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Characterizations and Photothermal Properties of Narrow Bandgap Conjugated Polymer Nanoparticles

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Abstract Photothermal therapy (PTT) is a minimally invasive treatment that kills cancer cells by converting photon energy into heat. The past few decades have witnessed the booming development of photothermal materials, mainly focusing on precious metal nanomaterials and carbon nanomaterials, such as nanogold and silver and nanocarbon materials for near-infrared (NIR) light-triggered PTT. As precious metals are expensive and potentially harmful to humans, exploration and development of a new type of photothermal materials has become a research hotspot in this field. Herein, we report narrow bandgap conjugated polymer nanoparticles (PDPP NPs) based on pyrrolo[3,4-*c*]pyrrole-1,4-dione (DPP) with intense NIR absorption at 900 nm, as well as a photothermal energy conversion efficiency of 75%. This polymer nanoparticle is essentially non-toxic, as the cell viability of mouse remained more than 90%, even when the concentration of PDPP NPs was at 0.5 mg·mL⁻¹.

Keywords Conjugated polymer; Nanoparticle; Photothermal property; Biotoxicity

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INTRODUCTION

The photothermal therapy (PTT) method relies on the use of a photothermal material to convert light energy into heat under an external light source (usually near-infrared light).^[1] The heat generated by converting light energy kills cancer cells. Since the transparent window of the biological tissue is located in the NIR region, an ideal photothermal agent should have strong NIR absorption and can effectively transfer the absorbed light energy into the heat.^[2,3] PTT also has a good development prospect for its obvious therapeutic effect, minimally invasive, and little or no side effects.^[4–9] Various nanomaterials with good photothermal properties, such as graphene oxide nanosheets,^[10] carbon nanomaterials,^[11] gold nanostructures,^[12–14] CuS,^[15] and transition metal disulfide, have been widely used in PTT. However, inorganic nanomaterials tend to have poor biodegradability and are difficult to be discharged from the body. Metal nanoparticles are potentially very toxic to human. In view of this, there is an urgent need to search for other suitable photothermal materials that are nontoxic, biocompatible, and highly efficient for use in PPT.

Conjugated polymers with intense absorption in the NIR

spectral region can be dissolved or dispersed in aqueous medium for biological applications and PTT. Pyrrolo[3,4-*c*]pyrrole-1,4-dione (DPP) moiety, which has excellent stability and high molar extinction coefficient, has been proven to be a strong electron withdrawing unit and was used to construct donor-acceptor (D-A) polymers for PTT.^[16–25] Yang *et al.* designed and synthesized three NIR absorption compounds based on thieno[2,3-*b*]indole (TI) and DPP, while only DPP-TI possessed excellent effective photothermal conversion efficiency (15.8% under the induction of 660 nm laser) as nanoparticles in water.^[17] Cai *et al.* synthesized DPP derivatives grafting hyaluronic acid (DTPP-HA) involving complicated synthesis steps.^[18] In this work, in order to further improve the efficiency of photothermal conversion, a narrow band gap conjugated polymer containing DPP moiety was designed and synthesized through Suzuki coupling reaction and its nanoparticles were prepared and characterized.

EXPERIMENTAL

Materials

3,6-Bis-(5-bromothiophen-2-yl)-2,5-bis-(2-hexyldecyl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (**M1**) and 2,5-bis(2-hexyldecyl)-3,6-bis(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)thiophen-2-yl)-2,5-dihydropyrrolo[3,4-*c*]pyrrole-1,4-dione (**M2**), tri(*o*-tolyl)phosphine (P(*o*-tol)₃), tris(dibenzylideneacetone) dipalladium (Pd₂(dba)₃), and polyether F-127 were purchased

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from Energy Chemical. Toluene and K_3PO_4 were purchased from Tianjin Chemical Co., Ltd., China. All of the reagents were of analytical grade and used as received without purification. The resistivity of water used in all experiments was 15 M Ω -cm.

