

RESEARCH ARTICLE

Rodlike nanomaterials from organic diradicaloid with high photothermal conversion capability for tumor treatment

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Abstract

Organic diradicaloids with unique open-shell structures and properties have been widely used in organic electronics and spintronics. However, their advantageous optical properties have been explored less in the biomedical field. In this work, the photothermal conversion behaviors of a boron-containing organic diradicaloid (BOD) are reported. BOD can assemble with 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol) to form rodlike nanoparticles (BOD NPs). These as-prepared BOD NPs exhibit high photothermal conversion capability and robust photothermal stability. Notably, they possess morphological superiority, which guarantees the effective photothermal therapy of tumors. This work thus demonstrates the promise of organic diradicaloids as efficient photothermal agents for biomedical applications.

KEYWORDS

boron-containing organic diradicaloid, high photothermal conversion efficiency, photothermal therapy, rodlike nanostructures

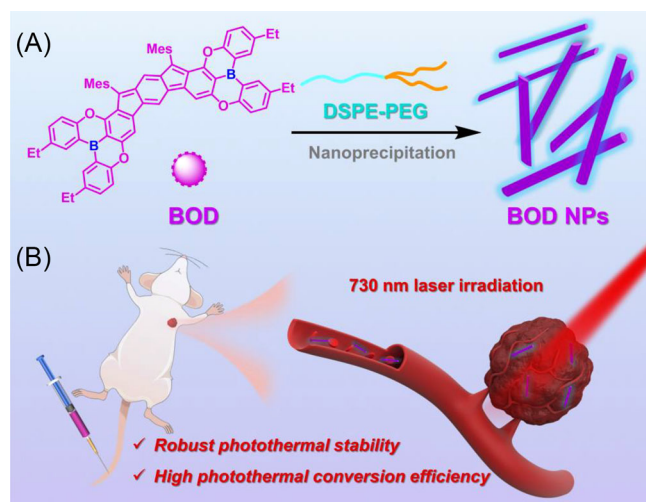
1 | INTRODUCTION

Exploring more efficient treatment modalities for cancer therapies remains a great challenge in the biomedical field. Photothermal therapy (PTT) is a novel therapeutic mode, which is based on the nonradiative decay process of photothermal agents (PTAs) under laser irradiation.^[1–5] Much attention has been paid to PTT because of its attractive features, such as minimal invasiveness, facile manipulation, and high specificity.^[6–10] To boost the therapeutic effect of PTT, researchers are working on the development of various PTAs, including inorganic and organic ones.^[11–16] Compared with inorganic ones, organic PTAs are featured with the merits of good biocompatibility and flexibility for structure design and modulating light absorption, thus becoming ideal alternatives for PTT.^[17–19]

To increase the penetration depth of laser and improve the PTT effect of PTAs, intense absorption in the near-infrared (NIR) region and high photothermal conversion efficiency

(PCE) are desirable. In addition, the morphologies of the assemblies also play a significant role in the therapeutic outcome. Rodlike nanomaterials have aroused great interest in drug delivery because of their preference in cellular uptake and tumor accumulation resulted from the specific aspect ratio.^[20–25] Interestingly, rodlike nanostructures can also be quantitatively tuned after synthesis (e.g., by ultrasound).^[26] However, the reported rodlike nanostructures are mainly based on inorganic materials.^[27–29] Hence, the preparation of PTAs with rodlike morphologies may be the icing on the cake of the unceasing efforts to boost the PCE of organic PTAs.

