Significant Inhibition of Tumor Growth following Single Dose Nanoparticle-Enhanced Photodynamic Therapy

Shih-Hsun Cheng,1,2 Chia-Hui Chu,1 Nai-Tzu Chen,1,2 Jeffrey S. Souris,2 Chin-Tu Chen,2 and Leu-Wei Lo1

1 Institute of Biomedical Engineering and Nanomedicine, National Health Research Institutes, Zhunan, Miaoli County 35053, Taiwan
2 Department of Radiology, The University of Chicago, Chicago, IL 60637, USA

Correspondence should be addressed to Leu-Wei Lo; lwlo@nhri.org.tw

Received 25 February 2014; Accepted 1 June 2014; Published 7 August 2014

Academic Editor: Quan-Guang Zhang

Copyright © 2014 Shih-Hsun Cheng et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Photodynamic therapy (PDT) for cancer treatment involves the pathology’s uptake of photosensitizers, which produce cytotoxic reactive oxygen species by photoirradiation. The use of nanoparticles as carriers of photosensitizers is one promising approach to this endeavor, owing to their small size, unique physicochemical properties, and easy/diverse functionalization. In the current work, we report on the in vivo assessment of PDT efficacy of these nanoconstructs in a murine model of human breast cancer, following a single (one-shot) nanoparticle dose and photoirradiation. Palladium-porphyrin (PdTPP) was administered intratumorally via injection of aqueous suspensions of either free PdTPP or MSN-conjugated PdTPP (MSN-PdTPP) at a dose of 50 μg. Mice were then exposed to a single photoirradiation session with total energy of 80 J. One month after one-shot PDT treatment, significantly greater reductions in tumor growth were observed in MSN-Pd treated animals than in PdTPP cohorts. Electron microscopy of tumor specimens harvested at various timepoints revealed excellent MSN-PdTPP uptake by cancer cells while immunohistologic analysis demonstrated marked increases in apoptotic response of MSN-PdTPP treated animals relative to PdTPP controls. Taken together, these findings suggest that considerable improvements in PDT efficacy can readily be achieved via the use of nanoparticle-based photosensitizers.

1. Introduction

Photodynamic therapy (PDT) has evolved considerably in recent years to treat a number of medical conditions that include wet macular degeneration, dermatologic disorders (e.g., acne, rosacea, and psoriasis), and several types of cancer including lung and esophageal cancer [1–6]. It is intrinsically a focal therapy far less invasive than surgical approaches and with fewer adverse effects than systemic chemotherapy. The most prevalent forms of PDT in use today are based on the photogeneration of cytotoxic, reactive oxygen species within tumor tissue via the optical stimulation of exogenous photosensitizing agents (PSs). The photosensitizer, in its ground (singlet) state, absorbs a photon that promotes the PS to a short-lived excited singlet state. Because the excited singlet state is so short-lived, the PS has little opportunity to transfer either energy or electrons to other molecules nearby and instead undergoes intersystem crossing to leave it in an excited, much longer-lived, triplet state. As the PS subsequently decays back to its initial ground (singlet) state, it transfers energy to nearby ground (triplet) state molecular oxygen, raising the latter to its first excited singlet state [7, 8]. Most photosensitizers in clinical use have been optimized/selected for their high, excited singlet state quantum efficiency and recursively generate substantial amounts of singlet oxygen from molecular oxygen rather than undergoing thermal decay (via internal conversion) or fluorescent emission [9, 10].

The most widely employed PSs at present are porphyrins. Photoirradiation of porphyrins at selective wavelengths of visible light (450–700 nm) leads to the photochemical conversion of molecular oxygen (O2) into singlet oxygen (1O2),...
an especially efficacious cytotoxic agent that leads to apoptosis or necrosis [11]. In the conventional PDT of cancer, a photosensitizer drug is administered to the patient, which then passively accumulates in the tumor tissue, though a few PSs have intrinsic proclivities for targeting the endothelial cells of vasculature. The involved tissue is then selectively illuminated with light which activates the photosensitizer inducing cell death. Photosensitizers have evolved over the years to maximize their potency and simultaneously minimize their systemic toxicity. Unfortunately, to date, the overwhelming majority of photosensitizers still possess multiple limitations that include high hydrophobicity, significant self-aggregation, and poor tumor selectivity, each limiting PDT efficiency and clinical benefit [12, 13]. As such, considerable effort has gone into developing compounds with improved “deliverability.”

