ACS APPLIED BIO MATERIALS

Article

Cite This: ACS Appl. Bio Mater. 2019, 2, 2872−2878

www.acsabm.org

Phototoxicity Generated by Silicon Quantum Dot Nanoparticles on Zebrafish Embryos

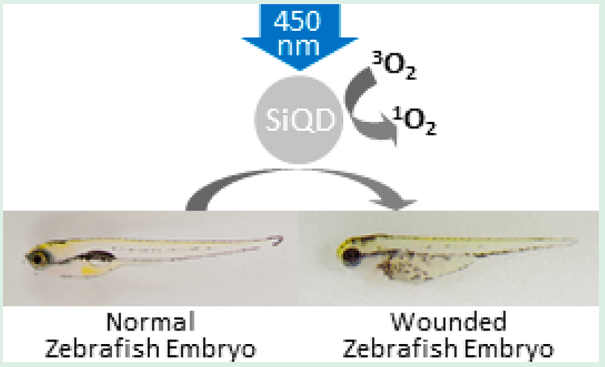
Prateek K. Srivastava,[†] Shanshan Han,[‡] Chang-Ching Tu,*[,‡] and Lili Jing*[,†]

[†]Engineering Research Center of Cell & Therapeutic Antibody, Ministry of Education, and School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, China

[‡]University of Michigan-Shanghai Jiao Tong University Joint Institute, Shanghai Jiao Tong University, Shanghai 200240, China

Supporting Information

ABSTRACT: We demonstrate phototoxicity generated by silicon quantum dot nanoparticles (SiQDNPs) using zebrafish as an animal model. Having long exciton lifetime, the SiQDNPs can function as photosensitizers which absorb incident optical light and transfer the energy to oxygen molecules in close proximity, generating cytotoxic singlet oxygens. First, the zebrafish embryos were soaked in the SiQDNP suspension in E3 medium, while being illuminated under blue light or kept in the dark for 6 h. Through neutral red staining immediately afterward, the illuminated embryos showed more prominent injuries at their head, yolk sac and tail parts than those in the dark. Furthermore, prolonged observation after the treatment revealed that the illuminated embryos had mortality rates significantly higher than those without illumination, clearly showing the phototoxicity effect generated by the SiQDNPs. However, adverse effect due to the immersion of whole embryos in the SiQDNP suspension was also observed. To alleviate this issue, minute amounts of the SiQDNPs were microinjected to the embryos, followed by blue light illumination. By acridine orange staining subsequently, cell apoptosis localized near the microinjection site was revealed, whereas no apoptosis was found for those also microinjected with the SiQDNPs but without illumination. The phototoxicity effect demonstrated on zebrafish embryos in this work manifests the potential of using the SiQDNPs as a photosensitizer for photodynamic therapy.



KEYWORDS: *phototoxicity, singlet oxygen, silicon quantum dot, zebrafish embryo, photodynamic therapy*

INTRODUCTION

Photodynamic therapy (PDT) is an emerging treatment strategy for dermatological diseases and certain types of cancers, owing to its low invasiveness and cytotoxicity.[1,2] Upon photoexcitation, a photosensitizer is excited into a singlet state, followed by relaxation to a relatively long-lived triplet state which further undergoes triplet energy transfer to a nearby oxygen molecule, causing the oxygen molecule to be excited from its ground triplet state ($^3O_2$) to excited singlet state ($^1O_2$). As a form of reactive oxygen species (ROS), the singlet oxygens, although not radicals, are highly reactive with biomolecules and destructive to cell organelles. However, due to their short diffusion length, which is estimated to be less than 0.1 μm,[3] the phototoxicity of singlet oxygens is only effective in localized regions where both the photosensitizer and photoexcitation are present. In other words, in the absence of light, a photosensitizer is generally considered benign. To date, the U.S. Food and Drug Administration (FDA) has approved the photosensitizer, porfimer sodium or Photofrin, for PDT treatment of some skin and organ cancers. Besides, several other PDT agents and their applications are being evaluated in clinical trials.[4,5]

Organic dye molecules with tetrapyrrole backbones, mainly porphyrins or porphyrinic derivatives, have been used as PDT photosensitizers for decades. Nevertheless, extensive research is still undergoing to further improve their chemical stability, enable targeted delivery, and to reduce the side effect of prolonged photosensitization, especially at the skin and eyes, as a result of long elimination half-life.[4,5] Meanwhile, some organic photosensitizers, such as phthalocyanines, although capable of generating singlet oxygens efficiently, are relatively hydrophobic due to their inherent planar conjugated π bond structures. Therefore, various nanovehicles, consisting of liposomes, micelles and nanoemulsions,[6−8] and inorganic nanostructured hosts, such as silica nanorods and gold nanoparticles,[9−11] have been used for improving their water solubility. Besides, the photosensitizers loaded in the nanovehicles have a higher tendency of accumulation in tumors, due to the enhanced permeability and retention (EPR) effect resulting from the nanoscale particle size.[11]

Received: March 28, 2019
Accepted: June 4, 2019
Published: June 3, 2019

Downloaded via SHANGHAI JIAO TONG UNIV on July 19, 2019 at 03:47:31 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

