Tin-Doped Near-Infrared Persistent Luminescence Nanoparticles with Considerable Improvement of Biological Window Activation for Deep Tumor Photodynamic Therapy

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ABSTRACT: The photodynamic therapy (PDT) as a promising antitumor therapy technique is greatly hampered by the low tissue penetration of light and the photothermal effect of prolonged irradiation. Near-infrared (NIR) persistent luminescence nanoparticles (NPLNPs) possess the potential for application in next-generation PDT. However, owing to the low re-excitation efficiency of NPLNPs in deep tissue, the current PDT nanoplatform based on NPLNPs is faced with the disadvantage of decreased PDT efficiency induced by persistent luminescence (PersL) decay at the lesion site. Herein, NPLNPs, Zn$_{1.3}$Ga$_{1.4}$Sn$_{0.3}$O$_4$:Cr$^{3+}$ (ZGS), with small particle sizes and excellent optical properties are synthesized via a simple acetylacetonate combustion method. The ZGS can be repeatedly excited by the biological window (659 nm) light to produce a strong NIR (700 nm) PersL. The response efficiency of ZGS to the wavelength in the biological window has been greatly improved due to their excellent NIR persistent luminescence (PersL) property. This unique property can further promote the application of NPLNPs in deep tissue applications and will further promote the application of NIR PLNPs in the biomedical field.

KEYWORDS: near-infrared, persistent luminescence, nanoparticle, tumor, photodynamic therapy

1. INTRODUCTION
Photodynamic therapy (PDT) is considered a promising treatment method in the field of anticancer therapy owing to its minimal invasiveness, repeatability without cumulative toxicity, and specific spatiotemporal selectivity. PDT depends on the intracellular singlet oxygen ($^{1}$O$_2$) induced by the photochemical reactions of photosensitizers (PSs). However, most clinically available PSs for PDT are activated primarily by using short-wavelength ultraviolet (UV) or visible (vis) light, which leads to limited penetration depth in tissues and low therapeutic efficacy for deep lesions. Recently, upconversion nanoparticles (UCNPs) have been utilized as light sources to excite PSs for PDT, which greatly increase the penetration depth of PDT. Although UCNPs' excited light at 808 nm exhibit less overheating than those excited by light at 980 nm, continuous excitation with a high-power laser in the process of PDT still cannot completely eliminate overheating and tissue damage. Therefore, a new PDT strategy that overcomes these drawbacks is urgently needed.

Recently, NIR persistent luminescence nanoparticles (NPLNPs) may open new doors for a new generation of PDT. NPLNPs are emerging fluorescent probes that have great advantages in high-sensitivity biosensing and bioimaging. Among the various NPLNPs, Cr$^{3+}$-doped gallate-based NPLNPs (ZnGa$_2$O$_4$:Cr$^{3+}$ and Zn$_3$Ga$_2$Ge$_2$O$_{10}$:Cr$^{3+}$) have received extensive attention from researchers in bioimaging due to their excellent NIR persistent luminescence (PersL) property. The PersL of NPLNPs can last for hours or even days after the irradiation is stopped. This unique property can further promote the application of NPLNPs in deep tissue applications.
optical property provides a new possibility for tackling the drawbacks of UCNPs in PDT. Recently, Cr3+/Pr3+-doped gallate-based NPLNPs, Zn2.78Ga1.68GeO8:Cr3+, Pr3+, were synthesized and used in PDT.24 In this process, the NPLNPs were pre-excited by UV light in vitro followed by PersL excitation of the PSs, which caused the generation of O2 radicals for continuous PDT. However, with the increase in PDT time, the PersL of NPLNPs continuously decay, resulting in the continuous decrease in PDT efficiency, which makes it difficult to achieve longer durations and higher efficiencies for PDT in vivo.