One approach to enhancing PS delivery is that of nanoparticle- (NP-) mediated transport. When compared to conventional unshielded delivery, NP-assisted conveyance of PSs has the added advantage of providing an exoplatform for the conjugation of polymers to prolong PS circulation half-lives and thereby increase PS accumulation in the leaky vasculature of tumors via the enhanced permeability and retention effect (EPR) [14–19]. Encapsulation of PSs within NPs also circumvents PS self-aggregation and enables high density PS delivery. NP conveyance of PSs also permits the incorporation of efficient, light-absorbing chromophores in close proximity to PSs, so as to greatly promote the energy transfer to PSs and thereby significantly enhance overall photodynamic efficiency [20–26]. One form of nanoparticles in particular has recently gained considerable interest for use both in cancer chemotherapy and in PDT: mesoporous silica nanoparticles (MSNs) [27–32].

MSNs are biocompatible, readily endocytosed, and easily modified after synthesis for the conjugation of targeting moieties on their outer surfaces [33–39]. The unique topology of MSNs also provides them with three distinct domains that can be independently functionalized: the silica framework, the hexagonal nanochannels/pores, and the nanoparticle’s outermost surface. As such, MSNs are especially well suited to the task of incorporating the essential capabilities of a combined diagnostic/therapeutic (i.e., theranostic) platform in a single particle, with (1) separate domains for contrast agents that enable traceable imaging of PS targeting, (2) PS payloads for therapeutic intervention, and (3) biomolecular ligands for highly targeted PS delivery.

Previously, we reported the development and in vitro characterization of MSNs that were sequentially functionalized with the fluorescent contrast agent ATTO647N embedded within the MSN’s silica framework for tracking, the cRGDyK peptide conjugated onto the MSN’s exterior for targeting the αVβ3 integrin expression, and the photosensitizer Pd-porphyrin (PdTPP) covalently linked within the MSN’s protective nanochannels for photodynamic therapy [40–42]. Herein we report the results of our in vivo PDT studies of MSNs-PdTPP using a murine model of human breast cancer and one-shot photoexcitation (Scheme 1).

2. Methods

2.1. Materials. Tetraethoxysilane (TEOS), cetyltrimethylammonium bromide (CTAB), ethanol, ammonium hydroxide (30%), N,N-dimethylformamide (DMF), and 3-Aminopropyltrimethoxysilane (APTS) were purchased directly from Acros. Di(N-succinimidyl) carbonate (DSC) and N,N-diisopropylethylamine were obtained from Sigma Chemical Co.

2.2. Preparation of MSNs. The sol-gel co-condensation of TEOS to synthesize MSNs was as follows. First, CTAB (0.58 g) was dissolved in NH₄OH (0.51 M, 300 mL) at 40°C. After stirring for 1 h, diluted TEOS (0.2 M in 5 mL ethanol) was added and the solution was stirred for an additional 4 h. Then, high concentration of TEOS (1.0 M in 5.0 mL of ethanol) was added under vigorous stirring for an additional hour. The solution was aged in darkness at 40°C for 20 h. Samples were subsequently collected by centrifuging at 12,000 rpm for

![Scheme 1: The illustration of in vivo PDT studies using MSN-PdTPP with single photoirradiation on a murine model bearing human breast cancer cells.](image-url)
20 min, washing, and redispersing the solids with deionized water and ethanol repeatedly. Surfactant templates were removed by extraction in 0.3 g of NH\textsubscript{4}NO\textsubscript{3} and 50 mL of ethanol solution under reflux at 65°C for 12 h.

### 2.3. Preparation of MSN-PdTPP

As the surfactant templates were being removed to yield MSNs, 50 mg PdTPP and 150 mg di(N-succinimidyl) carbonate (DSC) were mixed with 100 µL N,N-diisopropylethylamine (99.5%) in N\textsubscript{2}-dimethylformamide (DMF) (20 mL) solution for 2 h. The activated PdTPP was then reacted with 200 µL APTS for 1 h and purified by a PD-10 column (Amersham Biosciences). The amount of PdTPP loading in MSNs was determined by measuring the absorbance at 400 nm, the Soret band of PdTPP. The amounts of PdTPP in MSNs were thereby determined to be 5.5% w.t.