© 2019 American Chemical Society

DOI: 10.1021/acsabm.9b00264
ACS Appl. Bio Mater. 2019, 2, 2872−2878

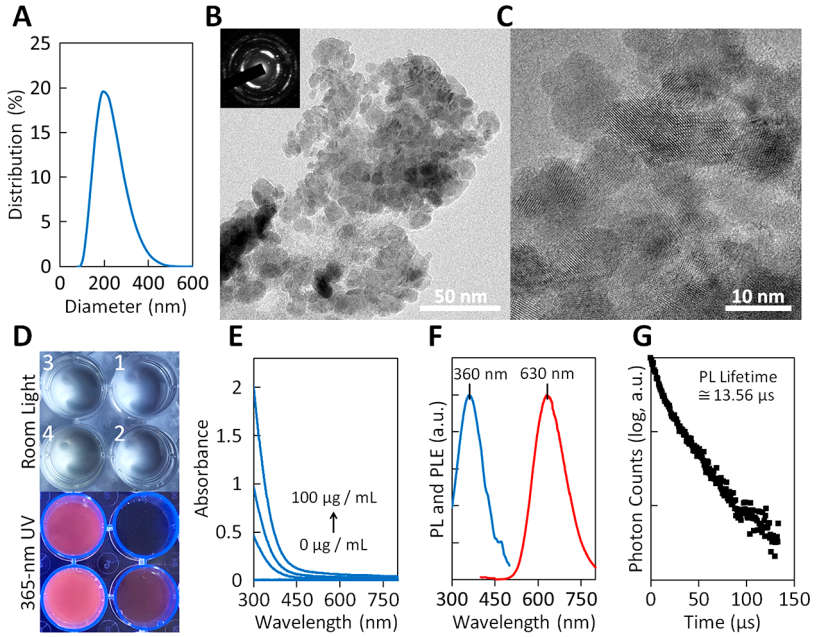


Figure 1. (A) Particle size distribution of the SiQDNPs dispersed in water, obtained by a dynamic light scattering method. (B) Transmission electron microscope (TEM) image of one SiQDNP, which has irregular shape and mesoporous surface. The inset shows the electron diffraction pattern of the entire SiQDNP. (C) High resolution transmission electron microscope (HRTEM) image taken at the SiQDNP surface. Clusters of crystalline silicon quantum dots of diameters about 5 nm are clearly visible in the image. (D) Photographs of the SiQDNP suspensions in E3 medium with different concentrations (0, 25, 50, and 100 μg/mL, denoted as 1, 2, 3, and 4, respectively) under room light or 365 nm UV light. (E) Absorbance spectra of the SiQDNP suspensions in E3 medium with different concentrations (0, 25, 50, and 100 μg/mL, from bottom to top). (F) Photoluminescence (PL, red line) and photoluminescence excitation (PLE, blue line) spectra of the SiQDNP suspension in water (100 μg/mL). (G) Time-resolved photoluminescence decay of the SiQDNP suspension in water (100 μg/mL, excited by 375 nm).

On the other hand, some nanostructures themselves can function as photosensitizers, such as fullerenes, titanium oxide nanoparticles, and semiconductor quantum dots.[12−14] Compared to organic counterparts, inorganic semiconductor quantum dots as photosensitizers absorb excitation light more efficiently due to their larger transition dipole moments.[15] More importantly, because they are much less susceptible to photobleaching,[16] the photosensitization process can last longer under continuous illumination. However, the photoexcited excitons in direct bandgap semiconductors, such as CdSe and InP, tend to recombine rapidly, usually in tens of nanoseconds, which is unfavorable to the triplet energy transfer.[14,17] Furthermore, the heavy-metal elements of these compound semiconductor quantum dots are cytotoxic,[18] arousing the concern of dark toxicity. The aforementioned issues can be resolved by using silicon quantum dots. Owing to the indirect bandgap nature of silicon and the long-lived surface defect states of silicon quantum dots,[19−21] the exciton lifetimes of silicon quantum dots, as measured by their time-resolved photoluminescence decay, are relatively long, typically from 10 to 30 μs,[20,21] which is more beneficial to the triplet energy transfer. Moreover, silicon nanomaterials in general have shown exceptional biocompatibility.[22,23] With the help of large surface areas, various silicon quantum dots and porous silicon surfaces have demonstrated high efficiency of singlet oxygen generation.[17,24−26]

The in vitro cell viability assay, which generally measures a metabolic enzyme or an enzyme associated with the viable cell number, is one of the most widely used methods to evaluate the cytotoxicity effects of a drug.[27] However, a cellular response cannot reflect the whole spectrum of biological activities of a drug in a living animal. For example, the majority of intravenously injected quantum dots tend to end up being accumulated in the mononuclear phagocyte system organs, such as liver and spleen,[28] and such observation cannot be revealed merely by a cellular assay. Besides, due to the lack of biokinetics, results obtained from in vitro studies cannot be used directly to predict the responses of organisms to a drug exposure. Therefore, in vivo studies using animal models are the critical next step toward clinical trials.[29] In recent years, zebrafish has become a popular vertebrate animal model for preclinical or toxicological studies, owing to its unique characteristics, such as rapid embryonic development, optical transparency, inexpensive husbandry, high fecundity rates, short breeding cycles, and ease of genetic modifications.[30] Furthermore, zebrafish have genomes 70% analogous to human ones, and possess key physiological structures, such as digestive, nervous, and cardiovascular systems.[31] Lastly, drug efficacy and toxicity studies using a large population of zebrafish embryos can be easily achieved, involving much less cost and ethical concern than using other mammal models, such as mice or rats.[32] In addition, recent studies supported a strong correlation of lethality in zebrafish embryos to toxicity in adult fish,[33−35] hence zebrafish embryos have also been used as an alternative system to predict toxicity in adults.

In this work, we used zebrafish embryos as an animal model to study the phototoxicity of singlet oxygens generated by photosensitizing silicon quantum dot nanoparticles (SiQDNPs). The zebrafish embryos were either soaked in the SiQDNP suspension under photoexcitation or microinjected with the SiQDNP suspension followed by photoexcitation. Through histological staining or morphological observation, phenotypic abnormalities, such as skin wounds and swollen hearts, of the treated zebrafish embryos were