Diradicaloids refer to the open-shell polycyclic hydrocarbons with fully bonded π -electrons, which usually exhibit diradical-like behaviors, and thus can be defined as diradicaloids. Organic diradicaloids have been extensively studied because of their unique optical, electronic, and magnetic properties and promising applications in organic electronics and spintronics.^[30–38] It is notable that organic diradicaloids generally possess narrow energy bandgap and NIR



SCHEME 1 Schematic illustration of (A) the preparation and (B) photothermal therapy (PTT) application of boron-containing organic diradicaloid-based nanoparticles (BOD NPs).

absorption, which are very desirable for PTAs. However, the biomedical application of diradicaloids has rarely been explored, probably because the diradical nature can decrease their stability at ambient conditions. Very recently, Li et al. reported the utilization of a donor-acceptor type diradicaloid molecule in PTT of tumors, which is the only one report so far about the PTT applications of diradicaloids.^[39] Therefore, the development of stable and efficient diradicaloid-based PTAs may open a new path for PTT.

In our recent work, the borylation of antiaromatic polycyclic hydrocarbons was reported as a design strategy to construct organoborane diradicaloids. A series of boron-containing organic diradicaloids composed of two dioxabridged triphenylborane moieties and one indenofluorene π -skeleton were developed.^[40] These diradicaloids have sufficient Lewis acidity and thus can coordinate with Lewis bases to dynamically modulate (anti)aromaticity and diradical character. It is noteworthy that they show intense NIR absorption and excellent stability toward air and moisture. Therefore, we envisioned that they could be employed as PTA for the application in PTT. In this study, we report the photothermal conversion behaviors of a boron-containing organic diradicaloid (BOD). It has a polycyclic chemical structure and can assemble with 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol) (DSPE-PEG) to form attractive rodlike nanoparticles (BOD NPs) (Scheme 1A). This diradicaloid-based nanostructure possesses high photothermal conversion ability and photothermal stability. It is also important that the effective tumor accumulation of BOD NPs contributes to the in vivo therapeutic effect (Scheme 1B).

2 | RESULTS AND DISCUSSION

2.1 | Preparation and characterizations of BOD NPs

BOD with an open-shell singlet diradical structure was synthesized via a three-step reaction in our previous work (Figure S1).^[40] The excellent ambient stability of BOD is hard-won for open-shell boron-containing polycyclic hydrocarbons.^[36,37,41] BOD can be well dissolved in tetrahy-

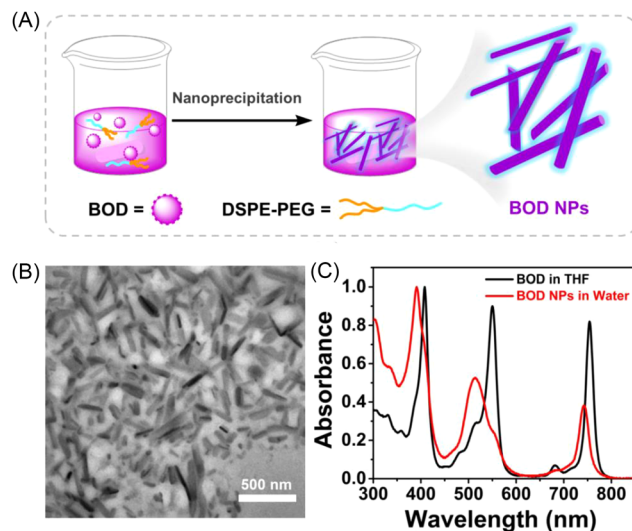


FIGURE 1 Preparation and characterizations of boron-containing organic diradicaloid-based nanoparticles (BOD NPs). (A) Preparation diagram of BOD NPs. (B) transmission electron microscopy (TEM) image of BOD NPs. (C) Normalized absorption spectra of boron-containing organic diradicaloid (BOD) in THF and BOD NPs in water.

drofuran (THF) and assemble with DSPE-PEG via the nanoprecipitation method (Figure 1A). The critical micelle concentration (CMC) of DSPE-PEG was measured to be 0.014 mg mL^{-1} with Nile red as the fluorescent probe (Figure S2). Notably, the assemblies in the transmission electron microscopy (TEM) image (Figure 1B) are rodlike nanoparticles (BOD NPs). Moreover, the electron paramagnetic resonance (EPR) spectrum of BOD NPs (Figure S3) is similar to that of BOD,^[40] verifying the diradical character of organic diradicaloids. To our knowledge, the rodlike nanostructures of organic diradicaloids have never been reported, let alone their biomedical applications. The rodlike morphology of BOD NPs may play a positive role in tumor therapy.

