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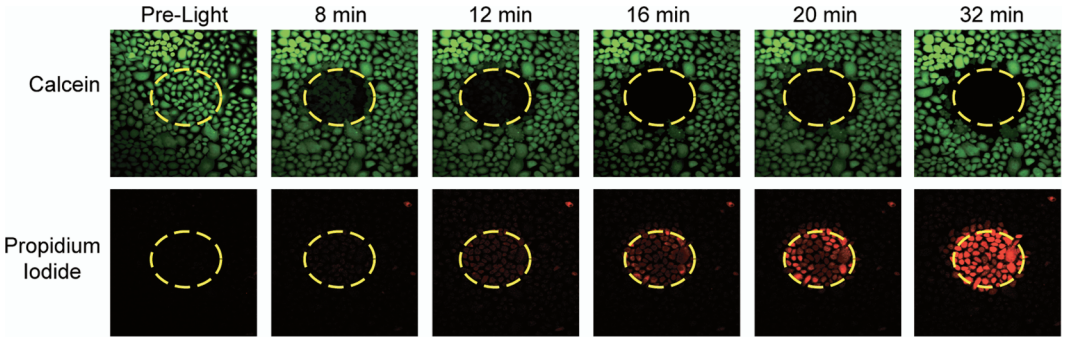

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ORIGINAL RESEARCH–HEAD AND NECK SURGERY

Polymeric micelle nanoparticles for photodynamic treatment of head and neck cancer cells

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

ABSTRACT

OBJECTIVE: To encapsulate 5,10,15,20-tetrakis(*meso*-hydroxyphenyl)porphyrin (mTHPP), a photosensitizer, into polymeric micelles; characterize the micelles; and test in vitro photodynamic therapy efficacy against human head and neck cancer cells.

STUDY DESIGN: A nanoparticle design, fabrication, and in vitro testing study.

SETTING: Polymer chemistry laboratory.

SUBJECTS AND METHODS: Micelles encapsulating mTHPP were produced, and micellar size was measured. Ultraviolet visible spectra and fluorescence spectroscopy were used to characterize the mTHPP-loaded micelles. In vitro cell culture using HSC-3 and HN-5 cancer cells was performed to test the photodynamic therapy efficacy of the micelles using confocal microscopy and method of transcriptional and translational (MTT) assay.

RESULTS: mTHPP was encapsulated with high loading efficiency (> 85%) and density (up to 17%) into micelles. Micelle size was 30.6 ± 3.3 nm by transmission electron microscopy and 30.8 ± 0.6 nm by dynamic light scattering. The absorption maximum for each sample was 418 nm, and fluorescent spectroscopy revealed quenching with maximal fluorescence at five percent loading. Significant cytotoxicity was observed with confocal microscopy when HSC-3 cells were treated with 10 percent mTHPP micelles, with 100 percent cytotoxicity within the zone of laser light exposure at 420 nm. Phototoxicity and dark toxicity against HSC-3 and HN-5 cells measured using the MTT assay with five and 10 percent loaded mTHPP micelles demonstrated greater than 90 percent cytotoxicity with photodynamic therapy and less than 10 percent dark toxicity at a micelle concentration of 25 μg/mL for both cell lines.

CONCLUSION: Micelles were able to encapsulate and solubilize mTHPP at high loading densities with uniform size distribution. These micelles exhibit fluorescence and photodynamic therapy mediated cytotoxicity against head and neck cancer cells in vitro.

Photodynamic therapy (PDT) is an effective treatment modality clinically used for the treatment of several different types of cancer including cancers of the head and neck.[1] Although a promising emerging modality, it is currently not part of standard therapy, especially for larger tumors of the head and neck. In PDT, a photosensitizer (PS) is administered and, when exposed to light, is excited to a triplet state, which can then subsequently lead to the generation of singlet oxygen ($^1O_2$) or free radicals. These reactive oxygen species can lead to significant cellular damage, destruction of tumor blood vessels, and stimulation of antineoplastic immunity.[2] The advantages of PDT include the ability to target cancer cells to minimize toxicity to normal tissues. Not only can the PS be encapsulated in nanocarriers to target tumor cells, but the activating energy source can also be directed at tumor tissue, confining the $^1O_2$ generation to the target. Compared with surgical resection, normal anatomic structures can be preserved, and, unlike radiotherapy, there is no cumulative toxicity to surrounding normal tissue.[1] In addition, the use of PDT does not compromise further therapy such as chemotherapy, radiation, or surgery.[3] Advances such as interstitial PDT allowing delivery of light to larger and deeper tumors, and the discovery of new PS agents, make PDT attractive as an adjuvant therapy to surgery, or as a stand-alone treatment for head and neck cancers.[4,5]