### 2.4. Characterization

The morphology of MSN samples was characterized via TEM (Hitachi, H-7650), operating at an acceleration voltage of 80 kV. Surface areas and pore sizes were determined by N\textsubscript{2} adsorption-desorption isotherm measurements at 77 K on a Micrometric ASAP 2010. Samples were outgassed at 10\textsuperscript{-7} Torr and 120°C for approximately 6 h prior to conducting adsorption experiments. Pore size distribution curves were obtained from analysis of the desorption portion of the isotherms using the BJH (Barrett-Joyner-Halenda) method. Steady-state absorption spectra were acquired with a DU800 UV spectrometer (Beckman). A Malvern Zetasizer Nano was used to measure the zeta potential of MSN samples in solution at pH 7.4.

### 2.5. Singlet Oxygen Measurement

We used a DPBF (8 mM) acetonitrile solution to measure single oxygen generation. 2 mL DPBF solution was added to the PdTPP and MSN-PdTPP samples and mixed thoroughly. The mixture was then irradiated at 532 nm via a laser operating at 250 ± 5 mW cm\textsuperscript{-2}. The absorption spectra of DPBF were detected at 400 nm every 20 seconds.

### 2.6. Cell Culture

MDA-MB-231 human breast carcinoma cells were maintained in RPMI-1640 medium (Gibco-BRL, NY, USA) with 10% heat-inactivated fetal bovine serum (FBS) (Hyclone, Utah, USA) and supplemented with 100 units/mL penicillin and 100 µg/mL streptomycin at 37°C with 5% CO\textsubscript{2}.

### 2.7. In Vitro PDT

A total of 5000 MDA-MB-231 breast cancer cells were seeded on a 96-well and incubated for 24 h. Cells were then treated with 10, 25, and 50 µg/mL of either PdTPP or MSN-PdTPP in a serum-free medium for 1 h at 37°C in darkness, respectively. Cell viability was estimated by WST-1 assay. In the WST-1 assay, tetrazolium salt WST-1 [4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolilo]-1, 3-benzene disulfonate] in live cells was cleaved by mitochondrial dehydrogenase to yield formazan. After treatment for 1 h, the supernatant was removed and added to 100 µL/well serum-free medium and 10 µL/well Cell Proliferation Reagent WST-1 (Roche). After incubation with WST-1 for 4 h, plates were shaken for 1 min and measured for optical absorbance at 450 nm using an ELISA reader ( Infinite M200, TECAN). Six specimens were run for each concentration, with each experiment being repeated three times. For the PDT, cells were irradiated using a 532 nm laser operating at 250 ± 5 mW cm\textsuperscript{-2} at energies of 1.2, 2.5, or 5 J after treatment of either PdTPP or MSN-PdTPP in a serum-free medium for 1 h at 37°C.

### 2.8. Tumor Xenograft Animal Model

MDA-MB-231 human breast carcinoma cells (1×10\textsuperscript{6} cells/200 µL sterile saline) were subcutaneously injected into the dorsal region of both thighs of male nude mice (nu/nu; 20–25 g; 6–8 weeks of age; BioLasco Taiwan Co., Ltd). Tumor growth curves were obtained using daily digital caliper measurement of tumor diameter once the bulge caused by the tumor cells at the injection site became visible (tumor size ~ 200 ± 50 mm\textsuperscript{3}). Tumor volume (mm\textsuperscript{3}) was calculated using the formula: 0.523 × (length × width × thickness) and assessed twice per week for one month. All experiments involving animals were performed in accordance with the guidelines of institutional animal care and use committee.

### 2.9. In Vivo Two-Photon Excited PDT

In vivo PDT experiments were performed when the tumor volume reached approximately 200 ± 50 mm\textsuperscript{3}. Mice were divided into groups of twelve animals to minimize variations in group weight and tumor size. Tumor-bearing nude mice were anaesthetized and treated by intratumoral injection of PdTPP or MSN-PdTPP at a concentration of 50 µg. One hour after injection, mice were subjected to 80 J of 532 nm laser irradiation such that the average power of light delivered to the skin was 235 mW. Control group mice received intratumoral injections of saline with or without irradiation, respectively. Mice were monitored for a maximum of 28 days. The length, width, and thickness of tumors were measured by digital calipers and assessed twice weekly for one month. All mice were euthanized at the end of the 28th day and tumors were removed for further study.

