One-pot solventless preparation of PEGylated black phosphorus nanoparticles for photoacoustic imaging and photothermal therapy of cancer

Caixia Sun a, b, Ling Wen b, Jianfeng Zeng b, Yong Wang b, Qiao Sun b, Lijuan Deng a, Chongjun Zhao a,**, Zhen Li b, *

a Key Laboratory for Ultrafine Materials of Ministry of Education, Shanghai Key Laboratory of Advanced Polymeric Materials, School of Material Science and Engineering, East China University of Science and Technology, Shanghai 200237, China
b Center for Molecular Imaging and Nuclear Medicine, School for Radiological and Interdisciplinary Sciences (RAD-X), Soochow University Collaborative Innovation Center of Radiation Medicine of Jiangsu Higher Education Institutions, Suzhou 215123, China

Article info
Article history:
Received 22 December 2015
Received in revised form 2 March 2016
Accepted 14 March 2016
Available online 17 March 2016

Keywords:
Black phosphorus
PEGylated nanoparticles
Photoacoustic imaging
Photothermal therapy

Abstract
Black phosphorus (BP) nanostructures such as nanosheets and nanoparticles have attracted considerable attention in recent years due to their unique properties and great potential in various physical, chemical, and biological fields. In this article, water-soluble and biocompatible PEGylated BP nanoparticles with a high yield were prepared by one-pot solventless high energy mechanical milling technique. The resultant BP nanoparticles can efficiently convert near infrared (NIR) light into heat, and exhibit excellent photo-stability, which makes them suitable as a novel nanotheranostic agent for photoacoustic (PA) imaging and photothermal therapy of cancer. The in-vitro results demonstrate the excellent biocompatibility of PEGylated BP nanoparticles, which can be accumulated in tumors through the enhanced permeability retention effect. The resultant BP nanoparticles can be further utilized for photothermal ablation of tumors by irradiation with NIR light. The in-vivo PA images demonstrate that these BP nanoparticles can be efficiently accumulated in tumors through the enhanced permeability retention effect. The resultant BP nanoparticles can be further utilized for photothermal ablation of tumors by irradiation with NIR light. The tumor-bearing mice were completely recovered after photothermal treatment with BP nanoparticles, in comparison with mice from control groups. Our research highlights the great potential of PEGylated BP nanoparticles in detection and treatment of cancer.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Phosphorus is an essential element for maintaining good health in humans. It has three main allotropes, including white phosphorus (WP), red phosphorus (RP), and black phosphorus (BP) [1], among which WP has a tetrahedral structure and is chemically unstable because of large bonding strains, RP has an amorphous structure and better chemical stability than WP, and the orthorhombic BP is thermodynamically the most stable allotrope, as well as being nonflammable and insoluble in most solvents. Compared with the other two allotropes, BP has not attracted much attention over the past century since it was discovered in 1914 due to the harsh preparation conditions and less applications [2]. Recently, layered bulk BP can be mechanically exfoliated into monolayer or few-layer black phosphorus (also known as phosphorene) [3] because of the weak van der Waals forces between stacked layers, which is similar to the behavior of other layered materials such as graphite [4,5] and the transition metal dichalcogenides (TMDCs: MX₂, M = Mo, W, Nb, and Ta; X = S, Se, and Te) [6,7]. In addition to mechanical exfoliation, there are some reports on the preparation of BP nanostructures by liquid exfoliation of bulk BP [8,9].

Different compared with other two-dimensional (2D) layered nano-materials such as graphene, graphene analogues [10] (e.g. silicene, germanene, and BN), and TMDCs, nanoscale BP distinguishes itself from them by its puckered structure along the armchair direction and bilayer configuration along the zigzag direction. This structural anisotropy leads to its unique properties, such as its electronic
conductivity [11], optical properties [12–14], thermoelectric properties [15], and topological features, as well as its unusual mechanical behavior [16,17] (e.g. a negative Poisson’s ratio). For example, BP nanostuctures exhibit an interesting layer-dependent bandgap, which can be tuned from 0.3 eV (bulk) to 2.0 eV (single layer) [18]. Zhang et al. experimentally verified the ultrafast nonlinear optical response of multi-layer BP nanoplates by Z-scanning measurement technique [19]. Subsequently, they found that Raman peaks of BP are red-shifted with the increase of layers, providing a fast and effective way to determine the thickness (layers) of phosphorene [20].