To overcome the drawback of decreased PDT efficiency caused by PersL decay of NPLNPs, a method of in situ re-excitation was proposed using a white LED, which achieved efficient and repeatedly excite ZGS to produce NIR PersL, with small particle sizes and excellent NIR PersL are synthesized via a simple acetylacetonate combustion method in a short time and at a low temperature. Importantly, compared with traditional ZnGa2O4:Cr3+ (ZGO), the response efficiency to the light of biological windows, which severely restricts their further application in deep tissue reactivated PDT in vivo. Therefore, there is a need to develop new NPLNPs with a high response efficiency to the light in biological windows for deep tissue reactivated PDT.

Herein, novel NPLNPs, Zn1.3Ga1.4Sn0.3O4:Cr3+ (ZGS), with small particle sizes and excellent NIR PersL are synthesized via a simple acetylacetonate combustion method in a short time and at a low temperature. Importantly, compared with traditional ZnGa2O4:Cr3+ (ZGO), the response efficiency to the light of biological windows (659 nm) of prepared ZGS showed a considerable improvement over the doping of Sn4+ into the ZnGa2O4:Cr3+. Based on prepared ZGS, we further proposed a strategy for reactivated PDT in deep tissue using 659 nm light as the excitation source (Scheme 1). The PDT nanoplatfom was constructed by modifying ZnPcS4 as a PS onto the surface of the ZGS. The 659 nm light can efficiently and repeatedly excite ZGS to produce NIR PersL, which served as the light source for PDT to overcome the drawback of decreased PDT efficiency induced by PersL decay in deep tissue. This novel PDT nanoplatfom provides a safe and effective method for future disease therapy in deep tissue.

2. METHODS

2.1. Materials. Dimethylformamide (DMF), NaOH, ethanol, and DMSO were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Ga(acac)3, Zn(acac)2, l3-diphenylisobenzofuran (DPBF), and (3-aminopropyl) triethoxysilane (APTES) were purchased from Aladdin (Shanghai, China). Sn(CH3COO)2, Cr(acac)3, Zn(acac)2, xH2O, and methyl thiazolyl tetrazolium (MTT) were purchased from Sigma (Saint Louis, MO, USA). ZnPcS4 (C32H16N8O12S4Zn) was purchased from J&K Scientific Ltd. (Beijing, China). Fetal bovine serum (FBS) and the RPMI 1640 medium were purchased from HyClone (Logan, UT, USA). Normal liver cells (L02) and human lung carcinoma cells (A549) were purchased from the Shanghai Institutes for Biological Sciences (SIBS, China). H22 tumor-bearing mice and normal Kunming mice were purchased from the Shanghai Slac Laboratory Animal Co., Ltd. (Shanghai, China). Animal experiments were conducted in accordance with the guidelines of the Animal Care and Use Committee of the Institute of Urban Environment, Chinese Academy of Sciences.

2.2. Synthesis of ZGS. The ZGS was synthesized using simple acetylacetonate combustion at a low temperature in air for a short time. In brief, the Ga(acac)3, Zn(acac)2, xH2O, Sn(CH3COO)2, and Cr(acac)3 powders were mixed and ground into homogeneous powders in an agate mortar with the following chemical formula: ZnGa2O4:Cr0.005 (ZGO), Zn1.3Ga1.4Sn0.3O4:Cr0.005 (ZGS). Then, the mixture was calcined at 800 °C for 3 h in air. After the mixture cooled, the final samples were obtained by wet grinding in an agate mortar for 30 min.

2.3. Surface Modifications of ZGS. The ZGS was modified following a previously described method.20,27 In brief, ZGS was dispersed in NaOH solution (5 mM) by sonication for 30 min and vigorously stirred for 24 h at room temperature. Then, the hydroxylated ZGS was obtained by washing and centrifugation. Thereafter, hydroxylated ZGS was dispersed in DMF (20 mL) followed by addition of the APTES (80 μL) under stirring. The mixture was continuously stirred at room temperature for 24 h. The aminated ZGS (ZGS–NH2) was obtained via washing and centrifugation. To conjugate ZnPcS4, 200 μg/mL ZnPcS4 was mixed with 1 mg/mL ZGS–NH2 at room temperature and stirred for 48 h. Finally, the ZGS–ZnPcS4 precipitate was separated by washing and centrifugation. The ZnPcS4 content was determined at 689 nm with a UV–vis spectrophotometer.