DOI: 10.1021/acsabm.9b00264
ACS Appl. Bio Mater. 2019, 2, 2872−2878

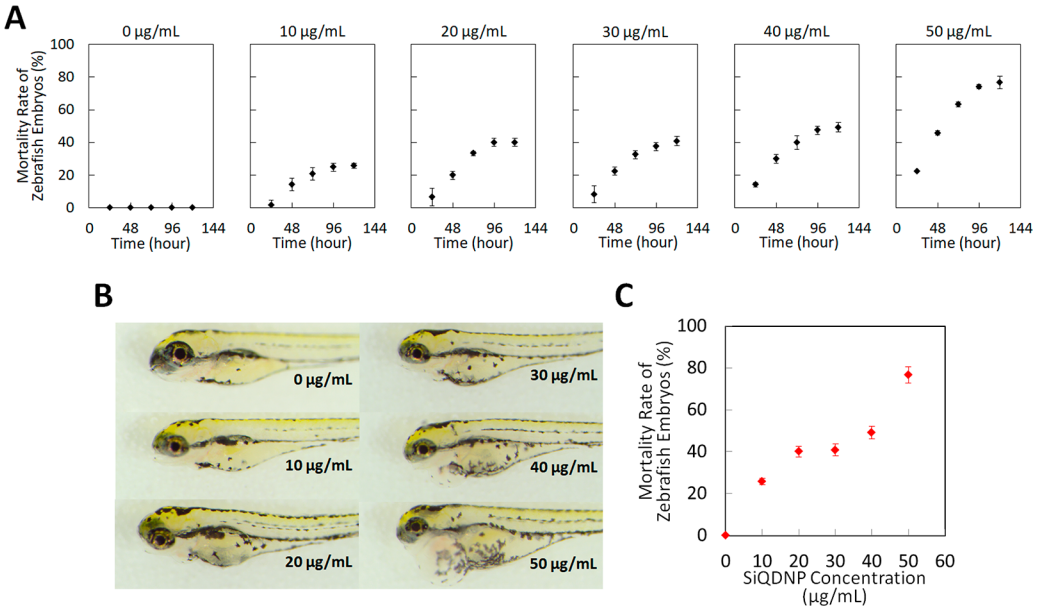


Figure 2. (A) Mortality rate of zebrafish embryos versus incubation time for different SiQDNP concentrations (0, 10, 20, 30, 40, and 50 μg/mL). (B) Representative malformed zebrafish embryos treated with different SiQDNP concentrations. The malformed embryos are mainly characterized by pericardial edema. All photographs were taken at the completion of 72 h of incubation. (C) Mortality rate at the completion of 120 h of incubation versus the SiQDNP concentration.

observed, clearly showing the phototoxicity effect generated by the SiQDNPs. Meanwhile, the general toxicity of the SiQDNPs in the absence of light illumination was also evaluated. Through restricted application, such as microinjection, the general toxicity of the SiQDNPs was minimized. Although phototoxicity against cancer cells has been demonstrated in vitro by using similar porous silicon nanoparticles as photosensitizers,[26] the present work provides more insights into how and where the SiQDNPs exerts their photosensitizing effect on a vertebrate animal model, further manifesting the potential of using the SiQDNPs as a photosensitizer for the PDT application.

EXPERIMENTAL RESULTS

The SiQDNPs were synthesized by using an electrochemical etching method developed previously (Supporting Information (SI) Figure S1).[36] The SiQDNPs with irregular shapes had an overall particle size distribution centered at around 200 nm (Figure 1A). Besides, the SiQDNPs were featured with mesoporous surfaces (Figure 1B), on which clusters of crystalline silicon quantum dots of diameters about 5 nm can be clearly observed (Figure 1C). Noteworthily, rather than the whole SiQDNP, only the silicon quantum dots on the SiQDNP surface with sizes close to the exciton Bohr radius of silicon (4.9 nm) are capable of emitting photoluminescence.[37] All SiQDNPs used in this work were functionalized with highly hydrophilic sulfonate groups (SI Figure S2), such that the SiQDNPs were able to form uniform and stable suspension in E3 medium, which the zebrafish embryos were either soaked in or microinjected with. While E3 medium is colorless, the SiQDNP suspension in E3 medium carried a pale yellow tint (Figure 1D), as a result of the suspension's relatively high absorption in the blue and ultraviolet (UV) wavelengths (Figure 1E). When under the illumination of 365 nm UV light, the SiQDNP suspension emitted bright orangish photoluminescence (PL) with a peak wavelength located at 630 nm (Figure 1F). After being stored at 4 °C for 10 days, the SiQDNP suspension maintained about 60% of its initial PL intensity (SI Figure S3). Furthermore, after 6 h of high intensity (34 mW/cm^2) 450 nm blue light illumination, which was the light treatment dosage used for the phototoxicity assay in this work, the SiQDNPs were still photoluminescent, retaining about 14% of its initial PL intensity (SI Figure S4). The PL mechanism of the SiQDNPs is briefly described as follows: the photoexcited electron−hole-pairs are first fast (in picoseconds) trapped to the oxide-related defect states located on the silicon quantum dot surface, and then recombined slowly (in microseconds) via the defect states to emit PL photons.[38] Consequently, the SiQDNPs have a long PL lifetime, about 13.56 μs (Figure 1G and SI Figure S5), which is more than 2 orders of magnitude longer than that of commonly used CdSe quantum dots which emit PL mainly through direct band-to-band recombination. The oxide-related defect states on the silicon quantum dot surface likely result from the formation Si−O−Si bonds,[19−21] and their presence can be indicated by the strong infrared absorption in the wavenumber range from 950 to 1300 cm^{-1} (SI Figure S2).

By taking advantage of the slow decaying PL of the SiQDNPs, time-gated fluorescence imaging, which removes nearly all background autofluorescence, has been achieved.[39] On the other hand, in this work we employed the SiQDNPs as photosensitizers to generate singlet oxygens, in view of the SiQDNPs' long exciton lifetime which favors the triplet energy transfer. The singlet oxygen generation quantum efficiency of the SiQDNPs was measured to be about 2.2% in ethanol, by using a white light emitting diode (LED) lamp as the excitation light source, 1,3-diphenylisobenzofuran (DPBF) as the singlet oxygen probe and Rose Bengal (RB) with a known quantum efficiency (86% in ethanol) as the reference (SI Figure S6). The white LED lamp was used because of its wide spectral range which covers the absorption spectra of both the SiQDNPs and RB. Furthermore, during the quantum efficiency measurement the white LED lamp was covered with a 455 nm long-pass filter for minimizing the absorption and photobleaching of DPBF. Recently, quantum efficiencies of 5−10% for similar porous silicon nanoparticles dispersed in ethanol have been reported.[26] Considering that the SiQDNPs can only be efficiently excited at wavelengths shorter than 500 nm, as revealed by the photoluminescence excitation (PLE) spectrum (blue curve in Figure 1F), the excitation light emitted from the white LED lamp at wavelengths longer than 500 nm, although being absorbed by the SiQDNPs, does not contribute to the generation of singlet oxygens effectively. Therefore, for the following animal studies, we used a 450 nm blue LED lamp as the photoexcitation source, with which the SiQDNPs can generate singlet oxygens more efficiently than under optical excitation at longer wavelengths (SI Figure S7). In addition,

DOI: 10.1021/acsabm.9b00264
ACS Appl. Bio Mater. 2019, 2, 2872−2878

compared to UV light, the blue light is less harmful to the zebrafish embryos.