Synthesis of the Polymer

The target polymer PDPP was synthesized by coupling monomers **M1** and **M2** in good yield (Scheme 1). In a typical Suzuki cross-coupling reaction, **M1** (136.1 mg, 0.15 mmol) and **M2** (150.0 mg, 0.15 mmol) were charged into a 50-mL flask. Aliquot 336, $P(o\text{-tol})_3$ (3.65 mg, 0.012 mmol), $Pd_2(dba)_3$ (2.75 mg, 0.003 mmol), toluene (10 mL), and 2 mol-L $^{-1}$ K_3PO_4 (5 mL) were then added. The mixture was first purged with nitrogen for 30 min, and gradually heated to 110 °C for 72 h. The mixture was then cooled to room temperature and the solution was added into methanol (50 mL) for precipitation. The polymer powder was first washed with acetone (24 h) and hexane (24 h) in Soxhlet extractor, and then extracted with chloroform (24 h). After concentrated, the chloroform solution was added dropwise to methanol. Then, the polymer was obtained by filtrated and dried under vacuum at 50 °C for 24 h. The polymer was obtained as black solid (157 mg, 70% yield).

Preparation of the PDPP NPs

PDPP (10 mg) and polyether F-127 (100 mg) were separately dissolved in THF (1 mL). Then, these two solutions (0.1 mL of each) were mixed. The mixture was quickly injected into pure water (10 mL) *via* syringe under ultrasonic, which was left in open air to let THF completely evaporate off. A small amount of precipitate was filtered off through a 0.45 μ m filter to obtain a clear stable solution of the nanoparticles. The filtered solution was then freeze-dried to yield a powdery product, which was stored at a low temperature.

Cell Culture

L929 cells were supplied by Sigma-Aldrich. The cells were cultured in a Roswell Park Memorial Institute (RPMI-1640) containing 10% fetal bovine serum (FBS), 2 mmol-L $^{-1}$ L-glutamine, and 0.086 μ mol-L $^{-1}$ doxorubicin hydrochloride. The cells were harvested *via* trypsin and resuspended in a fresh complete medium before plating. L929 cells were seeded onto a 96-well plate at a density of 4×10^3 cells per well. Cultures were incubated in RPMI-1640 supplemented with 10% FBS in a humidified 5% CO $_2$ /95% air incubator at 37 °C. The medium was replaced with 100 μ L of different concentrations of PDPP NPs for 24 h incubation after cells attachment. After treating these mouse cells with an 808 nm laser irradiation at the power of 1.5 W-cm $^{-2}$ for 5 min, the cell viability was determined quantitatively by CCK-8 analysis. The CCK-8 was added to the

wells and incubated for 2 h to measure the viability of cells.

Characterizations

The UV-Vis-NIR absorption spectra of PDPP film and PDPP NPs solution were recorded on a Shimadzu UV-3600 spectrophotometer. The morphologies and sizes of PDPP NPs were observed with transmittance electron microscopy (TEM, Phenom LE). The molecular weight of PDPP was measured by gel permeation chromatography (GPC) with polystyrene as standards and chloroform as eluent. Photothermal spectra of PDPP NPs under 808-nm irradiation were recorded on a Ti480 thermal imaging camera.