2.4. Characterization. The morphology and EDS elemental mapping of the ZGS were performed by using TEM (Talos F200S). The X-ray powder diffraction (XRD) of the ZGS was collected on an X-Ray diffractometer (PANalytical, Netherlands). The photoluminescence (PL), PersL decay curves, and PersL spectra were performed on an FLS920 spectrometer (Edinburgh, UK). The thermoluminescence (TL) curves of ZGS and ZGO were acquired on an OptistatDN-V2 TL instrument (Oxford Instruments, UK) with a heating rate of 10 °C min⁻¹. The ZGO and ZGS were precalcined for
2 h at 500 °C to empty the electrons in the trap and then pre-excited by using a 659 nm LED (30 W) for 5 min. The absorption spectra were measured using a UV–vis spectrophotometer (Thermo Scientific, USA). Fourier transform infrared spectra (FT-IR) were collected on a Nicolet iS10 FT-IR spectrometer (Thermo Scientific, USA). PersL imaging was obtained using an in vivo imaging system (PerkinElmer, USA).

2.5. In Situ Excited PersL Imaging. The ZGO and ZGS were dispersed in PBS solutions (1 mg/mL). Then, 50 μL of the solutions were subcutaneously injected into the abdomen of the mouse. A 659 nm LED (30 W) was used as the excitation source for in situ excited imaging. The PersL signals were obtained using an in vivo imaging system and the exposure time is 60 s. To study the depth of in situ excited imaging, 50 μg of ZGS was covered by pork tissue of different thicknesses. The 659 nm LED was used as the excitation sources for in situ excited imaging. PersL signals were obtained using an in vivo imaging system and the exposure time is 60 s.

2.6. Extracellular and Intracellular Singlet Oxygen (1O2) Detection. Extracellular 1O2 generation of ZGS–ZnPcS4 was measured with a DPBF probe. The ZGS–ZnPcS4 solution (1 mg/mL) was pre-excited using the 659 nm LED for 5 min. Then, the pre-excited ZGS–ZnPcS4 was added to the DPBF solution (30 μM) in the dark. The fluorescence spectra of DPBF were recorded every 5 min under 410 nm excitation. The generation of 1O2 was proved by the decrease in fluorescence in the DPBF at 483 nm.

Intracellular 1O2 generation was measured with a DCFH-DA probe. Briefly, the A549 cells were seeded in culture dishes and cultured in a CO2 incubator for 12 h. Then, the cells were exposed to the medium containing pre-excited (using the 659 nm LED for 5 min) ZGS (200 μg/mL), ZnPcS4 (6 μg/mL), and ZGS–ZnPcS4 (200 μg/mL) for 6 h. Thereafter, the cells were washed with PBS three times and incubated with DCFH-DA (10 μM) for 30 min at a CO2 incubator. Finally, the cell fluorescence signals were acquired on a Zeiss laser scanning confocal microscope.

2.7. Cytotoxicity Assay. Methyl thiazolyl tetrazolium (MTT) assay was performed to study the cytotoxicity of the ZGS. Briefly, the A549 and L02 cells were seeded onto 96-well plates. After 12 h, the cells were exposed to the medium containing various concentrations of ZGS. The cells were then cultured in a 5% CO2 incubator for 24 h. To determine cytotoxicity, MTT was added to each well and incubated in a CO2 incubator for 4 h. DMSO was then added to each well, and the cells were incubated on a plate shaker for 10–20 min. The absorbance of cells was measured using a microplate reader at 570 nm.