Prior to the phototoxicity studies, we first investigated the general toxicity of the SiQDNPs to zebrafish embryos. To do this, embryos at the age of 26 h post fertilization (hpf) were dechorionated and soaked in the SiQDNP suspensions in E3 medium with six different concentrations (0, 10, 20, 30, 40, and 50 μg/mL) for up to 120 h, during which the mortality and malformation rates were recorded every 24 h under a stereomicroscope. The embryos were kept in the dark constantly, and the SiQDNP suspensions were renewed every 24 h. For each dose, the experiment was repeated three times, with 40 embryos used each time. After the first 24 h of incubation, the mortality rate was from 2% to 12% as the concentration increased from 10 to 50 μg/mL (Figure 2A), and no obvious malformation of embryos was observed. When the incubation time was extended to more than 24 h, the mortality rates went up, and the appearance of malformed embryos, which are mostly manifested with pericardial edema, became apparent (Figure 2B). At the completion of 120 h, the mortality rate increased to 47% for the SiQDNP concentrations equal to 40 μg/mL (Figure 2C), which can be approximated as the 120 h LC_{50} for the SiQDNPs. Previous studies have shown that carbon-based nanomaterials, such as carbon nanotubes (CNT) and fullerene, which are generally considered biocompatible, have 48 h LC_{50} higher than 200 μg/mL.[40] When the SiQDNPs were incubated with E3 medium alone for 24 h at 28 °C, owing to the nanoparticles' highly hydrophilic surface passivation, the suspension was stable and no aggregation of the SiQDNPs occurred. In contrast, after the SiQDNPs were incubated with zebrafish embryos for 24 h at 28 °C, some SiQDNPs were found agglomerated and precipitated (SI Figure S8). Since during the incubation, the majority of the embryos tended to stay at the bottom of the plates where the agglomerated SiQDNPs were deposited, the actual SiQDNP concentrations experienced by the embryos could be higher than the concentrations originally prepared. This can be a major cause for the relatively low LC_{50} for the SiQDNPs.

To study the phototoxicity effect generated by the SiQDNPs, 26-hpf zebrafish embryos were soaked in the SiQDNP suspensions in E3 medium with five different concentrations (0, 10, 20, 30, and 40 μg/mL), while being kept either under 450 nm blue LED light illumination or in the dark for 6 h. Note that neither agglomeration nor precipitation of the SiQDNPs was observed after the 6 h incubation either under light or in the dark. Subsequently, all zebrafish embryos were carefully rinsed with fresh E3 medium, and then stained with neutral red which highlights the apoptotic epidermal cells.[41] Based on the magnitude and distribution of the red color marks, particularly located at the head, yolk sac, and tail parts, a neutral red staining level from 1 to 4 was determined for each zebrafish embryo (Figure 3A). For each SiQDNP concentration, the above experiment was repeated five times, with 40 embryos used each time. As a result, an average neutral red staining level was obtained for each SiQDNP concentration (Figure 3B). The effect of phototoxicity, as evidenced by the average staining level difference between the light and dark curves, became prominent when the SiQDNP concentration was higher than 30 μg/mL.

To further study the effects of the skin cell damages, after the same 6 h incubation with the SiQDNPs, the zebrafish embryos were carefully rinsed with fresh E3 medium and then transferred to E3 medium only, followed by morphological observation under the stereomicroscope every 12 h up to 96 h. During the observation, malformation and mortality rates of the zebrafish embryos were recorded (Figure 4A). At the SiQDNP concentrations equal to 0 and 20 μg/mL, the mortality rate was zero and almost no malformation was observed. In other words, the minor skin wounds on the zebrafish embryos, corresponding to the 20 μg/mL data in Figure 3B, did not lead to malformation and could eventually heal. When the SiQDNP concentration was increased to 40 μg/mL, the mortality rates of embryos incubated under light and in the dark increased to about 30% and 6% at 96 h, respectively. Furthermore, the rates of malformations (manifested as swollen hearts as shown in Figure 4B) reached the highest at 12 h and then gradually declined to about the same as the

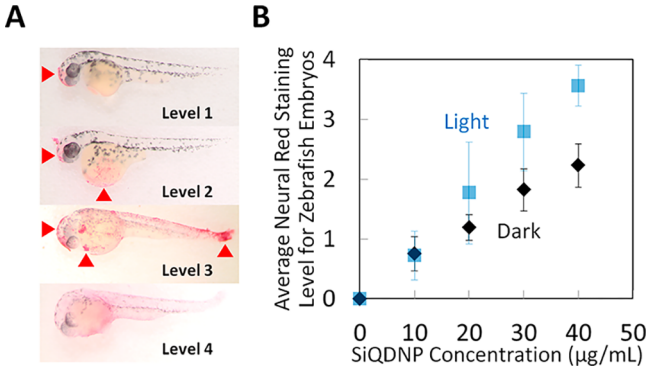


Figure 3. (A) Zebrafish embryos with different levels of neutral red staining which reflects different degrees of skin damages: at level 1, only the head is stained; at level 2, both the head and yolk sac are stained; at level 3, the head, yolk sac, and tail are all stained; at level 4, the whole body is stained, and the abnormal embryo usually dies shortly after the staining. (B) Average neutral red staining levels for zebrafish embryos which were incubated with the SiQDNP suspensions of different concentrations (0, 10, 20, 30, and 40 μg/mL), while being illuminated under 450 nm blue LED light with intensity equal to 34 mW/cm^2 (blue squares) or kept in the dark (black diamonds) for 6 h.

mortality rates. In other words, while some of the embryos with more serious skin wounds, corresponding to the 40 μg/mL data in Figure 3B, died shortly after the 6 h incubation, the rest of the embryos gradually recovered to normal. This recovery phenomenon indicates that the embryos have enough plasticity and regeneration abilities to restore the SiQDNP-induced impairments in case they are not too severe. When the SiQDNP concentration was further increased to 60 and 80 μg/mL, the zebrafish embryos incubated in 6 h of darkness behaved similarly to that at 40 μg/mL, except that the mortality rates became a little higher. However, for the embryos incubated under 6 h of light, the mortality rates drastically jumped to almost a hundred percent. At 96 h, the mortality rates difference between light and dark was the largest at 60 μg/mL (Figure 4C). Such distinguishable difference confirms the SiQDNP's efficacy to generate phototoxicity and potential as a photosensitizer for the PDT application. Noteworthily, in the absence of the SiQDNPs, the 6 h blue light illumination itself has resulted in no adverse effect to the embryos (as shown by the 0 μg/mL data in Figure 3B and Figure 4A).