As shown in Figure 1C, compared with the absorption spectrum of BOD in THF, BOD NPs in water show a blue-shifted and broadened absorption spectrum (λ_{abs} at 391/514/742 nm) due to the aggregation of BOD in the assemblies. The loading efficiency and content of BOD in BOD NPs are 87% and 59%, respectively, on the basis of the standard curve of BOD (Figure S4). Both the absorption spectra and the photos of BOD NPs after storage in water for 0, 7, and 14 days (Figure S5) demonstrate their good stability in water. Moreover, there is no fluorescence detected from either BOD in THF or BOD NPs in water, suggesting that the rodlike BOD NPs should have a good performance in photothermal conversion.^[42]

2.2 | Photothermal effect of BOD NPs

The photothermal conversion capability of BOD NPs was studied in detail. First, the temperature changes of different concentrations of BOD NPs under 730 nm laser irradiation (0.6 W cm^{-2}) were recorded with a thermocouple. As shown in Figure 2A,B and Figure S6, the higher the concentration of BOD NPs is, the higher the temperature can be reached after irradiation for 10 min. For a fixed concentration of BOD NPs ($20 \text{ } \mu\text{g mL}^{-1}$), the temperature increase turned out to be laser power density-dependent (Figure 2C and Figure

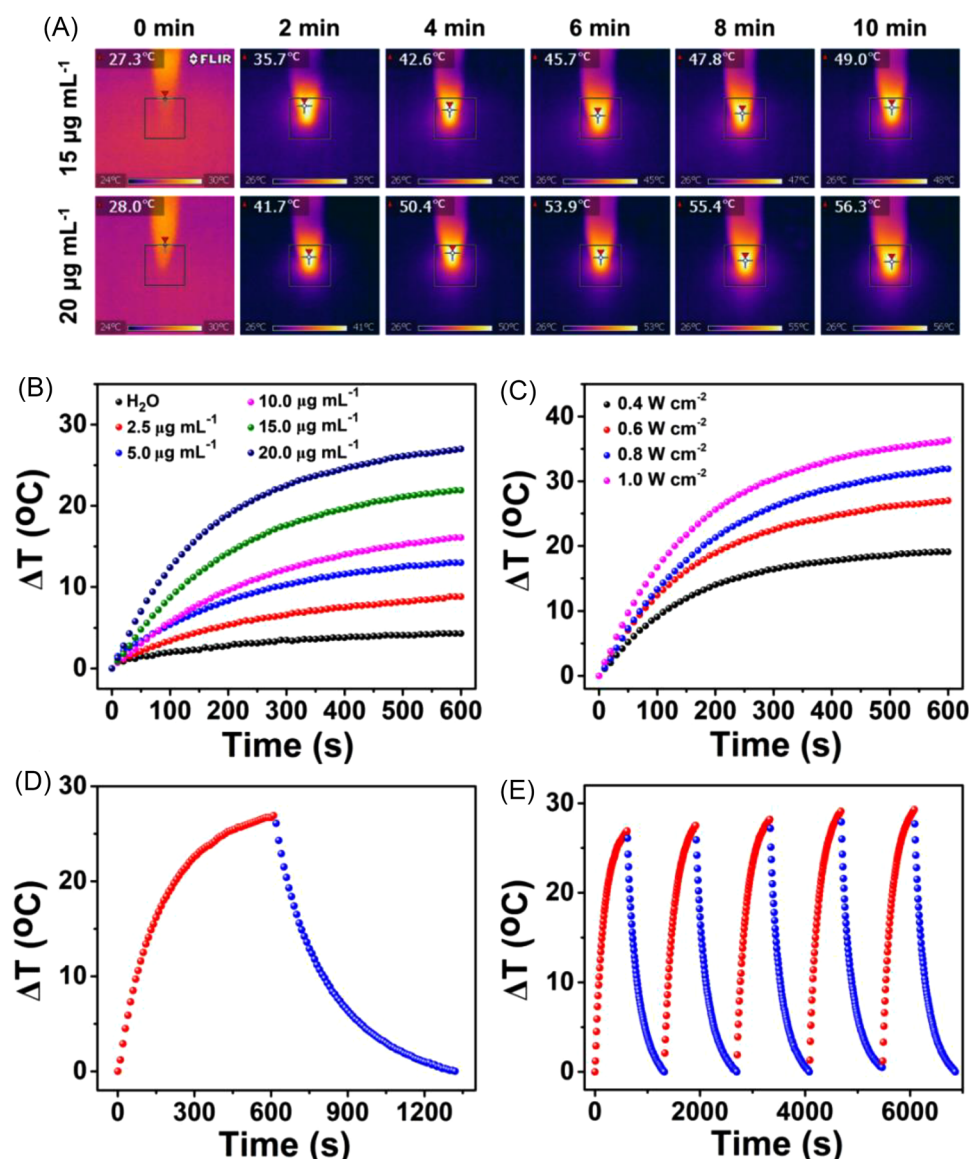


FIGURE 2 Photothermal effect of boron-containing organic diradicaloid-based nanoparticles (BOD NPs). (A) Infrared images of BOD NPs (15 and 20 $\mu\text{g mL}^{-1}$) under laser irradiation. Temperature variations of (B) various concentrations of BOD NPs under laser irradiation and (C) 20 $\mu\text{g mL}^{-1}$ of BOD NPs under laser irradiation of diverse power densities. (D) Temperature changes of BOD NPs (20 $\mu\text{g mL}^{-1}$) under laser irradiation (0.6 W cm^{-2}) for 10 min followed by removal of laser. (E) The heating and cooling curves of BOD NPs upon five cycles of laser on and off.