One potential challenge of PDT therapy is that many PS agents are lipophilic, making parenteral administration problematic. In addition, systemic administration of a PS leads to generalized photosensitivity and the temporary need to avoid light exposure. Various strategies to overcome these limitations have been investigated, including conjugation of PS agent to water soluble polymers and colloidal administration, as well as encapsulation in nanoparticle carriers such as micelles.[6,7] Recent progress has been made in the design of polymeric micelles for nanoscale therapeutic and diagnostic applications.[8-12] Polymeric micelles are composed of amphiphilic block copolymers that contain distinguished hydrophobic and hydrophilic segments. The

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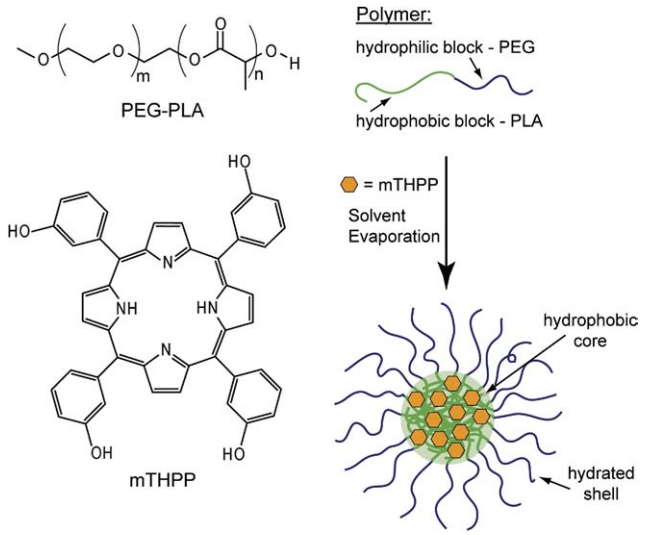

Figure 1 Chemical structures of PEG-PLA copolymer and mTHPP, along with a schematic representation of the preparation of mTHPP-loaded polymeric micelles, including their individual constituents.

distinct chemical nature of the two blocks results in thermodynamic phase separation in aqueous solution and formation of nanoscopic, supramolecular core/shell structures (Fig 1). This unique architecture enables the micelle core to serve as a nanoscopic depot for hydrophobic PS agents and the hydrophilic shell as a stabilizing corona. Sterically stabilized micelles have shown prolonged blood circulation and passive targeting to solid tumors through porous tumor vasculature leading to phase II clinical trials of several micellar systems in cancer patients.[13-15] In this study, we describe micelle encapsulation of a representative hydrophobic PS agent: 5,10,15,20-tetrakis(*meso*-hydroxyphenyl) porphyrin (mTHPP) (Fig 1).[16] High drug loading efficiency was observed that results in effective solubilization. The resulting mTHPP-loaded micelles also demonstrated PDT-mediated cytotoxicity against head and neck HSC-3 and HN-5 cells in vitro.

Materials and Methods

Preparation of mTHPP Micelles

This study was exempt from Institutional Review Board approval according to guidelines set forth by the UT Southwestern Medical Center Institutional Review Board. Poly-(ethylene glycol)-*co*-poly(D,L-lactic acid) (PEG-PLA; molecular weight [MW] = 10 kilodaltons [kD]) block copolymer was synthesized utilizing a ring-opening polymerization procedure as previously published.[14] The solvent evaporation method was used to encapsulate mTHPP in PEG-PLA micelles. Briefly, mTHPP (Frontier Scientific, Logan, UT) and PEG-PLA were first dissolved in tetrahydrofuran (THF) and added drop-wise to water under sonication by a Fisher Scientific Sonic Dismembrator 60 (Hampton, NH). Next, the THF solvent was allowed to evaporate overnight.[17]

Micelle size was determined using a Viscotek Dynamic Light Scattering (DLS) instrument (Houston, TX). Micelles were then further characterized by transmission electron microscopy (TEM) (JEOL, Peabody, MA). Ultraviolet (UV)-visible spectra (Vis) and fluorescence spectra of the micelles were obtained using a Perkin Elmer Lambda 20 UV-Vis Spectrophotometer (Fremont, CA). The excitation wavelength (λ_{ex}) for the fluorescence studies was 420 nm.