For the in vitro PDT, the A549 cells were seeded onto 96-well plates. After 12 h, the cells were exposed to three fresh mediums containing ZGS, ZnPcS4, and ZGS–ZnPcS4. The concentrations of ZnPcS4 (6 μg/mL) and ZGS (200 μg/mL) were set at the same level as those used in the ZGS–ZnPcS4 (200 μg/mL). Thereafter, the 96-well plates were covered by pork tissue of different thicknesses and irradiated with the 659 nm LED for 10 min. The cells were further incubated in a 5% CO2 incubator for 24 h. Finally, cell viabilities were measured by MTT assay.

2.8. In Vivo PDT Treatment. The mice were randomly divided into five groups (n = 5): (1) control group, (2) ZGS group, (3) ZnPcS4 group, (4) ZGS–ZnPcS4 group, and (5) ZGS–ZnPcS4 + 3 cm pork tissue group. For tumor PDT, the mice were intratumorally injected with 30 μL of PBS, ZGS (2 mg/mL), ZnPcS4 (60 μg/mL), and ZGS–ZnPcS4 (2 mg/mL) at day 0 and day 5. After 1 h, the tumors were irradiated using the 659 nm LED for 10 min. The mouse body weights were monitored every 2 days after PDT treatment. After 10 days, the tumors were collected and weighed. Finally, the tumors and the major organs were sectioned and then performed to H&E staining for histological analysis.

3. RESULTS AND DISCUSSION

3.1. Synthesis and Characterization of ZGS. TEM images show that the ZGS nanoparticles have diameters ranging from 10 to 20 nm (Figure 1A,B). The dynamic light scattering (DLS) result shows that the average hydrodynamic diameter of the ZGS is about 38 nm (Figure S1). The HR-TEM image of the ZGS shows that the lattice fringe spacing is 0.292 nm, which corresponds to the spacing of the (200) lattice planes (Figure 1C). The energy-dispersive X-ray spectrum (EDS) result confirms the presence of Zn, Ga, Sn, O, and Cr in the ZGS (Figure 1D). To confirm the elemental distribution, elemental mapping was performed. The results...
show that the Zn, Ga, Sn, O, and Cr elements are evenly distributed throughout the ZGS (Figure 1E–I). The XRD result shows that all peaks of ZGS are well matched with those of the cubic structure ZnGa$_2$O$_4$ phase (JCPDS 086-0413), and no peaks from other phases appear (Figure 1K), which indicates that Sn$^{4+}$ ions are successfully doped into the ZnGa$_2$O$_4$ host.

3.2. Optical Properties of ZGS. The optical properties of NPs are very important for biomedical applications. As shown in Figure 2A, the photoluminescence (PL) excitation spectrum of ZGS monitored at 696 nm has four bands covering a wide spectral range of 200–680 nm with peaks located at 260, 310, 422, and 565 nm, which are ascribed to $^4A_2 \rightarrow ^4T_1$ (te$^2$), $^4A_2 \rightarrow ^4T_1$ (t$^2$e) and $^4A_2 \rightarrow ^4T_2$ transition of Cr$^{3+}$, respectively. The ZGS shows an intense NIR PL emission peaking at 696 nm under 254 nm excitation. This NIR emission is ascribed to the $^2E \rightarrow ^4A_2$ transition of Cr$^{3+}$ in an octahedral crystal field. In order to understand the efficiency of PersL generated by different wavelengths of light, the relationship between the excitation light and the PersL intensity was studied. As shown in Figure 2B, the NIR PersL of ZGS can be achieved using excitation wavelengths of 200–680 nm, which indicates that the ZGS possesses a very wide excitation band. Importantly, we found a region of 650–680 nm in the PersL excitation spectrum of ZGS, which is located in the biological window (650–950 nm). To further study the excitation effect of the light in this region on NIR PersL of ZGS, a 659 nm LED was used to excite ZGS. As shown in Figure 2C, after being excited by a 659 nm LED, the ZGS exhibits a strong PersL emission peak at 696 nm, and its profile is the same as the PL emission spectrum, suggesting the NIR PersL of ZGS originates from the Cr$^{3+}$. Figure 2D shows the NIR PersL decay curve of ZGS monitored at 696 nm after being excited by a 659 nm LED. The NIR PersL of ZGS exhibits a slow decay, and the intensity of NIR PersL is still higher than the background signal after 3 h. Even after 24 h, an obvious NIR PersL from ZGS can still be detected (Figure S2). These results indicate that the ZGS possesses an excellent NIR PersL property after excitation by a 659 nm LED.