S7). The PCE of BOD NPs is determined to be 71% via a reported method (Figure 2D and Figure S8),^[43] which is higher than that of most reported organic PTAs, including the reported diradicaloid PTA.^[39,44–47] Furthermore, there is no decrease in the maximum temperature rise of BOD NPs after five repeating cycles of irradiation/cooling (Figure 2E), which indicates the good photothermal stability of BOD NPs. This property was also proved by the unchanged absorption spectra and color of BOD NPs before and after laser irradiation (Figure S9). The high PCE combined with the good photothermal stability and the rodlike structure portends the bright prospect of BOD NPs in PTT of tumors.

2.3 | Cytotoxicity of BOD NPs

The cytotoxicity of BOD NPs toward mouse colon carcinoma (CT26) cells without (BOD NPs) or with (BOD NPs

+ Laser) laser irradiation was studied. In Figure 3A, it is obvious that no cytotoxicity is observed when the CT26 cells are treated with BOD NPs alone. While phototoxicity could be detected from the CT26 cells treated with both BOD NPs and laser irradiation (0.6 W cm^{-2}). Especially for the cells incubated with a high concentration (20 $\mu\text{g mL}^{-1}$) of NPs, the viability decreases to 16% after laser irradiation. To verify this result, human cervical carcinoma (HeLa) cells were also employed, and similar outcomes were obtained (Figure 3B). To make it visible, propidium iodide (PI) and calcein-acetoxymethyl (calcein-AM) were utilized to distinguish dead (red) and live (green) cells. Whether for CT26 cells or for HeLa cells, bright red fluorescence was observed for those treated with BOD NPs followed by irradiation (BOD NPs + Laser, Figure 3C). However, treatment with BOD NPs or laser irradiation alone (BOD NPs or laser) has no influence on the cells, as demonstrated by the green fluorescence in Figure 3C.