RESULTS AND DISCUSSION

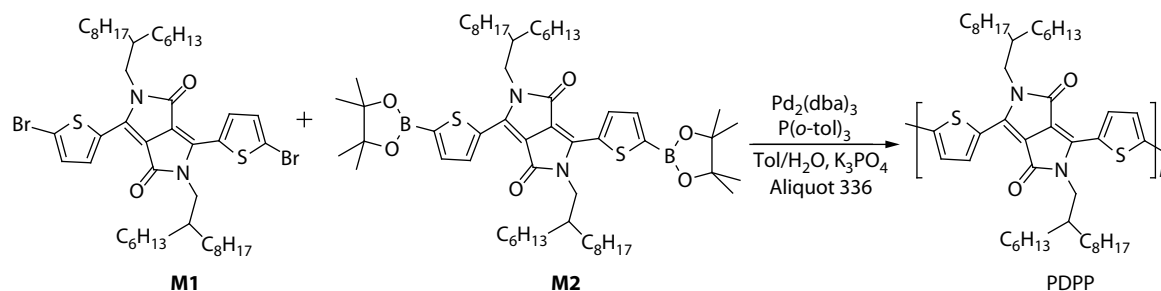
Synthesis and Characterization

The target polymer PDPP was synthesized by the procedure in Scheme 1. The molecular weight of PDPP was measured by gel permeation chromatography (GPC) with polystyrene as standards and chloroform as eluent. The number-average molecular weight (M_n) was 69.6 kg-mol $^{-1}$, while the polydispersity index (PDI) was 1.9. The UV-Vis-NIR absorption spectra of PDPP in chloroform are shown in Fig. 1. The dual-band π - π^* absorption peak was observed in the wavelength range of 360–450 nm. There was a low energy absorption with a maximum absorption at 928 nm and a shoulder at 820 nm, owing to the D-A charge transfer between the donor and acceptor units. The molar extinction coefficient (ϵ) was 26.3 cm $^{-1}$ ·g $^{-1}$ ·L.

The PDPP NPs were prepared through ultrasonic self-assembly method. There were some precipitates in the prepared nanoparticle aqueous dispersion, which became clear after filtration (Fig. 2). The particle size of the PDPP NPs measured by laser particle size analyser was in the range of 50–80 nm. The morphology of the PDPP NPs was observed by transmission electron microscopy (TEM). As shown in Fig. 3, the PDPP NPs appeared to be in a spherical shape, indicating that the nanoparticles were successfully prepared. The UV-Vis-NIR absorption spectra of PDPP NPs in chloroform are shown in Fig. 1. In comparison with the spectrum of PDPP in chloroform, the NPs show similar absorption profile over 650–950 nm but with a blue-shift, mainly due to the light scattering caused by the particles. The maximal absorption peak is at 900 nm, well covering the wavelength of typical 808-nm NIR laser (Fig. 1).

Photothermal Performance of PDPP NPs

The PDPP NPs aqueous dispersions (0.5 mL) with different



Scheme 1 Pd-catalyzed synthesis of PDPP.

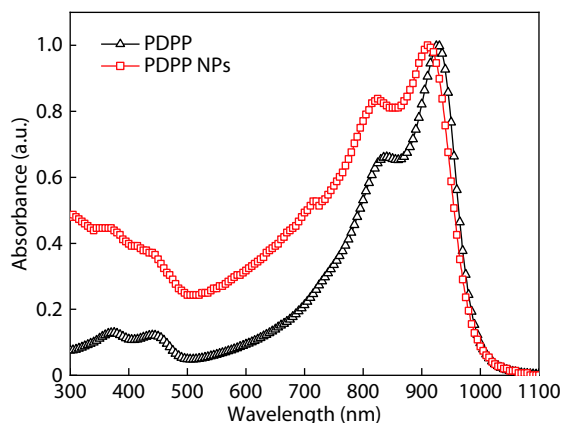


Fig. 1 UV-Vis-NIR absorption spectra of PDPP in chloroform and PDPP NPs in water at a concentration of 0.05 mg·mL⁻¹.

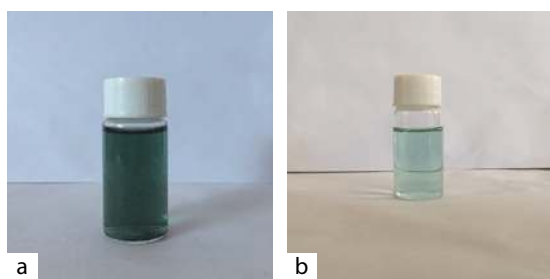


Fig. 2 The prepared solution of PDPP NPs (a) before and (b) after filtration.