Lastly, to study whether the observed phototoxicity resulted from singlet oxygens or localized heating, a control experiment using nonluminescent silicon nanoparticles (SiNPs) was conducted (SI Figure S9). The SiNPs, which were synthesized by a high energy ball milling method, had about the same particle size distribution and surface chemistry as the SiQDNPs, but without silicon quantum dots attached on the surfaces. Therefore, the SiNPs had no photoluminescence. Following identical experimental procedures, the malformation rates were found minimal for the zebrafish embryos incubated in the SiNP suspensions which absorbed even more amount of the 450 nm excitation light. Furthermore, the heating of the SiQDNP suspensions due to the light illumination was characterized (SI Figure S10). Temperature increases of less than 5 °C, which are usually well tolerable for the zebrafish embryos, were observed for all SiQDNP concentrations including E3 medium only. In other words, there was no correlation between the amount of temperature increase and the SiQDNP concentration. In view of the experimental results presented here (SI Figures S9 and S10) and the confirmation of singlet oxygen generation of the SiQDNPs (SI Figures S6 and S7), we may conclude that the phototoxicity observed in this work should mostly result from singlet oxygens rather than localized heating.

The above studies demonstrate that the SiQDNPs can generate cellular phototoxicity in a live animal, as evidenced by the increased skin damages and the subsequent enhanced mortality rates of

DOI: 10.1021/acsabm.9b00264
ACS Appl. Bio Mater. 2019, 2, 2872−2878

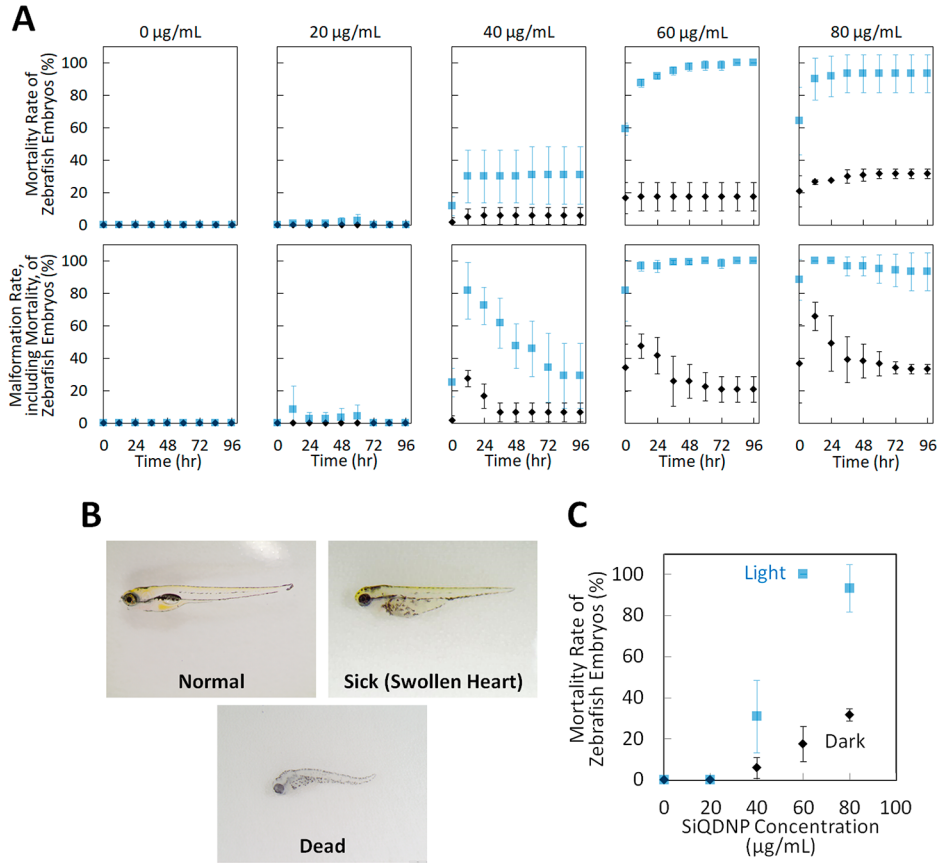


Figure 4. (A) Mortality rate (upper row) and malformation rate (lower row), including mortality, versus observation time for zebrafish embryos which were incubated with the SiQDNP suspensions of different concentrations (0, 20, 40, 60, and 80 μg/mL), while being illuminated under 450 nm blue LED light with intensity equal to 34 mW/cm^2 (blue squares) or kept in the dark (black diamonds) for 6 h. The time 0 is set as the time immediately after the 6 h incubation. (B) Representative normal, malformed and dead zebrafish embryos. (C) Mortality rates recorded at 96 h after the 6 h incubation with different SiQDNP concentrations (0, 20, 40, 60, and 80 μg/mL) under the blue light (blue squares) or in the dark (black diamonds).