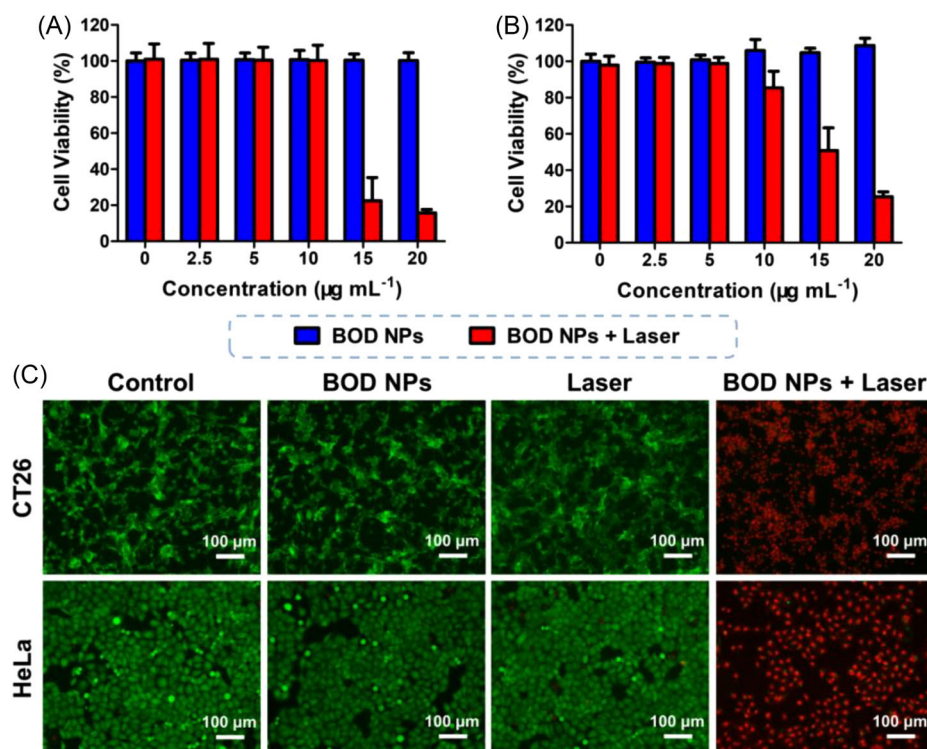


FIGURE 3 In vitro therapeutic effect of boron-containing organic diradicaloid-based nanoparticles (BOD NPs). Cytotoxicity of BOD NPs toward (A) CT26 and (B) HeLa cells with or without laser irradiation. (C) Live/dead staining images of CT26 and HeLa cells subjected to different treatments.