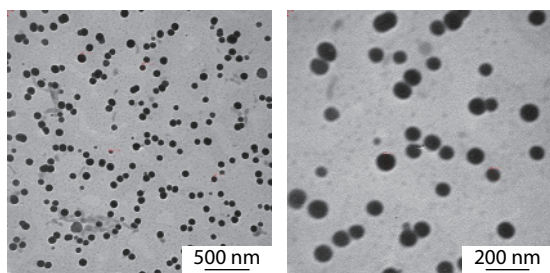


Fig. 3 TEM images of PDPP NPs.

concentrations (0.25, 0.5, and 1 mg·mL⁻¹) were irradiated with an 808 nm laser at the power of 1.5 W·cm⁻² for 5 min, while pure water was used as a control. The variation of the temperature was recorded on a Ti480 thermal imaging system with an accuracy of ± 0.1 °C. It can be seen from Fig. 4(a) that there was a temperature rise of pure water about 5 °C in 5 min. However, the temperature of the PDPP NPs dispersion increased to 50.4 °C, even at a concentration of 0.25 mg·mL⁻¹, which indicated that it had excellent photothermal performance. Furthermore, the photothermal characteristics of the PDPP NPs (0.5 mg·mL⁻¹, 0.5 mL) at different laser power densities (0.5, 1.0, and 1.5 W·cm⁻²) were also studied. The temperature of the PDPP NPs aqueous dispersions increased from 31.0 °C to 56.7 °C with the increase of laser power density respectively (Fig. 4b). This result shows that the increase in temperature of the NPs dispersions was also related to the intensity of the laser power, which further proved the excellent photothermal conversion performance.

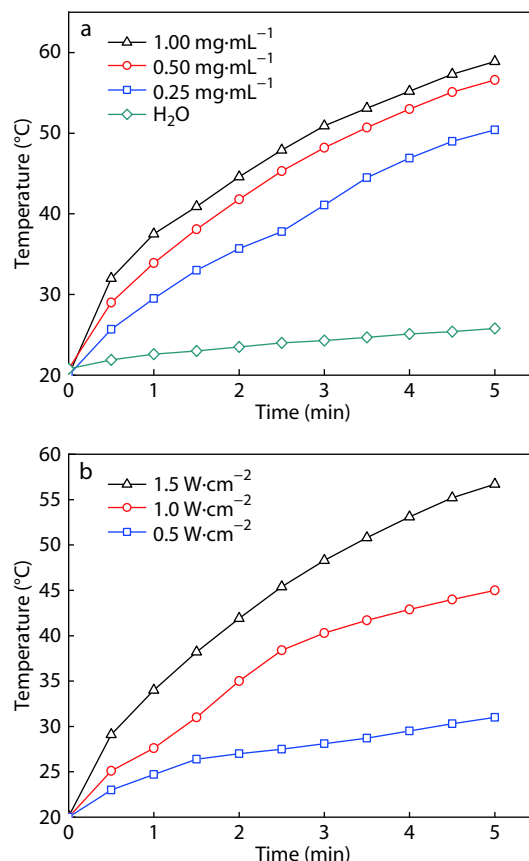


Fig. 4 Photothermal spectra of (a) PDPP NPs dispersions with different concentrations under 808 nm laser irradiation (1.5 W·cm⁻²) for 5 min, (b) PDPP NPs (0.5 mg·mL⁻¹) under 808 nm irradiation with different laser power densities for 5 min.

In order to determine the photothermal conversion capacity of the PDPP NPs, the temperature variation of the aqueous dispersion (0.5 mg·mL⁻¹) was recorded under 808 nm laser irradiation at the power of 0.5 W·cm⁻² (Fig. 5a). The photothermal conversion efficiency (η) was calculated according to the method reported by Roper *et al.*^[25] and the time-temperature curve (Fig. 5). The calculation equation is as follows:

$$\eta = \frac{hA(T_{\text{MAX}} - T_{\text{surr}}) - Q_{\text{dis}}}{I(1 - 10^{-A_{808}})} \quad (1)$$