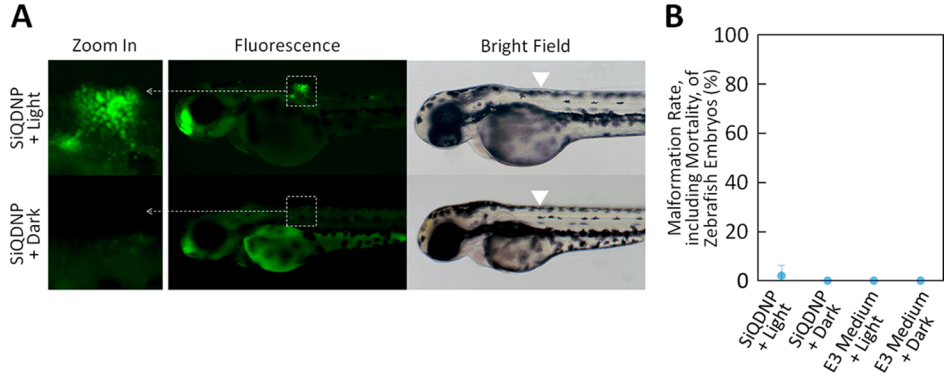


Figure 5. (A) Fluorescence and bright filed images of zebrafish embryos microinjected with the SiQDNP suspension at the dorsal trunk regions, followed by illumination under 450 nm blue LED light with intensity equal to 34 mW/cm^2 or keeping in the dark for 6 h. The white triangles indicate the microinjection sites. (B) Malformation rates, including mortality, of zebrafish embryos at 96 h, after microinjection of either the SiQDNP suspension or the E3 medium and then shining with blue light or keeping in the dark for 6 h.

zebrafish embryos soaked in the SiQDNPs along with blue light illumination. However, the toxicity in the absence of light illumination was also present and increased proportionally to the SiQDNP concentration. We speculate that this was due to the application of the SiQDNPs on whole embryos through the soaking method. To avoid full-body exposure, minute amounts (2 nL) of the SiQDNP suspension (1 mg/mL) were microinjected to zebrafish embryos, followed by shining with the same 450 nm blue LED light or keeping in the dark for 6 h. Immediately after the treatment, the embryos were stained with acridine orange to visualize the interior apoptotic cells.[42] Fluorescence microscopic examination revealed that the embryos with their dorsal trunk region microinjected with the SiQDNPs and then illuminated by the blue light showed obvious green fluorescent marks near the microinjection sites (Figure 5A, upper row). Whereas, the embryos with the same microinjection but without light illumination showed no fluorescence signal (Figure 5A, lower row). Moreover, the embryos microinjected with the SiQDNP suspension or the E3 medium showed nearly zero rate of malformation or mortality up to

DOI: 10.1021/acsabm.9b00264
ACS Appl. Bio Mater. 2019, 2, 2872−2878

120 h after the 6 h illumination (Figure 5B). These results support that a localized and minute amount of the SiQDNPs, although exerting prominent phototoxicity to the tissues around the injection site, brings negligible toxicity to the embryo as a whole organism.

CONCLUSION

In summary, we used zebrafish embryos as an animal model to demonstrate phototoxicity generated by photosensitizing SiQDNPs which had a long exciton lifetime of about 13.56 μs and singlet oxygen generation quantum efficiency of about 2.2% in ethanol. For the embryos continuously soaked in the SiQDNP suspension in E3 medium, the 120 h LC_{50} of the SiQDNPs was estimated to be about 40 μg/mL. To demonstrate the phototoxicity effect, the embryos are soaked in the SiQDNP suspensions of various concentrations, while being illuminated under 450 nm blue LED light or kept in the dark for 6 h. Through neutral red staining immediately after the 6 h incubation, which highlights apoptotic epidermal cells, the phototoxicity effect, as evidenced by the average neutral red staining level difference between light and dark, becomes prominent when the SiQDNP concentration is higher than 30 μg/mL. Furthermore, at 96 h after the 6 h incubation, the illuminated embryos show mortality rate about 5 times higher than those in the dark when the SiQDNP concentration is equal to 60 μg/mL. To lower the toxicity due to immersion of whole embryos in the SiQDNP suspension, minute amounts of the SiQDNPs are microinjected to the embryos, followed by shining with 450 nm blue LED light or keeping in the dark for 6 h. Immediately afterward, the embryos are stained with acridine orange to visualize the interior apoptotic cells. The illuminated embryos exhibit obvious green fluorescent marks around the microinjection sites, whereas no fluorescence signal is found for the embryos kept in the dark, indicating that the microinjected SiQDNPs, while being capable of generating phototoxicity, cause negligible toxicity to the embryos in the absence of light. The SiQDNP phototoxicity demonstrated on zebrafish embryos in this work manifests the potential of applying the SiQDNPs as a photosensitizer for the PDT application.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsabm.9b00264.

Synthesis process of the sulfonate-terminated SiQDNPs. FTIR−ATR spectra of the hydroxyl-terminated and the sulfonate-terminated SiQDNPs. Stability and photostability of the SiQDNPs. Estimation of PL lifetime of the SiQDNPs. Estimation of singlet oxygen generation quantum efficiency of the SiQDNPs. Comparison between singlet oxygen generation by the SiQDNPs under blue and red light. Comparison between E3 medium with and without incubation with zebrafish embryos. Malformation rate versus observation time for zebrafish embryos incubated with the SiNP suspensions of different concentrations. Time-resolved temperature analysis for the SiQDNP suspension under continuous illumination (PDF)

AUTHOR INFORMATION

Corresponding Authors

*(C.C.T.) Phone: +86-21-34206765 ×5081. E-mail: changching.tu@sjtu.edu.cn.

*(L.J.) Phone: +86-21-34204048; e-mail: lilijing@sjtu.edu.cn.

ORCID

Chang-Ching Tu: 0000-0002-9233-1928

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was financially supported by the Shanghai Pujiang Talent Program (18PJ1404900 and 16PJ1405500). We thank Prof. Chang-Ching Tu and Shanshan Han for proposing the original idea, synthesizing and analyzing the SiQDNPs and finalizing the manuscript. Prof. Lili Jing and Prateek K. Srivastava for conducting all the zebrafish experiments and composing the first manuscript draft.

REFERENCES

(1) Dougherty, T. J.; Gomer, C. J.; Henderson, B. W.; Jori, G.; Kessel, D.; Korbelik, M.; Moan, J.; Peng, Q. Photodynamic Therapy. *J. Natl. Cancer Inst.* **1998**, *90*, 889−905.

(2) Kalka, K.; Merk, H.; Mukhtar, H. Photodynamic Therapy in Dermatology. *J. Am. Acad. Dermatol.* **2000**, *42*, 389−413.

(3) Moan, J. On the Diffusion Length of Singlet Oxygen in Cells and Tissues. *J. Photochem. Photobiol., B* **1990**, *6*, 343−344.