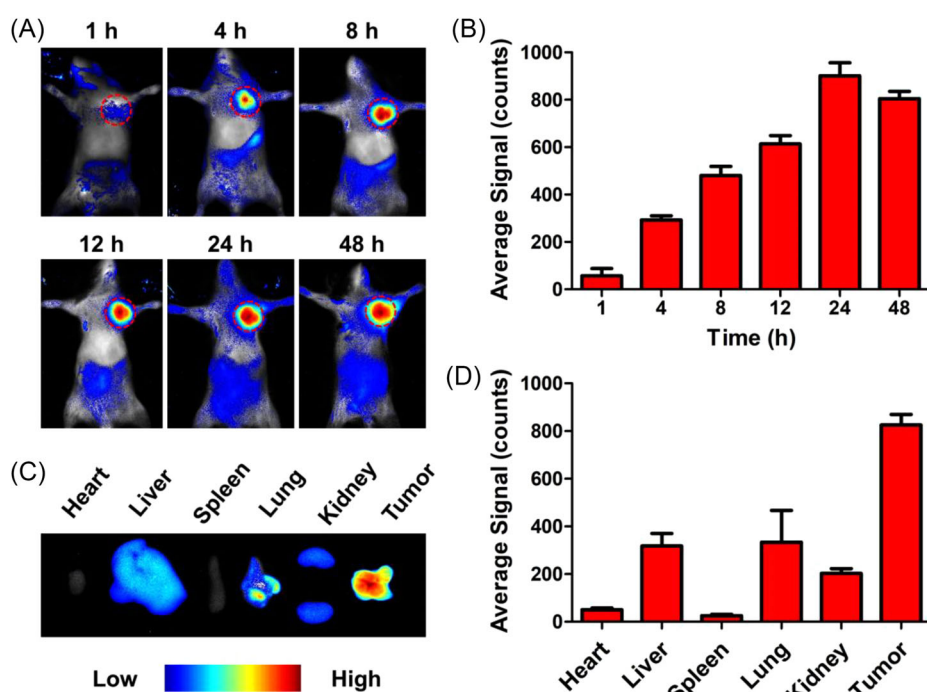


FIGURE 4 Fluorescence imaging of the labeled boron-containing organic diradicaloid-based nanoparticles (BOD NPs). (A) In vivo fluorescence images and (B) signal intensities of the tumor (the red dotted circles) after injection with the labeled BOD NPs for different times. (C) Fluorescence image and (D) signal intensities of the excised tissues and tumor after administration for 48 h. The results are presented as mean $\pm$ SD ($n = 3$).

2.4 | In vivo fluorescence imaging of BOD NPs

To explore the distribution of BOD NPs in tumors, they were labeled with IR780, and in vivo imaging was performed toward CT26 tumor-bearing BALB/c mice. BOD NPs were

administered via intravenous injection, and the fluorescence images of the mice at 1, 4, 8, 12, 24, and 48 h after injection were recorded. As displayed in Figure 4A,B, the labeled BOD NPs gradually accumulate in the tumors, and the fluorescence signals of the tumors reach the maximum 24 h post injection. In reality, a relative high accumulation of BOD