Compared with traditional ZnGa$_2$O$_4$:Cr$^{3+}$ (ZGO), the ZGS shows a better NIR PersL property owing to the doping of Sn$^{4+}$. Figure 3A is the PersL decay curve of ZGS and ZGO recorded at 696 nm after being excited by a 659 nm LED for 5 min. The NIR PersL of ZGS can be excited repeatedly by a 659 nm LED, and the NIR PersL intensity of ZGS is much higher than that of ZGO. According to the result of Figure 3A, we calculated the ratio of the PersL intensity of ZGS and ZGO. As shown in Figure 3B, the PersL intensity of ZGS is 55–15 times higher than that of ZGO, which suggests that the ZGS has a higher response efficiency in the biological window than traditional ZGO, permitting ZGS for diagnosis and treatment of diseases in deep tissue.

To further investigate the mechanism, the thermoluminescence (TL) of ZGO and ZGS was measured after being excited by a 659 nm LED. The TL curves of ZGS possess an obvious broad band at room temperature compared with those of ZGO (Figure 3C), which indicate that the Sn$^{4+}$ ion doping can create numerous traps in the original host. In addition, Sn$^{4+}$ doping can cause band gap narrowing and absorption redshift, which are also a factor that leads to an improvement of response efficiency. The possible mechanism is shown in Figure 3D, the ground-state
electrons of Cr\(^{3+}\) are promoted to the \(^{4}T_{2}\) levels under the excitation of the 659 nm LED, and the traps created by Sn\(^{4+}\) doping are filled via the tunneling process from the energy-matched Cr\(^{3+}\) energy levels. After the excitation is stopped, the reverse tunneling recombination produces strong NIR PersL.

3.3. Deep Tissue Re-Excitation of ZGS. In comparison with UV and white light, the light of the biological window exhibits low tissue injury and high tissue penetration, which is suitable for deep tissue re-excitation. To study the in vivo re-excitation potential of ZGS, the ZGO and ZGS were injected subcutaneously into the normal mice. As shown in Figure 4A, the ZGO and ZGS located in subcutaneous tissue can be excited repeatedly using a 659 nm LED. The NIR PersL signal of ZGS is much higher than that of ZGO, indicating that the ZGS has better re-excitation ability in vivo. We then examined the ability of the 659 nm LED to excite ZGS by covering the pork tissue with different thicknesses. As shown in Figure 4B, the NIR PersL of ZGS can be activated by a 659 nm LED under different thicknesses of pork tissue. The ZGS still shows a strong NIR PersL even when the thickness of pork tissue reached 3 cm. We further compared the excitation of 659 nm LED on ZGO and ZGS by covering the pork tissue with different thicknesses. As shown in Figure 4C, the NIR PersL signal of ZGS is much higher than that of ZGO through different thicknesses of tissue, indicating that the ZGS has better deep tissue re-excitation ability in vivo. This deep tissue re-excitation ability of ZGS is far superior to the previously reported Cr\(^{3+}\)-doped gallate-based NPLNPs, suggesting potential applications in bioimaging and PDT in deep tissue.