Because of its novel properties, BP nanostructures prepared by both mechanical and liquid exfoliation methods show great promise in various areas, including field-effect transistors [21–23], lithium-ion batteries [24,25], demodulators, memory devices [26], diodes [27,28], and photodetectors [29–31]. Their application in the biomedical field, however, has been rarely studied, and there are few reports on the bio-application of BP nanoparticles or nano-sheets due to the difficulties in large-scale preparation of these water-soluble and biocompatible nanostructures [32]. Recently, Xie and co-workers demonstrated exfoliated BP nanosheets to be photosensitizers suitable for the generation of singlet oxygen for photodynamic therapy [33]. Yu et al. also used liquid exfoliation to prepare BP quantum dots for investigation of their cytotoxicity [34]. The negligible toxicity of BP nanodots towards different types of cells suggests their potential in bio-applications.

Most currently available BP nanostructures are exfoliated from bulk crystalline BP [35], which was prepared under harsh conditions (i.e. high pressure, high temperature, and the use of complicated catalysts). Herein, we prepare water-soluble and biocompatible polyethylene glycol treated (PEGylated) BP nanoparticles from stable red phosphorus by the one-pot solventless high energy mechanical milling (HEMM) approach [36,37]. The resultant BP nanoparticles were successfully utilized for photoacoustic (PA) imaging and photothermal therapy (PTT) of cancer.

PA imaging is a newly emerging bioimaging modality that can overcome the drawbacks of ultrasound imaging (e.g. speckle artifacts) and optical imaging (e.g. limited penetration depth), and integrate their merits [38,39]. It can generate high spatial resolution images with optical contrast in a region up to 5–6 cm deep in biological tissues, and provides inherently background-free detection [40]. PTT is a minimally invasive therapeutic approach using near-infrared (NIR) light-absorbing agents to convert photons into heat for ablation of cancer cells. The combination of PTT and PA imaging could provide a perfect solution for accurate diagnosis and treatment of cancer, because they both could use the same NIR absorbing material as a theranostic agent. There has been no report, however, on application of BP nanostructures for in-vivo PA imaging and PTT of cancer, which will be investigated in this article [41].

2. Materials and methods

2.1. Materials

Red phosphorous (RP) was purchased from Sinopharm Chemical Reagent Co., Ltd and polyethylene glycol (PEG, Mn = 6000) was purchased from PegBio, Suzhou, China. All cell-culture related reagents were purchased from Hyclone.

2.2. Preparation of PEGylated BP nanoparticles

BP nanoparticles were prepared from RP by the solventless high energy mechanical milling (HEMM) technique. In a typical synthesis, 1 g of RP powder (High Purity Chemicals, >99%) and stainless steel balls (ball size: 6–25 mm in diameter) in a weight ratio of 100:1 were loaded into a hardened steel vial with a capacity of 100 mL. 4 g of PEG was added into the jar, and the mixture was milled for 72 h to produce a black powder, which was dispersed in 30 mL H2O. The resultant dispersion was centrifuged with a speed of 11,000 rpm for 20 min. The supernatant was further dialyzed against Milli-Q water for 72 h to remove free PEG to obtain the PEGylated BP nanoparticle solution.

2.3. Characterization of BP nanoparticles

The purified BP nanoparticles were characterized with state-of-the-art facilities, including X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM), etc. One droplet of nanoparticle solution was dropped onto an ultrathin carbon-coated holey carbon support film deposited on a 300 mesh copper grid for TEM characterization. TEM images were collected on a FEI Tecnai G20 microscope working with an accelerating voltage of 200 kV. Ultraviolet-visible (UV–VIS) absorption spectra were recorded at room temperature on a Shimadzu UV–VIS–NIR Spectrophotometer UV-3600. The hydrodynamic size was measured at 25 °C with a Malvern Zetasizer Nano ZS90 equipped with a solid-state He–Ne laser (λ = 633 nm). XRD patterns were collected on a Shidamiu XRD-6000 with Cu Kα1 radiation (λ = 0.15405 nm). XPS spectra were collected in an ultra-high-vacuum system with Mg Kα (1253.6 eV) as excitation source, and the binding energies of elements were calibrated with C 1s at 284.6 eV.