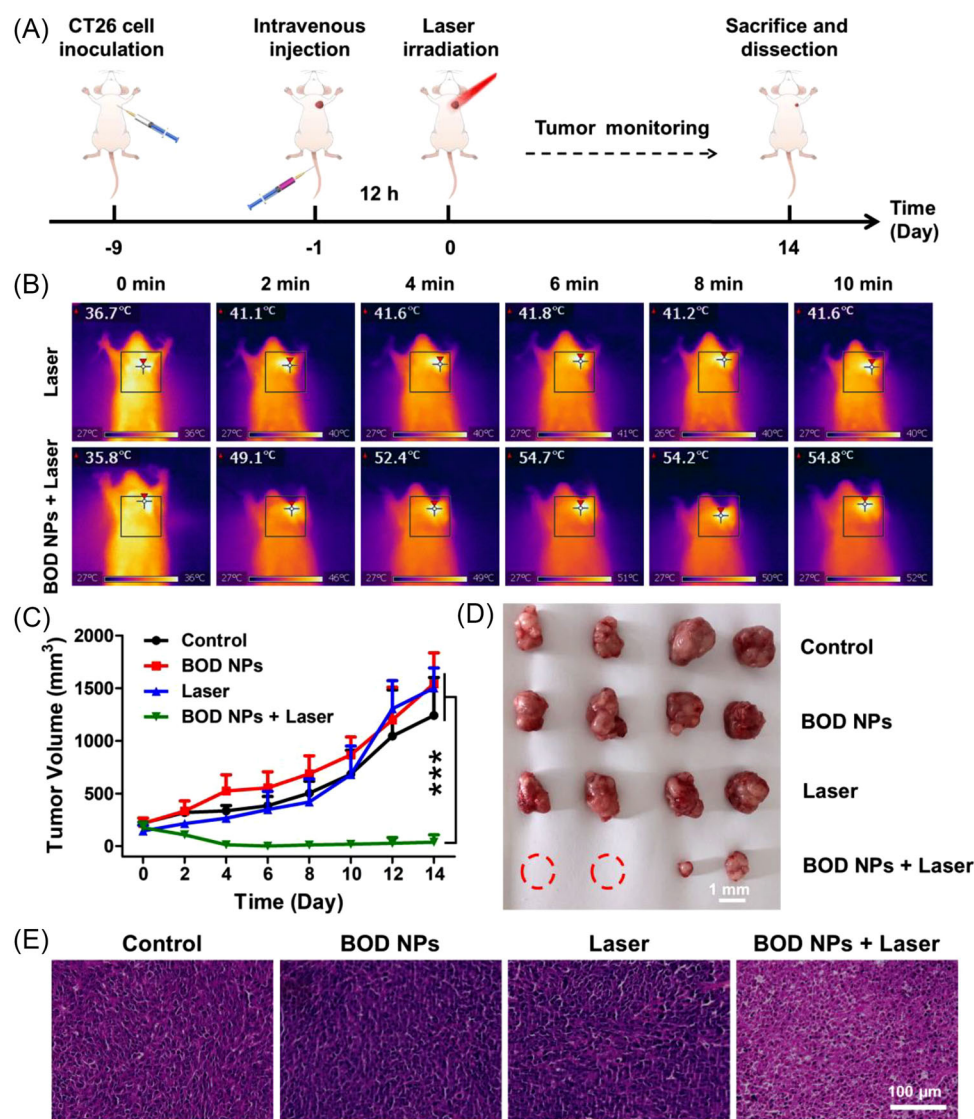


FIGURE 5 Photothermal therapy (PTT) effect of boron-containing organic diradicaloid-based nanoparticles (BOD NPs) in vivo. (A) Schematic presentation of the process of the animal experiment. (B) Infrared images of the irradiated mice with or without treatment with BOD NPs. (C) The changes in tumor volumes of the mice in the four groups (** $p < 0.001$, $n = 4$). (D) A photograph of the excised tumors. The completely ablated tumors are indicated by circles. (E) H&E staining of the tumors after different treatments.

NPs was presented 12 h after injection, thus laser irradiation toward tumors could be initiated. After administration for 48 h, the mice were sacrificed, followed by the excision of the tumors and the main organs. In the ex vivo fluorescence images, the tumors show the highest fluorescence intensity (Figure 4C,D), indicating that BOD NPs can rapidly accumulate in the tumor and keep retention for a long time.

2.5 | In vivo PTT effect of BOD NPs

The PTT effect of BOD NPs toward CT26 tumor-bearing BALB/c mice was further studied. According to different treatments to the mice, they were randomly divided into four groups. The mice without any treatment belonged to the Control group, and those treated with BOD NPs (6 mg kg^{-1}) or laser irradiation (0.6 W cm^{-2} , 10 min) alone, or BOD NPs (6 mg kg^{-1}) followed by laser irradiation (0.6 W cm^{-2}) for 10 min were assigned to the groups of BOD NPs, Laser, and BOD NPs + Laser, respectively. The entire process of the animal experiment is illustrated in Figure 5A. After CT26 cell

inoculation for 8 days, BOD NPs were intravenously injected into the mice in BOD NPs and BOD NPs + Laser groups. Twelve hours after administration, 730-nm laser irradiation was performed on the mice in Laser and BOD NPs + Laser groups. The temperature changes of the tumors during irradiation were recorded, as shown in Figure 5B and Figure S10. A much higher temperature elevation could be observed from the tumors in BOD NPs + Laser group than those in Laser group, which ulteriorly proved the accumulation and the photothermal effect of BOD NPs in tumors. After different treatments, the tumor sizes were measured every 2 days for 14 days. The mice were sacrificed, and the tumors were excised. The decreased tumor volumes (Figure 5C) or even disappeared tumors (Figure 5D) in BOD NPs + Laser group compared with the gradually growing tumors in the other three groups leave no doubt about the significant ablation effect of the rodlike BOD NPs under 730-nm laser irradiation toward tumors. Hematoxylin and eosin (H&E) staining images further confirm that the combination of BOD NPs and 730-nm laser irradiation is highly effective for PTT of tumors (Figure 5E).