3.4. Cytotoxicity and Surface Functionalization of ZGS. Cytotoxicity testing of the ZGS is essential for its in vitro and in vivo application. Thus, we first evaluated the cytotoxicity of ZGS at different concentrations using an MTT assay. As shown in Figure S3, the cell viabilities of A549 and LO2 cells were very similar after 24 h of incubation with ZGS of different concentrations, which suggests a low cytotoxicity of ZGS. To construct the PDT nanoplatform based on ZGS, the photosensitizer ZnPcS4 was modified onto the ZGS surface. First, hydroxyl groups were formed on the ZGS surfaces after erosion by NaOH. Subsequently, the APTES was modified on the ZGS surface via a condensation reaction. Finally, ZnPcS4 was modified onto the surface of ZGS via an electrostatic interaction. The actual loading concentration of ZnPcS4 on the ZGS was determined to be 3% by weight using UV−vis spectroscopy. As shown in Figure S4, the NIR PersL spectrum of ZGS and the absorption spectrum of ZnPcS4 have a good overlap, which provides a probability to generate \(^1\)O\(_2\) with PersL of ZGS as the excitation light source. The surface modified of ZGS was confirmed by FT-IR. The FT-IR absorption bands at 1050, 2920, and 2850 cm\(^{-1}\) originating from Si–O–Si and –CH\(_2\) stretching vibrations, respectively (Figure S5), which confirms the successful functionalization of the APTES. The appearance of the absorption bands at 1030, 1110, 1200 (sulfo group –SO\(_2\)– stretching vibration), and 1631 cm\(^{-1}\) (C≡N stretching vibration) confirms the successful modification of ZnPcS4 to the ZGS surface. In addition, a significant reduction of PersL intensity and the slight increase in a hydrodynamic diameter indicate the successful modification of ZnPcS4 (Figures S6 and S7).

Figure 3. (A) PersL decay curve of ZGS and ZGO recorded at 696 nm after being excited by the 659 nm LED for 5 min. (B) Ratio of the PersL intensity of ZGS and ZGO at 696 nm after being excited by 659 nm LED for 5 min. (C) TL curves of ZGS and ZGO recording at 696 nm in temperatures ranging from 0 to 200 °C. The ZGS and ZGO were pre-excited with the 659 nm LED for 5 min. (D) Schematic representation of the NIR PersL mechanism of ZGS.
3.5. In Vitro PDT of ZGS. The $^{1}\text{O}_2$ generation of the ZGS−ZnPcS$_4$ was examined via a DPBF fluorescence probe. The fluorescence of DPBF can be quenched by $^{1}\text{O}_2$; the quenched fluorescence of DPBF is a good indicator of $^{1}\text{O}_2$.
generation. As shown in Figure 5A, the pre-excitation of ZGS and free ZnPcS₄ using a 659 nm LED did not lead to significant fluorescence quenching of DPBF. In contrast, ZGS−ZnPcS₄ pre-excited by a 659 nm LED lead to a persistent fluorescence quenching of DPBF. Moreover, through tissues of different thicknesses, the ZGS−ZnPcS₄ pre-excited by 659 nm LED can still lead to fluorescence quenching of DPBF (Figure S8). These results indicate that the PersL of ZGS induced by the 659 nm LED can continuously excite ZnPcS₄ to produce 1O₂. Intracellular 1O₂ generation was also measured with a DCFH-DA probe in A549 cells. As shown in Figure 5B, after exposure of A549 to pre-excited ZGS−ZnPcS₄, the DCFH-DA fluorescence significantly increased compared to the control, ZGS, and ZnPcS₄ groups, which confirmed that the PersL of ZGS induced by the 659 nm LED can continuously excite ZnPcS₄ to produce ¹O₂ for in vitro PDT.