### 2.10. Tissue Preparation for Histopathology and Immunohistochemistry

At the end of experiments, a thorough necropsy of mice was carried out immediately after euthanasia. Samples of the tumor mass were embedded in OCT (Tissue-Tek) and stored in −80°C freezer. Samples were sectioned at 10 µm thickness and then stained with hematoxylin and eosin (H&E), caspase-3 (abcam), and TUNEL stain (Roche, in situ cell death detection kit) for examination by light microscopy.

For H&E staining, sections from cryopreserved tissues were fixed in 4% paraformaldehyde and stained with hematoxylin and eosin. Following staining, sections were dehydrated, mounted, and observed.

For caspase-3 staining, sections from cryopreserved tissues were incubated with 3% (v/v) hydrogen peroxide in methanol for 15 min and in 3% BSA for 1 h to block endogenous peroxidases and nonspecific binding. Sections were then incubated with polyclonal antibodies to active caspase-3 (1:500) in diluting buffer (1% bovine serum albumin,
0.1% Tween-20 in PBS) overnight at 4°C. Sections were next incubated with goat anti-rabbit HRP secondary antibodies (Jackson, 1:10000) in PBS for 1 h. Detection of caspase-3 positive cells was undertaken via Diaminobenzidine (DAB) immunohistochemistry, followed by counterstaining with Gills Haematoxylin.

For TUNEL staining, sections were fixed in 4% paraformaldehyde and blocked in 3% hydrogen peroxide in methanol for 15 min. Next specimens were incubated in permeabilization solution (0.1% triton X-100 and 0.1% sodium citrate in PBS) for 2 min on ice. TUNEL reaction mixture was added and incubated for 1 h at 37°C in the dark. After PBS rinsing, samples were incubated with converter-POD for 30 min at 37°C. Detection of apoptosed cells was achieved using DAB immunohistochemistry, followed by counterstaining with Gills Haematoxylin.

2.11. Transmission Electron Microscopy Imaging of Tumor Tissue. For electron microscopy, tumor tissue specimens were fixed overnight in glutaraldehyde, buffered (2.5%) with PBS. Tissues were then washed with 3 changes of PBS and postfixed for 1 h in a solution containing OsO₄ buffered (2%) with PBS. Samples were next washed in 3 changes of dH₂O and dehydrated stepwise in ethanol. Tissues were polymerized using Spurr resin at 68°C for 15 hours. The embedded specimens were then thin-sectioned at 70 nm and viewed on a Hitachi H-7650 transmission electron microscope operating at 80 kV.