Measurement of mTHPP Loading Properties in PEG-PLA Micelles

Free mTHPP was removed from the micelle solution by three cycles of centrifugal filtration at 4°C (Amicon Ultra [Millipore Corporation, Billerica, MA]; MW cutoff = 10 kD). Total concentration of free mTHPP in the combined filtrate was then determined by obtaining its UV-Vis absorbance (λ_{max} = 418 nm, ε = 549.2 mL/cm · mg). Micelle solutions were then freeze-dried, weighed, dissolved in THF, and analyzed via UV-Vis spectrophotometry to determine the total amount of encapsulated mTHPP. Micelle yield, mTHPP loading efficiency and loading density were determined utilizing the following equations:

$$\%\ \text{micelle yield} = \frac{\text{total micelle weight} - \text{free mTHPP weight}}{\text{theoretical micelle weight}} \times 100$$

$$\%\ \text{net mTHPP loading efficiency} = \frac{\text{mTHPP weight}}{\text{theoretical micelle weight}} \times 100$$

$$\%\ \text{mTHPP loading density} = \frac{\text{mTHPP weight}}{\text{theoretical micelle weight} - \text{free mTHPP weight}} \times 100$$

Cell Culture

The HSC-3 cell line derived from a patient with squamous cell carcinoma of the tongue was obtained from the Japan Health Sciences Foundation, Health Science Research Resources Bank (JCRB0623) in August of 2008, and the HN-5 cell line was acquired from frozen master stock in the laboratory of Michael Story at UT Southwestern with their identification being confirmed by short tandem repeats profiling within the last year. Cells were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10 percent heat inactivated fetal bovine serum, 50 U/mL penicillin, and 50 μg/mL streptomycin and incubated at 37°C in a five percent CO_2 environment.

In Vitro Cytotoxicity Study of mTHPP Micelles

To analyze the PDT efficacy of the mTHPP micelles, a modified version of a previously published protocol was used.[18] Briefly, HSC-3 cells were seeded on dishes with glass cover slips and allowed to grow for 24 hours before

the study. Cells were treated for two hours in complete media with micelles at a final mTHPP concentration of 0.18 mg/mL. After treatment, the cells were washed with Dulbecco's phosphate-buffered saline (PBS) three times. The cells were then incubated in a staining solution for 30 minutes that contained Calcein acetoxymethylester (AM) (0.1 μmol/L), propidium iodide (PI; 10 μmol/L), and 10 mmol/L HEPES dissolved in Hank's balanced salt solution. Live cell confocal laser scanning microscopy was used to visualize the laser-induced PDT effect. After incubation in the proper solutions, cells were placed in a live cell imaging chamber affixed to a Nikon C1 confocal microscope (Nikon Instruments Inc., Melville, NY). The chamber was kept at 37°C and five percent CO_2 atmosphere for the duration of the study. Pre-exposure images were taken using a 20× objective in the Calcein and PI channels. Calcein was excited at 488 nm after conversion from Calcien-AM, and PI was excited at 515 nm with the detection of their fluorescence in the green and red channels, respectively. The cells were exposed for two minutes at the PDT absorption wavelength of 420 nm using the 60× objective. To monitor cell death, images at 20× were taken at eight, 12, 16, 20, and 32 minutes postexposure while including the area excited by the 420 nm light.

Phototoxicity and dark toxicity were measured using a method of transcriptional and translational (MTT) assay. Cells were seeded onto 96-well plates at a density of approximately 1.2×10^4 cells per well and allowed to attach overnight. The medium was replaced with fresh medium containing mTHPP-loaded micelles and incubated for 4 hours. The cells were then washed with PBS, and the fresh DMEM medium was added. Cells were illuminated with 532-nm continuous wavelength laser via an optical fiber, the total light dose of 12 J/cm^2 as at 20 mW/cm^2. The response of the cells to mTHPP micelle PDT and dark toxicity of the cells to mTHPP micelles is evaluated by MTT assay. The interval between irradiation and performance of the MTT assay was 24 hours.

Results

mTHPP Micelle Characterization

mTHPP loaded PEG-PLA micelles were characterized by TEM and DLS for their size and distribution. Data showed that the micelles were mostly monodisperse with a narrow size distribution. Figure 1A shows a representative TEM image of mTHPP micelles with 20 percent theoretical loading density after staining with two percent phosphotunstic acid (PTA) solution, confirming the core-shell morphology of the micelles. An image acquired at a higher magnification is shown in the inset. The average diameter of the micelles obtained from a TEM analysis was 30.6 ± 3.3 nm (n = 50), which was confirmed by a DLS analysis (30.8 ± 0.6 nm) (Fig 2B).