(4) Zhang, J.; Jiang, C.; Longo, J. P. F.; Azevedo, R. B.; Zhang, H.; Muehlmann, L. A. An Updated Overview on the Development of New Photosensitizers for Anticancer Photodynamic Therapy. *Acta Pharm. Sin. B* **2018**, *8*, 137−146.

(5) Abrahamse, H.; Hamblin, M. R. New Photosensitizers for Photodynamic Therapy. *Biochem. J.* **2016**, *473*, 347−364.

(6) Sadasivam, M.; Avci, P.; Gupta, G. K.; Lakshmanan, S.; Chandran, R.; Huang, Y. Y.; Kumar, R.; Hamblin, M. R. Self-Assembled Liposomal Nanoparticles in Photodynamic Therapy. *Eur. J. Nanomed.* **2013**, *5* (5), 115−129.

(7) Gibot, L.; Lemelle, A.; Till, U.; Moukarzel, B.; Mingotaud, A.-F.; Pimienta, V.; Saint-Aguet, P.; Rols, M.-P.; Gaucher, M.; Violleau, F.; Chassenieux, C.; Vicendo, P. Polymeric Micelles Encapsulating Photosensitizer: Structure/Photodynamic Therapy Efficiency Relation. *Biomacromolecules* **2014**, *15*, 1443−1455.

(8) Day, R. A.; Estabrook, D. A.; Logan, J. K.; Sletten, E. M. Fluorous Photosensitizers Enhance Photodynamic Therapy with Perfluorocarbon Nanoemulsions. *Chem. Commun.* **2017**, *53*, 13043−13046.

(9) Yang, G.; Gong, H.; Qian, X.; Tan, P.; Li, Z.; Liu, T.; Liu, J.; Li, Y.; Liu, Z. Mesoporous Silica Nanorods Intrinsically Doped with Photosensitizers as a Multifunctional Drug Carrier for Combination Therapy of Cancer. *Nano Res.* **2015**, *8*, 751−764.

(10) Cheng, Y.; Meyers, J. D.; Broome, A. M.; Kenney, M. E.; Basilion, J. P.; Burda, C. J. Deep Penetration of a PDT Drug into Tumors by Noncovalent Drug-Gold Nanoparticle Conjugates. *J. Am. Chem. Soc.* **2011**, *133*, 2583−2591.

(11) Lucky, S. S.; Soo, K. C.; Zhang, Y. Nanoparticles in Photodynamic Therapy. *Chem. Rev.* **2015**, *115*, 1990−2042.

(12) Mroz, P.; Xia, Y.; Asanuma, D.; Konopko, A.; Zhiyentayev, T.; Huang, Y. Y.; Sharma, S. K.; Dai, T.; Khan, U. J.; Wharton, T.; Hamblin, M. R. Intraperitoneal Photodynamic Therapy Mediated by a Fullerene in a Mouse Model of Abdominal Dissemination of Colon Adenocarcinoma. *Nanomedicine* **2011**, *7*, 965−974.

(13) Li, Z.; Mi, L.; Wang, P.-N.; Chen, J. Y. Study on the Visible-Light-Induced Photokilling Effect of Nitrogen-Doped TiO_2 Nanoparticles on Cancer Cells. *Nanoscale Res. Lett.* **2011**, *6*, 356.