where h is the coefficient of heat transfer, A is the superficial area of the receptacle, T_{MAX} and T_{surr} represent the maximal temperature (45 °C) (Fig. 5a) and room-temperature (25 °C), I represents laser power density (0.5 W·cm⁻²), and A_{808} represents the absorbance of PDPP NPs at the wavelength of 808 nm. Q_{dis} is the heat generation power after the solvent absorbs light, and it indicates how much heat can be generated per unit time. Generally, Q_{dis} of pure water is 14 mW, and hA is calculated according to the Eq. (2):

$$\tau_s = \frac{m_D C_D}{hA} \quad (2)$$

where m_D is the mass (0.5 g) of pure water while C_D is the heat capacity (4.2 J·g⁻¹). τ_s was calculated according to Eq. (3):

$$t = -\tau_s (\ln \theta) \quad (3)$$

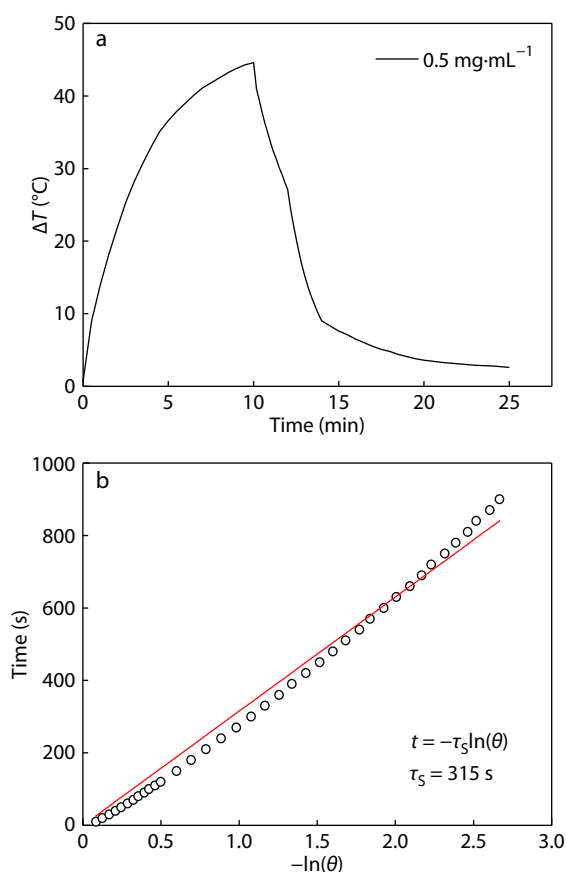


Fig. 5 (a) Photothermal response of PDPP NPs (0.5 mg·mL⁻¹) upon irradiation with 808 nm light (0.5 W·cm⁻²) for 10 min and then allowed to cool naturally. (b) Relationship of temperature and time during the cooling off period in (a).

where θ is the ratio of ΔT to ΔT_{MAX} , and τ_s is calculated from Fig. 5(b).

It was further determined through measurement and calculation that η of the PDPP NPs was about 75%, which was higher than the values of most previously reported PTT reagents such as Au nanorods (21.0%),^[26] Dpa-melanin CNSs (40.0%),^[27] and C16 pBDP@HSA (37.5%).^[28] Such a high photothermal conversion efficiency further illustrated that the PDPP NPs had a good application prospect in NIR PTT.

Stability of the PDPP NPs

To further investigate the storage stability of the nanoparticles, the aqueous dispersion of PDPP NPs (0.5 mg·mL⁻¹) was examined at different storage time. No visible changes were observed after the aqueous dispersion was stored under room-temperature conditions for 30 days (Fig. 6). This result confirmed the good stability of PDPP NPs.

The photostability of the PDPP NPs aqueous dispersion (0.5 mg·mL⁻¹) is illustrated in Fig. 7. The aqueous dispersion was irradiated with an 808 nm laser for 10 min and then cooled to room-temperature naturally. From Fig. 7, it can be seen that the photothermal conversion property of the PDPP NPs had no obvious decrease after five cycles, which indicated that PDPP NPs could withstand repeated long-term exposure and exhibited excellent photostability.