Loading content of mTHPP micelles with different theoretical loading densities were analyzed, and the results are

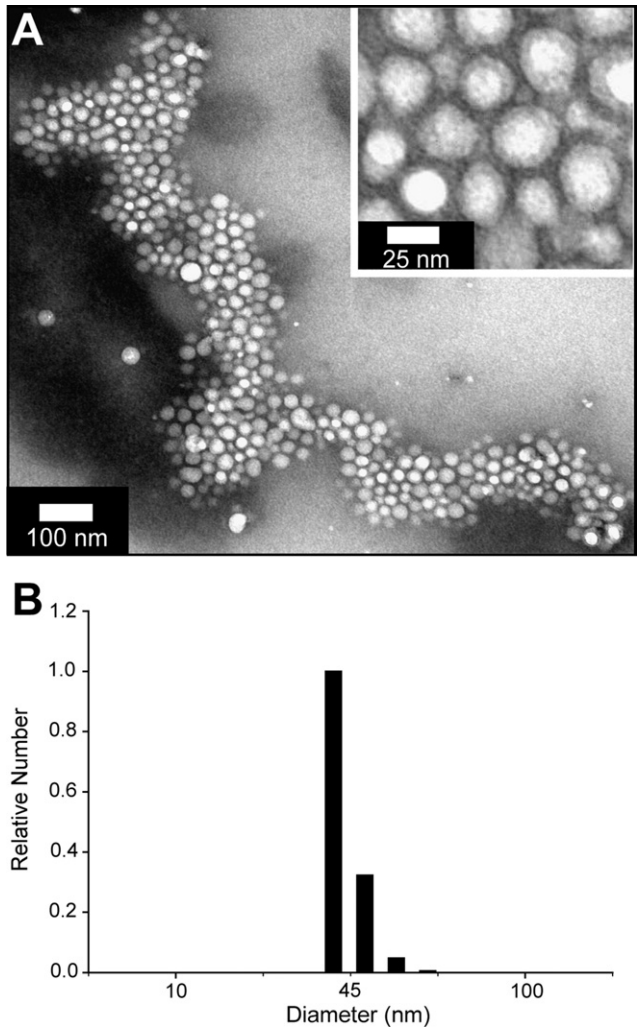

Figure 2 (**A**) TEM image of mTHPP micelles with two percent phosphotunstic acid (PTA) counterstain. (**B**) Histogram depicting mTHPP micelle size distribution determined by DLS analysis.

shown in Table 1. Drug loading density, loading efficiency, and mTHPP and micelle yields of the micelles with five, 10, and 20 percent theoretical loading densities were calculated. Results show that mTHPP was effectively loaded into PEG-PLA micelles, with high loading efficiency in all formulations. mTHPP loading densities for five, 10, and 20 percent theoretical loading were 4.9 ± 0.6, 10.0 ± 0.1, 17.5 ± 0.3 percent, respectively. Net loading efficiencies of five and 10 percent theoretical loading were quantitative, whereas that of 20 percent theoretical loading was 87.6 ± 1.5 percent. The mTHPP and micelle yields were also sufficiently high to confirm micelle encapsulation of mTHPP as an effective strategy for solubilization of the PS. mTHPP micelles demonstrate the maximum UV-Vis absorbance (λ_{max}) at 418 nm with absorbance correlating directly with loading (Fig 3A). The maximum fluorescence emission was observed at 645 nm (λ_{ex} 420 nm) of micelle solutions (Fig 3B). A decrease in fluorescence intensity was observed with increasing mTHPP loading density in the polymeric micelles.

Table 1
mTHPP micelle fabrication procedure with resulting yield and drug loading parameters*

Micelle fabrication method	Theoretical loading (%)	Micellar yield (%)	mTHPP yield (%)	Net loading efficiency (%)	Loading density (%)
Solvent evaporation	5	93.2 ± 1.1	93.1 ± 0.01	99.8 ± 1.1	4.9 ± 0.6
	10	94.5 ± 1.2	94.8 ± 0.01	100.3 ± 1.3	10.0 ± 0.1
	20	94.8 ± 1.6	83.0 ± 0.09	87.6 ± 1.5	17.5 ± 0.3

*The mTHPP micelle fabrication procedure was repeated three times to obtain the average values represented in the table.

Cytotoxicity of mTHPP Micelles In Vitro

The cellular uptake and resultant efficacy of the mTHPP micelles were assessed in HSC-3 cells. Cell viability and cytotoxicity were monitored using Calcein and PI fluorescence after incubation with mTHPP micelles and PDT treatment at 420 nm. Calcein-AM is a cell permeant dye that is nonfluorescent until converted into green-fluorescent Calcein after the hydrolysis of acetoxymethyl ester by intracellular esterases and was used as a cell viability marker pre- and post-PDT. Simultaneously, PI was used to mark cells that were dead or dying by staining cell nuclei only when the plasma membrane was compromised. Live cell confocal laser scanning microscopy was used to obtain sequential images of the treated cells before and after exposure to two minutes of 420 nm light. Figure 4A shows composite images taken before exposing the cells to light and eight, 12, 16, 20, and 32 minutes after light exposure. The prelight exposure image confirms that all cells were viable, exhibiting green fluorescence before the exposure to light. After light exposure, a decrease in Calcein fluorescence was observed at the earliest time point of eight minutes. This loss of Calcein fluorescence was limited to cells within the zone of PDT wavelength light exposure (Fig 4A, dashed circle). PI staining of dead cells was also apparent at the earliest time point and gradually increased to involve all of the cells within the zone of PDT wavelength light exposure.