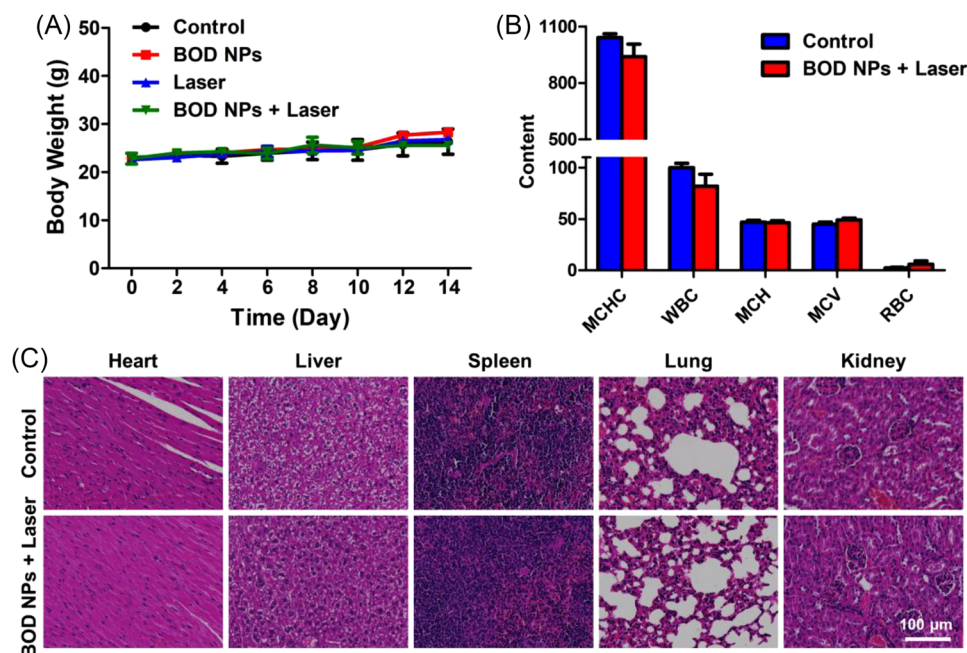


FIGURE 6 Biosafety of boron-containing organic diradicaloid-based nanoparticles (BOD NPs). (A) Changes in bodyweights of the mice. (B) Hematological parameters (MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cell; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; and RBC, red blood cell) and (C) Hematoxylin and eosin (H&E) staining of the main organs in BOD NPs + Laser and Control groups.

2.6 | Biosafety of BOD NPs

After different treatments, the bodyweights of the mice were also recorded. The slight increase of bodyweights in Figure 6A shows that the growth of mice was not affected by the above treatments. As shown in Figure 6B, the mice in BOD NPs + Laser and Control groups showcase negligible differences in all the detected hematological parameters. The H&E staining images of the major organs further validate the long-term biosafety of the treatment with both BOD NPs and 730-nm laser irradiation (Figure 6C).

3 | CONCLUSION

In conclusion, BOD NPs with favorable rodlike nanostructures have been prepared and applied in PTT of tumors. The obtained BOD NPs exhibit a high PCE of 71%, which is higher than that of most of the reported organic PTAs. Moreover, the effective accumulation and long retention of BOD NPs in the tumors lay a solid foundation for tumor therapy. After administration of BOD NPs and laser irradiation, the tumors can be efficiently suppressed or even completely ablated, whereas there was no obvious *in vivo* toxicity detected. This work not only expands the application of organic diradicaloids in the biomedical field, but also provides an effective strategy to develop PTAs with high performance via morphology engineering of the assemblies.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All animal treatment and maintenance protocols were approved by the Animal Ethics Committee of Changchun Institute of Applied Chemistry, Chinese Academy of Sciences (Approval No. 20220005).

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SUPPORTING INFORMATION

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