3. Results and Discussion

The intrinsic flexibility of MSN surface chemistry readily affords postsynthesis, application-specific modification, for
example, tailoring for optimal drug release, dispersion stability, cellular uptake, and attachment of imaging agents and targeting ligands. For the current application, the porphyrin-based photosensitizer PdTPP was attached via the addition of amino groups to the MSN's outer surfaces and nanochannel walls. Amino modification of MSN surfaces also served to increase the overall surface charge of the nanoparticle (zeta potential of MSN-PdTTP: +28.5 mV), thereby enhancing cell uptake and minimizing self-aggregation (and thus self-quenching). Di(N-succinimidyl) carbonate (DSC) coupling was used to encapsulate PdTTP to the MSNs. MSN-PdTTP morphology was characterized by combined transmission electron microscopy (TEM), N$_2$ adsorption-desorption isotherm measurement, and UV-Vis spectroscopy. TEM studies demonstrated well-ordered, hexagonal porous structures characteristic of mesoporous silica, with average particle diameters of 80 nm (Figures 1(a) and 1(b)). Surface area and pore size of MSNs and MSN-PdTPPs were determined by N$_2$ adsorption-desorption isotherm measurements (Figures 1(c) and 1(e)). Following PdTTP incorporation, nanoparticle surface areas decreased from 985 m$^2$ g$^{-1}$ (MSN) to 632.06 m$^2$ g$^{-1}$ (MSN-PdTTP). Measured UV absorption spectra of MSN-PdTTP and PdTTP showed MSN conjugation greatly increasing PdTTP solubility in water via possessing only slight differences in methanol solutions but significant differences in DI water (Figure 1(d))—a consequence of the solubility of PdTTP in water being poor, and its aggregation broadening the Soret band spectra. Quantization of the PdTTP content in water for comparison (10.4 μg mL$^{-1}$) with these promising findings in hand, we then sought to characterize the in vivo utility of our nanosystems for PDT by using male nu/nu nude mice bearing MDA-MB-231 xenografts. MDA-MB-231 and MDA-MB-231-GFP tumor cells (1 × 10^6 cells/200 μL sterile saline) were injected subcutaneously into the dorsal region of the right and left thigh, respectively (Figure 4). The fluorescent MDA-MB-231-GFP tumor cells were inoculated for validation of tumor location and better visualization of growth. After tumors had grown to a volume of approximately 200 ± 50 mm$^3$, we conducted comparative PDT efficacy studies by first dividing animals into four groups (n = 12), so as to minimize intergroup weight and tumor size variation. Next two control groups (with/without photoirradiation) were intratumorally injected with 120 μL sterile saline solution (0.9% NaCl) while the remaining two groups were intratumorally injected with either 50 μg of MSN-PdTTP or the same PdTTP-concentration of free PdTTP. One hour after administration, one control group and both free PdTTP or MSN-PdTTP prior to photoirradiation. With these promising in vitro findings in hand, we then sought to characterize the in vivo utility of our nanosystems for PDT by using male nu/nu nude mice bearing MDA-MB-231 xenografts. MDA-MB-231 and MDA-MB-231-GFP tumor cells (1 × 10^6 cells/200 μL sterile saline) were injected subcutaneously into the dorsal region of the right and left thigh, respectively (Figure 4). The fluorescent MDA-MB-231-GFP tumor cells were inoculated for validation of tumor location and better visualization of growth. After tumors had grown to a volume of approximately 200 ± 50 mm$^3$, we conducted comparative PDT efficacy studies by first dividing animals into four groups (n = 12), so as to minimize intergroup weight and tumor size variation. Next two control groups (with/without photoirradiation) were intratumorally injected with 120 μL sterile saline solution (0.9% NaCl) while the remaining two groups were intratumorally injected with either 50 μg of MSN-PdTTP or the same PdTTP-concentration of free PdTTP. One hour after administration, one control group and both free PdTTP or MSN-PdTTP experimental groups were anesthetized and their tumors irradiated at 532 nm for total energy deposition.

![Figure 2: Decay curve of DPBF absorbance of 1$^2$O$_2$ generated from PdTTP and MSN-PdTTP upon photoirradiation. Green line denotes DPBF with photoirradiation (control).](image-url)
of 80 J. Tumor size and body weight were subsequently monitored on a daily basis, for the next 28 days. As illustrated in Figure 5(a), a single intratumoral administration of MSN-PdTPP and exposure to a single-shot photoirradiation were significantly more efficacious in tumor volume reduction than either single-shot photoirradiated free PdTPP or saline/control (Figure 5(a)). We postulate that the observed marked increase in PDT efficacy afforded by MSN protected delivery of the PS arose primarily from nanoparticle-enhanced endocytosis and consequent retention of the PS-combining to delay PS clearance from the tumor site as well as to avoid environmental degradation.

Twenty-eight days after PDT treatment, mice were sacrificed and the tumors were removed for measurement (Figure 5(b)). No differences in the long-term PDT benefit were found between the 2 saline control groups (with/without photoirradiation) and the free PdTPP experimental groups (Figure 5(a)), with mean tumor volumes, at the study’s end, measuring 10280 ± 1648 mm$^3$ (saline, no irradiation, $n = 12$), 9850 ± 525 mm$^3$ (saline, with irradiation, $n = 12$), and 10160 ± 656 mm$^3$ (free PdTPP, with irradiation, $n = 12$). After intratumoral injection, free PdTPP molecules had the propensity to drain away from the tumor and thus imposed nonobservable PDT effect on tumor following localized
irradiation. The MSN-PdTTP group, however, demonstrated dramatic PDT benefit at 28 days after irradiation, with a mean tumor volume of $780 \pm 567 \text{mm}^3 (n = 12)$ as can also be seen from a representative specimen in Figure 5. By contrast, the MSN-PdTTP group without irradiation demonstrated no significant PDT effect, whose tumor volume averagely increased 6-fold 15 days after injection and 13.5-fold 28 days after injection, respectively $(n = 5)$. It illustrated a similar tumor growth curve to that of the saline control group.