DOI: 10.1021/acsabm.9b00264
ACS Appl. Bio Mater. 2019, 2, 2872−2878

(14) Samia, A. C. S.; Chen, X.; Burda, C. Semiconductor Quantum Dots for Photodynamic Therapy. *J. Am. Chem. Soc.* **2003**, *125*, 15736−15737.
(15) Bakalova, R.; Ohba, H.; Zhelev, Z.; Ishikawa, M.; Baba, Y. Quantum dots as photosensitizers. *Nat. Biotechnol.* **2004**, *22*, 1360−1361.
(16) Resch-Genger, U.; Grabolle, M.; Cavaliere-Jaricot, S.; Nitschke, R.; Nann, T. Quantum Dots versus Organic Dyes as Fluorescent Labels. *Nat. Methods* **2008**, *5*, 763−775.
(17) Kovalev, D.; Fujii, M. Silicon Nanocrystals: Photosensitizers for Oxygen Molecules. *Adv. Mater.* **2005**, *17*, 2531−2544.
(18) Derfus, A. M.; Chan, W. C. W.; Bhatia, S. N. Probing the Cytotoxicity of Semiconductor Quantum Dots. *Nano Lett.* **2004**, *4*, 11−18.
(19) Godefroo, S.; Hayne, M.; Jivanescu, M.; Stesmans, A.; Zacharias, M.; Lebedev, O. I.; Tendeloo, G. V.; Moshchalkov, V. V. Classification and Control of the Origin of Photoluminescence from Si Nanocrystals. *Nat. Nanotechnol.* **2008**, *3*, 174−178.
(20) Dohnalová, K.; Poddubny, A. N.; Prokofiev, A. A.; Boer, W. D. d.; Umesh, C. P.; Paulusse, J. MJ; Zuilhof, H.; Gregorkiewicz, T. Surface Brightens Up Si Quantum Dots: Direct Bandgap-Like Size-Tunable Emission. *Light: Sci. Appl.* **2013**, *2*, e46.
(21) Kůsová, K.; Cibulka, O.; Dohnalová, K.; Pelant, I.; Valenta, J.; Fučíková, A.; Žídek, K.; Lang, J.; Englich, J.; Matějka, P.; Štěpánek, P.; Bakardjieva, S. Brightly Luminescent Organically Capped Silicon Nanocrystals Fabricated at Room Temperature and Atmospheric Pressure. *ACS Nano* **2010**, *4*, 4495−4504.
(22) Park, J.-H.; Gu, L.; Maltzahn, G. v.; Ruoslahti, E.; Bhatia, S. N.; Sailor, M. J. Biodegradable Luminescent Porous Silicon Nanoparticles for In Vivo Applications. *Nat. Mater.* **2009**, *8*, 331−336.
(23) Erogbogbo, F.; Yong, K.-T.; Roy, I.; Hu, R.; Law, W.-C.; Zhao, W.; Ding, H.; Wu, F.; Kumar, R.; Swihart, M. T.; Prasad, P. N. In Vivo Targeted Cancer Imaging, Sentinel Lymph Node Mapping and Multi-Channel Imaging with Biocompatible Silicon Nanocrystals. *ACS Nano* **2011**, *5*, 413−423.
(24) Portolés, M. J. L.; Gara, P. M. D.; Kotler, M. L.; Bertolotti, S.; Román, E. S.; Rodríguez, H. B.; Gonzalez, M. C. Silicon Nanoparticle Photophysics and Singlet Oxygen Generation. *Langmuir* **2010**, *26*, 10953−10960.
(25) Lapkin, A. A.; Boddu, V. M.; Aliev, G. N.; Goller, B.; Polisski, S.; Kovalev, D. Photo-Oxidation by Singlet Oxygen Generated on Nanoporous Silicon in a LED-Powered Reactor. *Chem. Eng. J.* **2008**, *136*, 331−336.
(26) Xiao, L.; Gu, L.; Howell, S. B.; Sailor, M. J. Porous Silicon Nanoparticle Photosensitizers for Singlet Oxygen and Their Phototoxicity against Cancer Cells. *ACS Nano* **2011**, *5*, 3651−3659.
(27) Kepp, O.; Galluzzi, L.; Lipinski, M.; Yuan, J.; Kroemer, G. Cell Death Assays for Drug Discovery. *Nat. Rev. Drug Discovery* **2011**, *10*, 221−237.
(28) Choi, H. S.; Liu, W.; Misra, P.; Tanaka, E.; Zimmer, J. P.; Ipe, B. I.; Bawendi, M. G.; Frangioni, J. V. Renal Clearance of Quantum Dots. *Nat. Biotechnol.* **2007**, *25*, 1165−1170.
(29) Yong, K.-T.; Law, W.-C.; Hu, R.; Ye, L.; Liu, L.; Swihart, M. T.; Prasad, P. N. Nanotoxicity Assessment of Quantum Dots: from Cellular to Primate Studies. *Chem. Soc. Rev.* **2013**, *42*, 1236−1250.
(30) Kimmel, C. B.; Ballard, W. W.; Kimmel, S. R.; Ullmann, B.; Schilling, T. F. Stages of Embryonic Development of the Zzebrafish. *Dev. Dyn.* **1995**, *203*, 253−310.
(31) Kettleborough, R. N. W.; Busch-Nentwich, E. M.; Harvey, S. A.; Dooley, C. M.; Bruijn, E. d.; Eeden, F. v.; Sealy, I.; White, R. J.; Herd, C.; Nijman, I. J.; Fényes, F.; Mehroke, S.; Scahill, C.; Gibbons, R.; Wali, N.; Carruthers, S.; Hall, A.; Yen, J.; Cuppen, E.; Stemple, D. L. A Systematic Genome-Wide Analysis of Zebrafish Protein-Coding Gene Function. *Nature* **2013**, *496*, 494−497.
(32) Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the Protection of Animals Used for Scientific Purposes.
(33) Embry, M. R.; Belanger, S. E.; Braunbeck, T. A.; Galay-Burgos, M.; Halder, M.; Hinton, D. E.; Leonard, M. A.; Lillicrap, A.; Norberg-King, T.; Whale, G. The fish embryo toxicity test as an animal alternative method in hazard and risk assessment and scientific research. *Aquat. Toxicol.* **2010**, *97*, 79−87.
(34) Strähle, U.; Scholz, S.; Geisler, R.; Greiner, P.; Hollert, H.; Rastegar, S.; Schumacher, A.; Selderslaghs, I.; Weiss, C.; Witters, H.; Braunbeck, T. Zebrafish embryos as an alternative to animal experiments-a commentary on the definition of the onset of protected life stages in animal welfare regulations. *Reprod. Toxicol.* **2012**, *33*, 128−132.
(35) Knöbel, M.; Busser, F. J. M.; Rico-Rico, Á.; Kramer, N. I.; Hermens, J. L. M.; Hafner, C.; Tanneberger, K.; Schirmer, K.; Scholz, S. Predicting adult fish acute lethality with the zebrafish Embryo: relevance of test duration, endpoints, compound properties, and exposure concentration analysis. *Environ. Sci. Technol.* **2012**, *46*, 9690−700.
(36) Tu, C.-C.; Chen, K.-P.; Yang, T.-A.; Chou, M.-Y.; Lin, L. Y.; Li, Y.-K. Silicon Quantum Dot Nanoparticles with Antifouling Coatings for Immunostaining on Live Cancer Cells. *ACS Appl. Mater. Interfaces* **2016**, *8*, 13714−13723.
(37) Proot, J. P.; Delerue, C.; Allan, G. Electronic Structure and Optical Properties of Silicon Crystallites: Application to Porous Silicon. *Appl. Phys. Lett.* **1992**, *61*, 1948−1950.
(38) Dohnalová, K.; Kůsová, K.; Pelant, I. Time-Resolved Photoluminescence Spectroscopy of the Initial Oxidation Stage of Small Silicon Nanocrystals. *Appl. Phys. Lett.* **2009**, *94*, 211903.
(39) Tu, C.-C.; Awasthi, K.; Chen, K.-P.; Lin, C.-H.; Hamada, M.; Ohta, N.; Li, Y.-K. Time-Gated Imaging on Live Cancer Cells Using Silicon Quantum Dot Nanoparticles with Long-Lived Fluorescence. *ACS Photonics* **2017**, *4*, 1306−1315.
(40) Haque, E.; Ward, A. C. Zebrafish as a Model to Evaluate Nanoparticle Toxicity. *Nanomaterials* **2018**, *8*, 561.
(41) Peng, G.; He, Y.; Zhao, M.; Yu, T.; Qin, Y.; Lin, S. Differential Effects of Metal Oxide Nanoparticles on Zebrafish Embryos and Developing Larvae. *Environ. Sci.: Nano* **2018**, *5*, 1200−1207.
(42) Deng, J.; Yu, L.; Liu, C.; Yu, K.; Shi, X.; Yeung, L. W. Y.; Lam, P. K. S.; Wu, R. S. S.; Zhou, B. Hexabromocyclododecane-Induced Developmental Toxicity and Apoptosis in Zebrafish Embryos. *Aquat. Toxicol.* **2009**, *93*, 29−36.

DOI: 10.1021/acsabm.9b00264
ACS Appl. Bio Mater. 2019, 2, 2872−2878