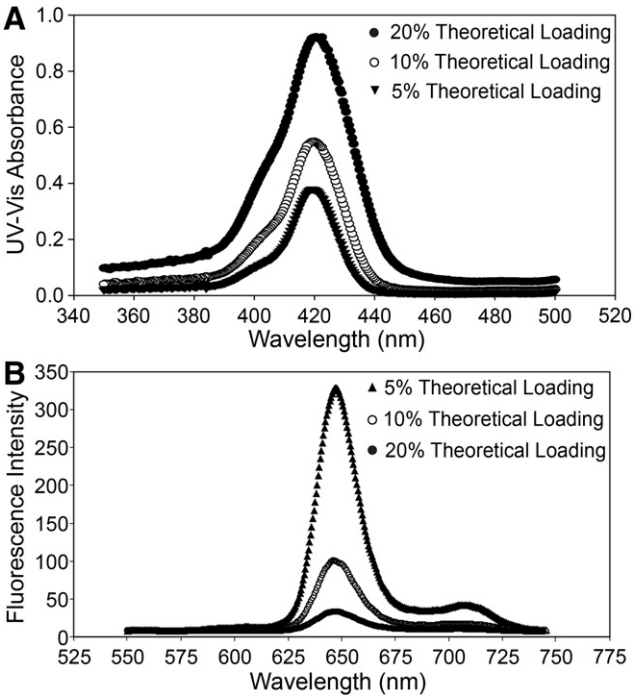

Figure 3 (**A**) UV-Vis absorption spectra for five, 10, and 20 percent theoretically loaded mTHPP encapsulated micelles on a per micelle basis. (**B**) Fluorescence spectra for five, 10, and 20 percent theoretically loaded mTHPP encapsulated micelles on a per micelle basis.

Control experiments were conducted simultaneously to demonstrate PDT activation of mTHPP micelles with 420 nm light is the mechanism for cytotoxicity. The first three columns in Figure 4B show images of HSC-3 cells under various experimental conditions used as controls: (1) no mTHPP micelle treatment and no exposure to 420 nm light; (2) mTHPP micelle treatment but no exposure to 420 nm light; (3) no mTHPP micelle treatment with exposure to 420 nm light. No loss of Calcein and gain of PI fluorescence were observed in the control experiments indicating that exposure to 420 nm light alone or exposure to mTHPP micelles alone was not sufficient for cell death.

Phototoxicity and dark toxicity were also measured using an MTT assay. Using five and 10 percent loaded mTHPP micelles, cell cytotoxicity for HSC-3 and HN-5 cells was measured for increasing concentrations of mTHPP (Figs 5 and 6). Even for mTHPP concentrations less than 25 μg/mL, significant cytotoxicity was observed for both cell lines with greater than 90 percent of cells being killed, with maximal cell death being observed for both formulations, for both cell lines at 9 μg/mL. Conversely, the dark toxicity of the micelles was less than 20 percent even at concentrations of 200 μg/mL.

Discussion

PDT is a promising emerging modality that can potentially be used for the treatment of a diverse group of malignant and nonmalignant conditions.[19] The efficiency with which a PS leads to the generation of $^1O_2$ and tissue damage is based on a variety of factors including triplet state quantum yield, triplet state lifetime, quantum yield of $^1O_2$, lifetime of $^1O_2$, and stability of PS.[20] The advantages of porphyrin-based PS compounds include their ability to efficiently absorb a wide