2.4. Cell viability

4T1 murine breast cancer cells were cultured in a 96-well plate (8 × 10^4 cells/well) filled with Dulbecco’s Modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, and 1% penicillin and streptomycin. The cells were cultured in a humidified incubator with 5% carbon dioxide at a constant temperature of 37 °C. After 12 h incubation, the medium was replaced with 100 µL of fresh DMEM containing PEGylated BP nanoparticles with concentrations of 4.5, 9, 18, 35, 70, and 140 µg/mL, respectively. Six wells were designated for each sample. The cells were then incubated for another 12 h. (3-4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) was dissolved in phosphate-buffered saline (PBS) solution at a concentration of 5 mg/mL and filtered through a 0.22 μm membrane to sterilize and remove insoluble residues, and then stored in amber vials at 4 °C. The cell viability was assayed by adding 10 µL of MTT PBS solution (5 mg/mL) into each well. After the cells were incubated with MTT at 37 °C for 2 h, the MTT solution was removed from all the samples, and 100 µL of dimethyl sulfoxide (DMSO) was added to dissolve the formed formazan crystals. The absorbance correlated with the number of viable cells in each well was measured by a Thermo Reader at a wavelength of 570 nm. The cell viability was calculated by Eq. (1).

\[\text{Cell viability}(\%) = \frac{A_T}{A_C} \times 100\% \]  

where \( A_T \) is the mean absorbance of treatment group, and \( A_C \) is the mean absorbance of control group without BP nanoparticles.

2.5. Photothermal ablation of cancer cells

To evaluate the potential of PEGylated BP nanoparticles for PA imaging and PTT therapy, different concentrations of PEGylated BP nanoparticles were irradiated by 808 nm light with a power density...
of 1.0 W/cm² for 10 min. The temperature of the BP nanoparticle solution was recorded with an infrared (IR) thermal camera. The photostability of BP nanoparticles was tested for 5 cycles of irradiating and cooling processes.

For photothermal ablation of cancer cells, 4T1 cells were incubated with 4.5, 9, 18, 35, 70, and 140 μg/ml PEGylated BP nanoparticles for 12 h, and then irradiated with a laser (808 nm, 1.0 W/cm²) for 10 min. After incubation for another 12 h, MTT assays were used to evaluate the cell viability.

2.6. Tumor model

Female BALB/c mice were purchased from Nanjing Sikerei Biological Technology Co Ltd and used under protocols approved by the Soochow University Laboratory Animal Center. 2 × 10⁶ 4T1 cells suspended in 50 μL PBS were subcutaneously injected into the right leg of each female BALB/c mouse. The mice bearing 4T1 tumors were treated when the tumor volume reached ~60 mm³.

2.7. Photoacoustic (PA) imaging

All in-vitro phantom and in-vivo mouse imaging experiments were performed using a real-time multispectral optoacoustic tomographic imaging system (MSOT, inSight/inVision 256, iThera Medical GmbH). The phantom is made of polyurethane, specially designed to mimic the shape, size, and optical properties of a mouse, containing two inner cylindrical channels for holding the control medium (Milli-Q water) and the contrast agent solution. In-vitro PA images of PEGylated BP nanoparticles with different concentrations were acquired with an excitation wavelength of 680 nm.

For in-vivo PA studies, a nude mouse bearing subcutaneous tumors was anesthetized by 1% isoflurane delivered via a nose cone, and then the PEGylated BP nanoparticles (200 μL, 2 mg/mL) were injected via the tail vein. In-vivo PA images were acquired before injection and at different time points post injection (i.e. 0.5 h, 1 h, 2 h, 4 h, 6 h, and 24 h) using the multispectral optoacoustic tomography system at a wavelength of 680 nm. A region of interest (ROI) volume consisting of transverse slices with a step size of 0.3 mm, spanning through the tumor region, was selected by manual inspection of live MSOT images. The averaged PA signals of the tumor area were extracted using the multispectral optoacoustic tomography software.