We then performed hematoxylin and eosin (H&E) histological staining of excised tumor and injection site tissue sections, confirming the existence of extravasated blood that is characteristic of PDT (Figure 6(a)). To more precisely assess antitumor response to our nanoparticle PDT treatments, we compared terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and caspase-3 immunohistochemical staining for apoptosis of tumors harvested from mice that had been treated with MSN-PdTTP nanoparticles for 0 h and 1 h

---

**Figure 4:** White light (a) and fluorescence (b) images of nude mouse bearing xenografts comprised of GFP-expressing MDA-MB-231 cells on animal’s left thigh and nonfluorescent MDA-MB-231 cells on animal’s right thigh.

**Figure 5:** (a) 29-day comparative PDT efficacy studies of MDA-MB-231 s.c. xenograft nude mouse model subjected to a single intratumoral injection of (i) saline without photoirradiation (black squares), (ii) saline with photoirradiation (red circles), (iii) PdTTP with photoirradiation (green upward-pointing triangles), and (iv) MSN-PdTTP with photoirradiation (blue downward-pointing triangles)—showing MSN-PdTTP to be highly efficacious in tumor reduction. Datapoints denote mean of twelve mice per group. (b) Photographs of tumors harvested from mice 28 days after treatment with (A) saline (control), (B) PdTTP, and (C) MSN-PdTTP, all subjected to photoirradiation (scale bar: 1 cm).
Figure 6: (a) Histological staining of the excised tumor sections on treatment day 1 of (i) saline with no photoirradiation (control), (ii) MSN-PdTPP with photoirradiation at 0 h after injection, (iii) MSN-PdTPP with photoirradiation at 1 h after injection, and on treatment day 28 of (iv) MSN-PdTPP with photoirradiation at 1 h after injection. H&E staining; scale bars: 200 μm. (b) TUNEL staining of consecutive sections for each group at 200x magnification (upper, scale bars: 200 μm) and 1000x magnification (upper, scale bars: 50 μm). (c) Caspase-3 staining of consecutive sections for each group at 200x magnification (upper, scale bars: 200 μm) and 1000x magnification (upper, scale bars: 50 μm).

To further characterize our nanoparticle’s uptake by cancer cells in vivo, we conducted TEM of MDA-MB-231 tumor sections harvested 0, 1, and 6 h after intratumoral injection (Figure 7). As shown in Figures 7(a) and 7(b), the TEM images of tumors treated 1 h prior with MSN-PdTPP demonstrated greater nanoparticle uptake than those harvested immediately following nanoparticle administration, with nanoparticles mostly confined to the periphery of cells at 0 h and not yet significantly endocytosed. TEM images of tumors treated 6 h prior, however, revealed few nanoparticles remaining within harvested tumors. Taken together we posit that optimal endocytosis and thus optimal photoirradiation benefit strongly depended upon efficient nanoplatform endocytosis.
are likely to be somewhere between 1 and 6h following intratumoral injection of our nanoparticles.

4. Conclusions

In this work we describe significant enhancement of photodynamic therapy efficacy achieved via the use of nanoparticle-based photosensitizers, even when treatment is limited to a single photosensitizer dose and a single photoirradiation session. By covalently conjugating large numbers of the photosensitizer Pd-porphyrin onto the environmentally communicative nanochannel walls of mesoporous silica nanoparticles, we circumvent our hydrophobic photosensitizer’s tendency to self-aggregate and self-quench. The benefits of this protected conveyance of substantial quantities of photosensitizer, our nanoparticle’s proclivity for endocytosis, and thereby photosensitizer accumulation within tumor permit the use of much lower photosensitizer dose than free photosensitizer and with much greater antitumor response.

Current efforts are aimed at the surface functionalization of
our nanoplatform for pathology-specific targeting and further optimization of pathology-dependent timing/frequency of nanoparticle dose and photoradiation.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

**Acknowledgments**

The authors acknowledge financial support from the National Health Research Institutes of Taiwan (BN-103-PP-04 and NM-103-PP-01) and the National Science Council of Taiwan (NSC-100-2911-I-400-502 and NSC102-2113-M-400-001-MY3).

**References**


Submit your manuscripts at
http://www.hindawi.com