a 660 nm red light laser, resulted in a notable reduction in cell viability, with nearly all cells in the laser-treated area experiencing cell death, mirroring the outcomes observed in lung cancer cells. (B) Quantitative analysis of the dead cell area difference between the control sample and the laser-treated group with the 16:1 complex. Scale bar = 300 μm

Duplicating the experiment shown in Figure 4, this experiment is conducted to test the effectiveness of PPIX-chitosan nanoparticle complexes on SK-MEL-2 skin cancer cells. SK-MEL-2 is a cell line derived from metastatic melanoma, a type of skin cancer. Similar to the experiment on lung cancer cells,

when the 16:1 (chitosan: PPIX) complex was treated with a laser, almost all the cells in the laser-treated area died (Figure 5A). Approximately 670,000 pixels of dead cell area difference was observed compared to the sample only treated with a laser but without the nanoparticle complexes (Figure 5B). These results again indicate that PPIX-chitosan nanoparticle complexes effectively kill cancer cells when exposed to a 660 nm laser.

Discussion

First, we could reduce the particle size from 1300 kDa to 130~500 kDa by conducting Chitin deacetylation. Then, through ionotropic gelation, we could produce ~50 kDa chitosan nanoparticles from 130~500 kDa-sized collected chitosan. Therefore, we could successfully develop chitosan nanoparticles from chitin through deacetylation and ionotropic gelation of chitosan. After, chitosan nanoparticles are combined with a PPIX solution at four distinct molar ratios to assess the most effective complex for cancer treatment: 1:1, 4:1, 16:1, and 160:1 chitosan particle to PPIX ratio. According to the experimental result, excessive PPIX molecule concentration adversely impacts cell viability, particularly with a 1:1 and 1:4 chitosan to PPIX ratio. Therefore, we concluded the use of a 16:1 or 160:1 chitosan to PPIX ratio solution for the following experiments. Finally, when PPIX-chitosan nanoparticle complexes are treated on A549 lung cancer cells with 660 nm red light laser, approximately 70,500 pixels of dead cell area difference was observed compared to the control sample and one only treated with only laser, indicating that complexes effectively kill cancer cells when exposed to a 660 nm laser. Duplicating the experiment on lung cancer, when treated on SK-MEL-2 skin cancer cells, approximately 670,000 pixels of dead cell area difference was observed compared to the sample only treated with a laser but without the nanoparticle complexes. This proves that chitosan-PPIX nanoparticle complexes effectively kill cancer cells when exposed to a 660 nm laser.

The mode of cell death induced by the PPIX-chitosan complex appears to involve both apoptosis and necrosis. Apoptosis, a programmed form of cell death, may be initiated by the accumulation of ROS, which activates intrinsic apoptotic pathways. The oxidative stress damages mitochondrial membranes, releasing cytochrome c and activating caspases, enzymes that drive apoptotic cell death. In parallel, severe oxidative damage can overwhelm cellular repair mechanisms, leading to necrosis, where cells lose membrane integrity and die in an uncontrolled manner. Moreover, PPIX accumulates more in cancer cells than in normal cells due to reduced ferrochelatase activity, an enzyme that converts PPIX into heme. This selective accumulation enhances the susceptibility of cancer cells to ROS-mediated damage. Additionally, chitosan nanoparticles serve as efficient carriers for PPIX, improving its cellular uptake through electrostatic interactions between the positively charged chitosan and negatively charged cancer cell membranes.

Although the experiments have been successful, several aspects still require further investigation due to the innovative nature of the research. For instance, since we have only tested the nanoparticle complexes on two types of cell lines, A549 lung cells, and SK-MEL-2 skin cells, we need to treat more cell types in the future to establish the credibility of the complexes. Also, although we used a 660 nm laser in the experiment, expecting its best performance on cancer treatment as it penetrates the deepest, we can still use other wavelengths of lasers to compare effectiveness. Last, considering that the mechanism of cell death induced by the PPIX-Chitosan complex has not been researched enough, we can plan further experiments, such as RT-PCR, to analyze the gene expression level for investigating the molecular level of cell death signaling.

The study proposes using chitosan nanoparticles to deliver the photosensitizer protoporphyrin (PPIX) to cancerous tissues, which provides multiple impacts on cancer treatment. First, the research explores the application of PDT using 660 nm red light to activate the photosensitizer PPIX. This specific wavelength of red light is used for its ability to reach deeper-located cancers and penetrate tissues more effectively than shorter wavelengths. This approach can potentially improve the selectivity of PDT, ensuring that it primarily affects cancer cells, minimizing damage to healthy tissues while effectively treating cancerous cells, as shown in the laser testing on A549 lung and SK-MEL-2 skin cancer cells. Also, penetrating the body makes the treatment applicable to various cancer types. This versatility may broadly impact the cancer treatment landscape, providing an effective solution for multiple malignancies. In addition, considering that one of the significant challenges in cancer therapy is the side effects, the study suggests that using nanotechnology to target cancer cells can reduce the side effects of PDT. Using chitosan, which is naturally derived from shells and can be degraded in the body, can lead to a safer treatment process for patients, potentially improving their overall quality of life during and after treatment.

Another achievement of the study is the development of a cost-effective Chitosan-PPIX complex due to the shared nature of chitosan compared to other artificial substances composing nanoparticles. The research of the Chitosan-PPIX complex opens possibilities for massive production, emphasizing its practicality and feasibility. The treatment can be adopted in various healthcare settings if it proves effective on more samples. The cost-effective nature of the Chitosan-PPIX complex can lead to a reduction in the overall cost of cancer treatment, which can alleviate financial burdens on healthcare systems and patients, making advanced cancer therapies more economically feasible.

Therefore, this study enhances the understanding of photodynamic therapy (PDT) using chitosan-based nanoparticles, suggesting a promising avenue for clinical applications, particularly in treating cancers that are difficult to manage with traditional methods due to their location or the patient's overall health. The biocompatibility, environmentally friendly nature, and cost-effectiveness of chitosan make it suitable for broader clinical trials, focusing on optimizing delivery mechanisms to improve patient outcomes. Future research should explore its

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scalability and long-term outcome, aiming to establish this approach as a mainstay in cancer treatment.

Conclusion

Overall, the research emphasizes the development of nanotechnology-based methods in photodynamic therapy, leading to a deeper understanding of how nanoparticles can be effectively utilized in cancer treatment. In addition, as it is naturally derived, the environmentally friendly characteristics of chitosan nanoparticles can also have positive implications for both health and the environment. The potential for mass production may contribute to economic efficiency in healthcare. The research introduces an interdisciplinary approach combining nanotechnology and photodynamic cancer therapy. Still, further research is needed to elucidate the precise molecular pathways activated in different cancer types and how they may be modulated to enhance therapeutic efficacy. Additionally, investigating the potential synergies between chitosan's properties and the photodynamic effects could lead to even more promising cancer therapies. Therefore, this research's success can guide future research, encouraging scientists to explore similar innovative solutions to address challenges in cancer healthcare.

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