2.8. Photothermal therapy

Mice bearing 4T1 tumors were intratumorally injected with 50 μL of 2 mg/mL PEGylated BP nanoparticle solution. For control groups, mice were treated with the same volume of PBS. Mice injected with and without PEGylated BP nanoparticles were irradiated by an 808 nm NIR laser with a power density of 2 W/cm² for 5 min. The tumor sizes were measured with calipers every day and calculated as the volume = (tumor length) × (tumor width)²/2. Relative tumor volumes were calculated as V/V₀ (where V₀ is the initial tumor volume when the treatment was started).

2.9. Histological analysis

For hematoxylin and eosin (H&E) staining, major organs, including the liver, spleen, kidney, heart, and lung, were harvested, fixed in 10% neutral buffered formalin, processed routinely into paraffin, sectioned into thin slices, and stained with H&E for histological analysis.

3. Results and discussion

3.1. Preparation and characterization of PEGylated black phosphorus

The PEGylated BP nanoparticles were prepared from stable RP and PEG by a solventless one-pot high-energy ball-milling approach. Fig. 1a shows the photo image, XRD pattern of RP. The resultant black powder (inset in Fig. 1b) indicates the formation of BP, as the reactant mixture has a red color (inset in Fig. 1a). The crystal structure of BP nanoparticles was determined by X-ray diffraction (XRD), and Fig. 1b shows the XRD pattern of BP nanoparticles obtained with ball-milling for 2 h, which is consistent with that of standard orthorhombic black phosphorus (JCPDS No. 76–1957). The XRD patterns of BP nanoparticles obtained from different ball-milling time are shown in Figure S1 (supporting information), which demonstrates that BP nanoparticles become smaller and smaller with the increase of ball-milling time. Furthermore, the difference in their Raman spectra also proves the transformation of RP into BP nanoparticles (Fig. 1c). All Raman peaks of BP are much sharper than that of RP. Indicating that BP nanoparticles are well crystallized than RP. In addition, the broad peak around 387 cm⁻¹ in RP is disappeared after ball milling, and a new peak at 431.1 cm⁻¹ is appeared in BP. Three sharp peaks can be attributed to one out-of-plane phonon mode (A1g) at 357.6 cm⁻¹ and two in-plane modes (B2g and A2g) at 431.1 and 459.3 cm⁻¹, respectively [42,43].

Transmission electron microscopy (TEM) and atomic force microscopy (AFM) were employed to examine the morphology of BP nanoparticles. Their TEM image in Fig. 1d clearly shows uniform nanoparticles with a size of (3.2 ± 1.0) nm. The high-resolution TEM (HRTEM) image displays lattice fringes with an interplanar spacing of 0.217 nm, which is well consistent with the (002) planes of black phosphorous. The fast Fourier transform (FFT) image in Fig. 1d shows the single-crystal nature of BP nanoparticles. The typical AFM image of BP nanoparticles is shown in Fig. 1e, and the heights of nanoparticles labeled with line 1 and line 2 were measured to be 0.85, 1.15, and 1.53 nm (Fig. 1f). According to the statistical analysis of BP nanoparticles in AFM image (Fig. 1h), their average height is 1.2 ± 0.6 nm.

The resultant BP nanoparticles were further characterized by X-ray photoelectron spectroscopy (XPS). Figure S2 in the supporting Information and Fig. 1i show the binding energies of the elements in the sample after calibration with the binding energy of C 1s at 284.6 eV. The absence of other elements apart from C, O, and P indicates the high purity of BP nanoparticles. The two distinct peaks at 129.3 eV and 130.2 eV in the P 2p spectrum are assigned to 2p3/2 and 2p1/2 orbitals of zero-valent phosphorus (P0), respectively. The broad peak at 133.9 eV is attributed to oxidized phosphorus (i.e. P5⁺), which indicates the partial oxidation of P0 during sample preparation and purification, as the surface of BP nanoparticles is sensitive to oxygen and moisture [44]. The ratio of P5⁺/P0 in our BP nanoparticles was calculated to be 1:1, and the high ratio of P5⁺ is due to their extremely small size (3.2 ± 1.0 nm).