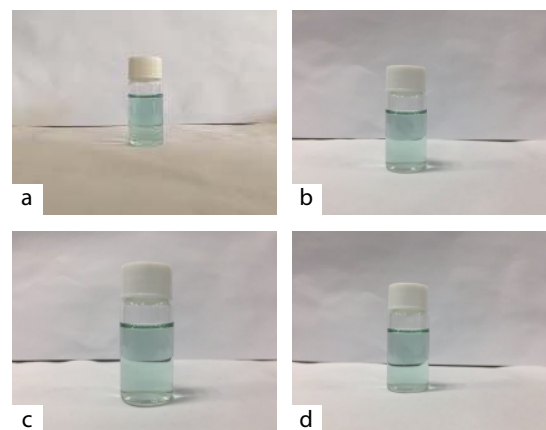


Fig. 6 Photographs of the PDPP NPs (0.5 mg·mL⁻¹) after different storage time of (a) 1 day, (b) 7 days, (c) 14 days, and (d) 30 days.

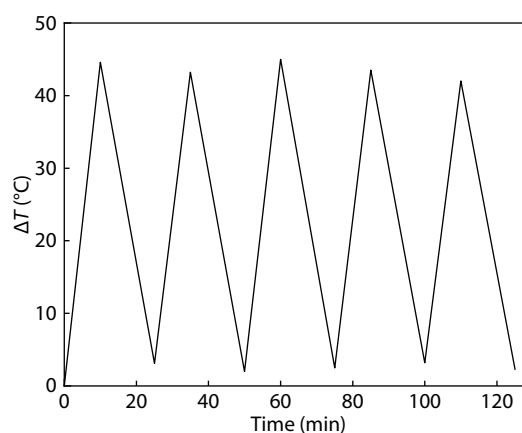


Fig. 7 Temperature variations over 5 irradiation/cooling cycles (PDPP NPs 0.5 mg·mL⁻¹, 808 nm light, 1.5 W).

Detection of Biotoxicity of the PDPP NPs

To further explore the feasibility of the PDPP NPs for PTT, the survival rate of mouse cells in different concentrations of the PDPP NPs was also explored (Fig. 8). CCK-8 analysis was used to quantitatively determine cell viability, after treating these mouse cells with PDPP NPs under an 808 nm laser irradiation

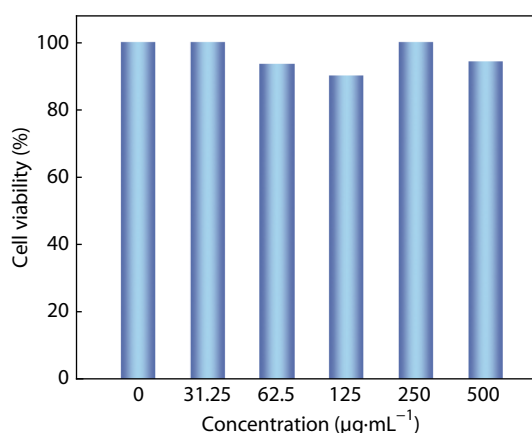


Fig. 8 Cell viability of mouse L929 cells when different concentrations of PDPP NPs were added.

at the power of $1.5 \text{ W}\cdot\text{cm}^{-2}$ for 5 min. Even when the concentration of the PDPP NPs was up to $500 \mu\text{g}\cdot\text{mL}^{-1}$, the cell viability still reached more than 90%. This preliminary result proved that PDPP NPs had good biocompatibility to mouse cells. Meanwhile, it also suggested great development prospects for PDPP NPs as PTT reagents.

CONCLUSIONS

In summary, we synthesized and characterized a new type of photothermal nanomaterials based on DPP conjugated polymer (PDPP). The PDPP NPs had broad UV-Vis-NIR absorption, high photothermal conversion efficiency (up to 75%), good photothermal stability, and no or very low toxicity to mouse cells, making it potentially suitable for use in NIR PTT.

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