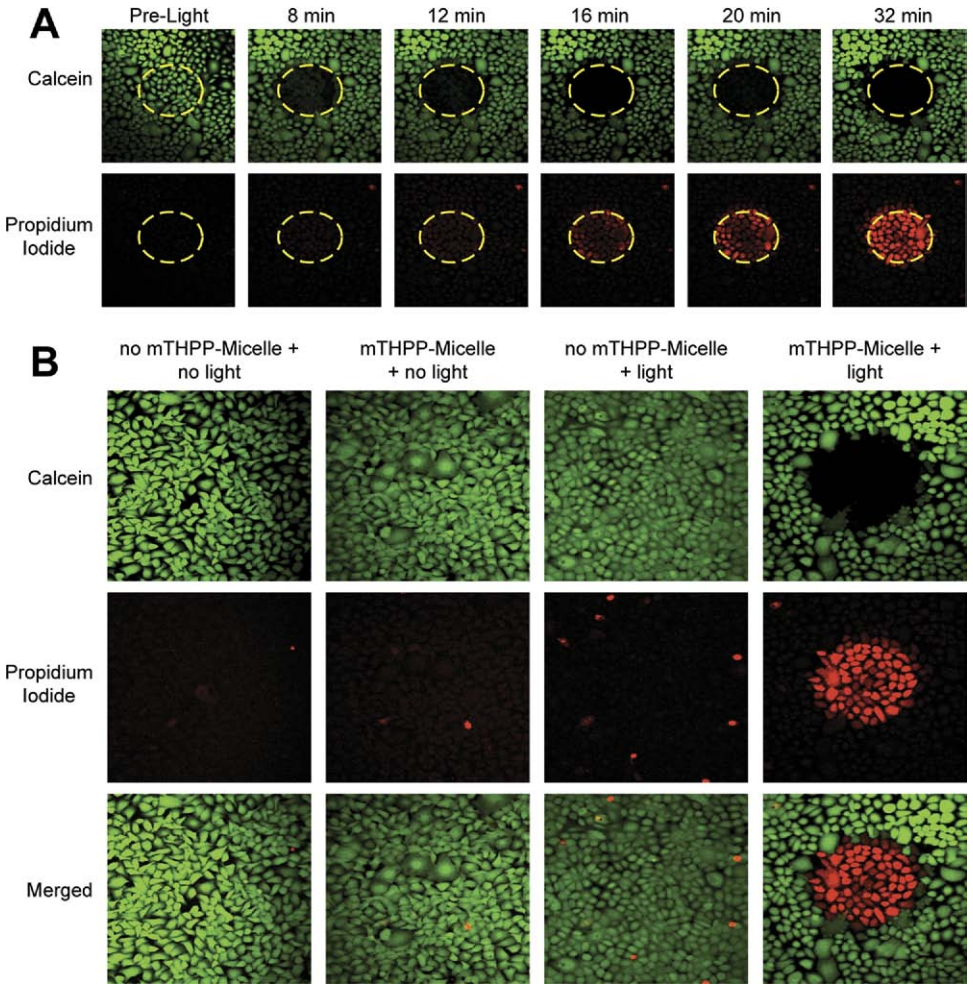

Figure 4 Confocal images of HSC-3 cells after treatment with a 0.18 mg/mL solution of 10 percent theoretically loaded mTHPP micelles. (**A**) Images were captured at a wavelength of 488 nm for Calcein and 568 nm for propidium iodide at the labeled time points using a 20× objective. The approximate area of the cells exposed to 420-nm light is outlined in the *hatched yellow circles*. (**B**) Images captured at 488 nm for Calcein, 568 nm for propidium iodide, and overlay images taken at 32 minutes after light exposure of four separate dishes of cells under different experimental conditions.

range of light spectra, especially red light, which has greater tissue penetration, as well as high quantum yield of $^1O_2$.[19]

The objective of the present study was to develop polymeric micelles that are able to efficiently encapsulate mTHPP, a porphyrin-based PS agent. One study showed that the lifetime of the triplet state for porphyrin PS was significantly increased when encapsulated into micelles versus when free in aqueous solution.[20] Other studies have also shown improved $^1O_2$ generation when porphyrin-based PS is incorporated into micelle carriers.[7] In addition to the possible enhancement of $^1O_2$ generation, other advantages to incorporating porphyrin-based PS agents into micelles include the ability to solubilize these generally hydrophobic agents, the small and uniform size of micelles, and potential for passive targeting of solid tumors via the enhanced permeation and retention effect decreasing systemic photosensitization.[6] Active targeting of tumors using micelles is also possible by chemically modifying the micelle surface with targeting ligands. The solvent evaporation method proved to be very efficient at encapsulating mTHPP into the PEG-PLA micelle formulation with minimal drug loss and uniform micelle size. Fluorescence properties of micelle samples with lower theoretical mTHPP loading were investigated, and the results showed greater fluorescence on a per dye molecule basis (1% theoretical loading, data not shown). This is likely due to fluorescent quenching of the mTHPP molecules when local concentration inside the micelle core is extremely high.

Calcein-AM is a nonfluorescent cell-permeant dye that is converted to the green fluorescent Calcein after acetoxymethyl ester hydrolysis by intracellular esterases in live cells. PI was concurrently used to confirm cell death because this dye is able to penetrate the cell membrane of dead cells but is excluded by live cells. Confocal microscopy was used to test the photodynamic cytotoxicity of mTHPP-loaded micelles in vitro in human squamous HSC-3 cells. Figure 4A shows that the green fluorescence in HSC-3 cells from Calcein was lost and replaced by the