During preparation, PEG was used to improve the water-solubility and biocompatibility of BP nanoparticles [45,46]. Fig. 1j displays the Fourier transform infrared (FTIR) spectra of BP nanoparticles, PEGylated BP nanoparticles, and pure PEG. The broad absorption bands centered at ~1000 cm⁻¹ and ~1200 cm⁻¹ can be ascribed to the P–O stretching and P–P–O linear stretching modes, while the small peak at ~1620 cm⁻¹ is assigned to the P–O stretching mode [31]. The FTIR results demonstrate that PEG molecules were chemically bound with BP nanoparticles through the formation of P–O–C bonds. To further prove the formation of
P–O–C bonds, ethylene glycol (EG) was used to replace PEG during particle preparation. The resultant EGylated BP nanoparticles were purified by the similar way and then characterized with FTIR. The FTIR spectrum of EGylated BP nanoparticles (Fig. S3) shows the characteristic vibration of P–O–C at 920, 1000 cm⁻¹, which indicates the formation of P–O–C bonds. Based on these results, it is safe to conclude that PEG molecules were chemically bound with BP nanoparticles.

The surface PEG coating and the partial oxidation ensure the excellent water-solubility and stability of the resultant BP nanoparticles, and their hydrodynamic size and zeta potential were determined to be 21.7 nm and −35.4 mV respectively, by dynamic light scattering (DLS) spectroscopy (Fig. S4). The negative zeta potential further supports the partial oxidation of BP nanoparticles and the formation of phosphate groups on the surface. The hydrodynamic size of PEGylated BP nanoparticles in water was further monitored for 8 days (Fig. S5). No significant increase in their hydrodynamic size shows no aggregation of BP nanoparticles occurred. The results demonstrate the excellent colloidal stability of BP nanoparticles.

3.2. NIR optical properties of the PEGylated black phosphorus

Fig. 2a shows the absorption of PEGylated BP nanoparticles solutions with different concentrations in the ultraviolet–visible–near infrared (UV–vis–NIR) range, and the inset contains their corresponding digital photographs. Similar to other 2D layered nanostructures such as graphene oxide (GO) [47] and WS₂ [48,49], PEGylated BP nanoparticles show a broad absorption band across the UV and NIR regions. According to the Lambert–Beer law in Eq. (2):

$$A/L = \alpha C$$

where A is the absorbance of a BP nanoparticles solution, L is the length of the cuvette, C is its concentration, and $\alpha$ is its extinction
one major concern about nanomaterials for bio-applications is their toxicity, and the cytotoxicity of PEGylated BP nanoparticles

A major concern about nanomaterials for bio-applications is their toxicity, and the cytotoxicity of PEGylated BP nanoparticles.
towards tumor cells was investigated with the standard MTT assays (Fig. 3). The nanoparticles were diluted with Dulbecco’s Modified Eagle’s medium (DMEM) and incubated with 4T1 cells in 5% CO2 at 37°C for 24 h. The cytotoxicity slightly decreases with increasing nanoparticles concentration. No significant cytotoxicity was observed in cells when the concentration of PEGylated BP nanoparticles was less than 140 μg/mL, and the cell viability remained above 85%. The results indicate that our as-synthesized BP nanoparticles have low cytotoxicity and good biocompatibility. Next, we further investigated PEGylated BP as the photothermal agent for in vitro cancer cell ablation under laser irradiation. 4T1 cells were incubated with PEGylated BP nanoparticles solution at 140 μg/mL for 4 h and then irradiated by the 808 nm laser. It should be noted that the photothermal ablation efficacy is dependent on the concentration of BP nanoparticles and the laser intensity (Fig. 3a). If the power intensity was increased to 2.0 W/cm², most cancer cells were killed with a solution of 70 μg/mL PEGylated BP nanoparticles, and no cell survived in the case of 140 μg/mL PEGylated BP nanoparticles. Staining the cells with a Live-Dead Cell Staining Kit further demonstrate their excellent photothermal therapy efficacy. All cells are dead (indicated by red color) and no living cell (indicated by green color) was found after they were incubated with 140 μg/mL PEGylated BP nanoparticles and exposed to 808 nm laser irradiation (2.0 W/cm²) (Fig. 3b). In contrast, only few cells died if they were not incubated with PEGylated BP nanoparticles. These results demonstrated that PEGylated BP nanoparticles could serve as a potential PTT agent for photothermal ablation of cancer cells.