3.6. In Vivo PDT of ZGS. Based on the above results of in vitro PDT, animal experiments were performed to further evaluate the tumor PDT effect of our nanoplatorm. H22 tumor-bearing mice and normal mice were randomly assigned into five groups: (1) control group, (2) ZGS group, (3) ZnPcS₄ group, (4) ZGS−ZnPcS₄ group, and (5) ZGS−ZnPcS₄ + 3 cm pork tissue group. For in vivo PDT, the mice were intratumorally injected with PBS, ZGS, ZnPcS₄, and ZGS−ZnPcS₄ at 0 and 5 days, respectively. After injection, the tumors were irradiated by 659 nm LED for 5 min. The tumors were collected and weighed after 10 days. As shown in Figure 6A,B, the (2) ZGS group has no obvious inhibition effect on the tumor. The (3) ZnPcS₄ group caused a limited inhibition effect on the tumor. In contrast, the (4) ZGS−ZnPcS₄ group shows a significant inhibition effect on the tumor. It is worth noting that the ZGS−ZnPcS₄ still shows an obvious tumor inhibition effect even through 3 cm of tissue, which is better than previously reported Cr³⁺-doped gallate-based NPLNPs. Furthermore, the results of H&E staining...
show that the tumor cells of control, ZGS, and ZnPcS₄ groups remain essentially in their normal morphology, while some tumor cells of ZGS–ZnPcS₄ and ZGS–ZnPcS₄ + 3 cm pork tissue groups are destroyed (Figure 6C). These results agreed with the above tumor inhibition data. The above results proved that the PDT nanoplatform based on ZGS can be repeatedly and effectively excited by the 659 nm LED for deep tumor PDT.

To evaluate the biological safety of the PDT nanoplatform and excitation source, we monitored the mouse body weights. As shown in Figure S5, the mouse body weights show no significant difference during the experiment, suggesting a low toxicity of the nanoplatform and excitation source. In addition, the major organs were collected for histology analysis. We found no significant damages or inflammatory lesions in any of the major organs (Figure 6D), which further confirm that the nanoplatform and excitation source has no noticeable toxicity in vivo. The above results indicate that the PDT nanoplatform based on ZGS is an effective and safe method for deep tumor therapy.

4. CONCLUSIONS

In summary, we synthesized a novel NPLNPs with a small particle size and excellent optical properties via a simple acetylacetonate combustion method in a short time and at a low temperature. We greatly improved the response efficiency of ZGS to the light of the biological window by doping Sn⁴⁺ into ZnGa₂O₄. Results showed that the response efficiency of ZGS to the light of the biological window (659 nm) is 55 times higher than that of ZGO. More importantly, the ZGS can be repeatedly excited by the 659 nm LED to produce a strong NIR PersL. Based on the optical properties of ZGS, we developed a new PDT nanoplatform by modifying specific photosensitizer molecules on its surface. The 659 nm light with high tissue penetration can repeatedly and efficiently excite the nanoplatform to generate strong NIR PersL, which can further excite the photosensitizer to generate ¹O₂ for high-efficiency and long-term PDT in deep tissue. In vitro tumor cell PDT experiments showed that the nanoplatform we constructed has a good potential for deep tumor cell killing. We further achieved continuous tumor PDT in vivo at a depth of 3 cm using repeated excitation by a 659 nm LED. This novel NPLNP largely solves the problem of the low re-excitation efficiency after NIR PersL decay of traditional PLNPs in deep tissue applications and opens a new avenue for further application of NIR PLNPs in the biomedical field.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsabm.0c00644.

Hydrodynamic diameter, NIR PersL images of ZGS, cell viabilities, NIR PersL spectrum of ZGS and absorbance spectrum of ZnPcS₄, FT-IR spectra, NIR PersL emission spectra of ZGS and ZGS–ZnPcS₄, quenching of DPBF fluorescence, and body weight of mice (PDF)

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Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (61705228, 21507129), the Natural Science Foundation of Fujian Province, China (2019 J05159, 2018 J05028), the Project of Major Science Technology Innovation Platform of Xiamen (no. 3502ZCQ20171002), and the Austrian Science Foundation (P28854, I3792, DK-MCD W1226 to TM).

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