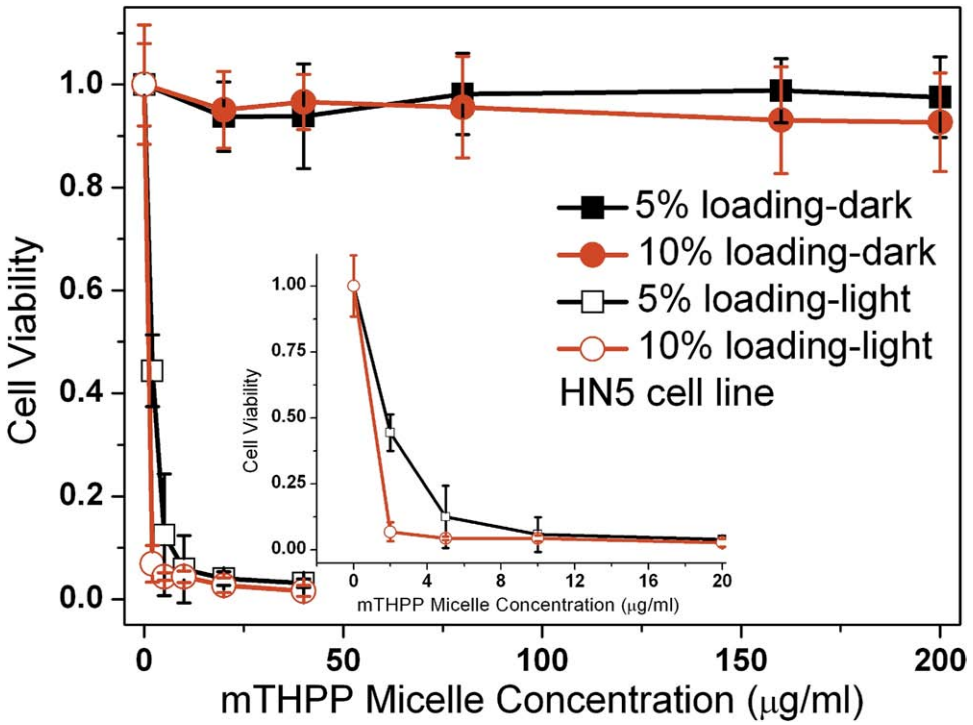

Figure 5 The response of HSC-3 cells to mTHPP micelle mediated photo and dark toxicity is evaluated by MTT assay.

red fluorescence of PI in a time-dependent manner in the area treated with light at 420 nm after the cells had been incubated with mTHPP micelles. All of the cells within the treated area are nonviable 32 minutes after light exposure. It is also evident that cell damage is only induced in the area where cells are exposed to light. The area outside of the dashed yellow circles (Fig 4A) remains green indicating that mTHPP micelles alone are not toxic. The images in Figure 4B further demonstrate that the combination of mTHPP-loaded micelles and light

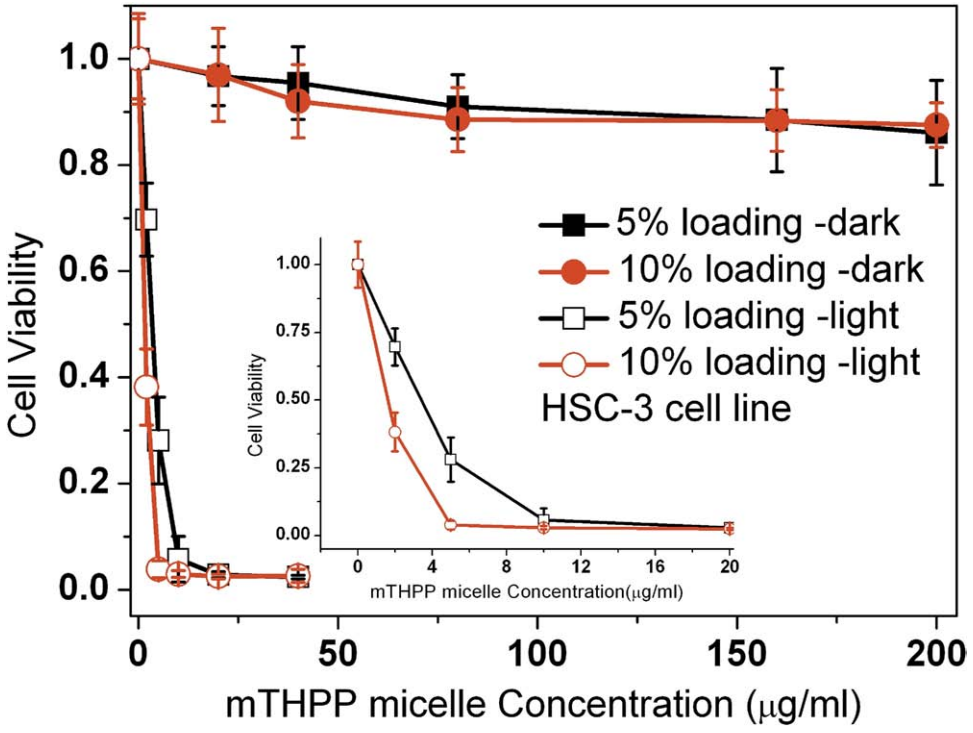

Figure 6 The response of HN5 cells to mTHPP micelle mediated photo and dark toxicity by MTT assay.

is required for cell death in vitro and that neither alone is toxic to the HSC-3 cells.

Further testing of the cytotoxicity of the mTHPP micelles was carried out using an MTT assay for cell viability as shown in the dose response curves in Figures 5 and 6. The mTHPP micelles exhibited significant cytotoxicity in the presence of light against both HSC-3 and HN-5 cells even at concentrations as low as 2 μg/mL, with almost no toxicity observed for the dark experiments, confirming the photosensitizing effect of the mTHPP.

In summary, this study shows that polymeric micelles are effective carriers to solubilize mTHPP, a hydrophobic PS, with high loading efficiency and loading density. The resulting micelle nanoparticles are spherical in shape and have a uniform size distribution. In vitro studies demonstrate that mTHPP-loaded micelles produce PDT-mediated cytotoxicity against head and neck cancer cells.

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Disclosures

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References

1. Biel MA. Photodynamic therapy in head and neck cancer. Curr Oncol Rep 2002;4:87–96.
2. Juarranz A, Jaen P, Sanz-Rodriguez F, et al. Photodynamic therapy of cancer. Basic principles and applications. Clin Transl Oncol 2008;10:148–54.
3. Hornung R, Walt H, Crompton NE, et al. m-THPC-mediated photodynamic therapy (PDT) does not induce resistance to chemotherapy, radiotherapy or PDT on human breast cancer cells in vitro. Photochem Photobiol 1998;68:569–74.
4. Lou PJ, Jones L, Hopper C. Clinical outcomes of photodynamic therapy for head-and-neck cancer. Technol Cancer Res Treat 2003;2:311–7.
5. Huang Z. A review of progress in clinical photodynamic therapy. Technol Cancer Res Treat 2005;4:283–93.
6. van Nostrum CF. Polymeric micelles to deliver photosensitizers for photodynamic therapy. Adv Drug Deliv Rev 2004;56:9–16.
7. Li B, Moriyama EH, Li F, et al. Diblock copolymer micelles deliver hydrophobic protoporphyrin IX for photodynamic therapy. Photochem Photobiol 2007;83:1505–12.
8. Adams ML, Lavasanifar A, Kwon GS. Amphiphilic block copolymers for drug delivery. J Pharm Sci 2003;92:1343–55.
9. Kwon GS. Polymeric micelles for delivery of poorly water-soluble compounds. Crit Rev Ther Drug Carrier Syst 2003;20:357–403.
10. Lukyanov AN, Torchilin VP. Micelles from lipid derivatives of water-soluble polymers as delivery systems for poorly soluble drugs. Adv Drug Deliv Rev 2004;56:1273–89.
11. Otsuka H, Nagasaki Y, Kataoka K. PEGylated nanoparticles for biological and pharmaceutical applications. Adv Drug Deliv Rev 2003;55:403–19.
12. Torchilin VP. Targeted polymeric micelles for delivery of poorly soluble drugs. Cell Mol Life Sci 2004;61:2549–59.
13. Danson S, Ferry D, Alakhov V, et al. Phase I dose escalation and pharmacokinetic study of pluronic polymer-bound doxorubicin (SP1049C) in patients with advanced cancer. Br J Cancer 2004;90:2085–91.
14. Shuai X, Ai H, Nasongkla N, et al. Micellar carriers based on block copolymers of poly(epsilon-caprolactone) and poly(ethylene glycol) for doxorubicin delivery. J Control Release 2004;98:415–26.
15. Matsumura Y, Hamaguchi T, Ura T, et al. Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin. Br J Cancer 2004;91:1775–81.
16. Bonnett R, White RD, Winfield UJ, et al. Hydroporphyrins of the meso-tetra(hydroxyphenyl)porphyrin series as tumour photosensitizers. Biochem J 1989;261:277–80.
17. Blanco E, Bey EA, Dong Y, et al. Beta-lapachone-containing PEG-PLA polymer micelles as novel nanotherapeutics against NQO1-overexpressing tumor cells. J Control Release 2007;122:365–74.
18. Tang W, Xu H, Park EJ, et al. Encapsulation of methylene blue in polyacrylamide nanoparticle platforms protects its photodynamic effectiveness. Biochem Biophys Res Commun 2008;369:579–83.
19. Dougherty TJ, Gomer CJ, Henderson BW, et al. Photodynamic therapy. J Natl Cancer Inst 1998;90:889–905.
20. Yu JH, Weng YX, Wang XS, et al. The triplet excited state changes of amphiphilic porphyrins with different side-chain length in AOT reverse micelles. J Photochem Photobiol A 2003;156:139–44.