3.4. In-vitro and in-vivo photoacoustic images

As mentioned previously, PTT agents could also serve as contrast agents for photoacoustic imaging. The in vitro PA imaging performance of PEGylated BP nanoparticles in a water bath at 37°C was evaluated on a multispectral optoacoustic tomography system. The upper frame in Fig. 4 shows the PA images of PEGylated BP nanoparticles solutions. With increasing nanoparticles concentration from 0 to 250 μg/mL, the enhancement of the PA signal is linearly dependent on the concentration of BP nanoparticles (Fig. S8). The above excellent in-vitro results suggest the great potential of PEGylated BP nanoparticles for in-vivo photoacoustic imaging and photothermal therapy. Tumor bearing mice were intravenously injected with PEGylated BP nanoparticles, and the PA images of their livers, kidneys, and tumors were recorded at different time intervals (Fig. 4). The pre-contrast images show weak signals in the liver, kidney, and tumor. After intravenous injection of BP nanoparticles, the contrasts in the liver, kidney, and tumor regions are

---

**Fig. 3.** In-vitro cell experiments. (a) Relative cell viabilities of 4T1 cells after incubation with various concentrations of PEGylated BP nanoparticles for 24 h and then irradiation with/without 808-nm laser light (1.0 W/cm², 2.0 W/cm², 10 min); (b) Live-Dead Cell Staining Kit stained images of 4T1 cells incubated with PEGylated BP nanoparticles after laser irradiation for 10 min at a power density of 1.0 W/cm² and 2.0 W/cm².

**Fig. 4.** In-vitro photoacoustic images of PEGylated BP nanoparticles solutions (first row), and in-vivo photoacoustic images of liver, kidney, and tumor obtained at different time intervals after intravenous injection of PEGylated BP nanoparticles, in comparison with the corresponding pre-contrast images.
remarkably enhanced with increasing circulation time, indicating the gradual accumulation of BP nanoparticles in these organs. 24 h after injection, the tumor retains higher signal intensity than the liver and kidney, suggesting that more BP nanoparticles remained in the tumor than in the liver and kidney. This result also indicates that PEGylated BP nanoparticles have a long retention time in the tumor, while they are easily excreted from the liver and kidney.

3.5. In-vivo photothermal therapy

In-vivo photothermal therapy of cancer by using PEGylated BP nanoparticles as photosensitizer was conducted on 4T1 tumor-bearing mice. To monitor the in-vivo photothermal effect of PEGylated BP nanoparticles, the changes in temperature in the tumor area were recorded using thermal imaging apparatus. It should be noted that the BP nanoparticles accumulated in the tumor through the enhanced permeability retention (EPR) effect after intravenous injection are not sufficient for photothermal therapy of the tumor [54]. Tumor-bearing mice were intratumorally injected with PEGylated BP nanoparticles solution (50 µL, 2 mg/mL), and exposed to 808 nm laser light with a power density of 2.0 W/cm². Under irradiation, the temperature of the tumor increased significantly from 34 °C to 59 °C within 5 min (Fig. 5a–b). In comparison, the temperature of the tumor without injection of BP nanoparticles only slightly increased by 6 °C (i.e., ΔT) under the same irradiation conditions.

The phototherapeutic efficacy of PEGylated BP nanoparticles was further investigated. Five tumor bearing mice were intratumorally injected with PEGylated BP nanoparticles solution (50 µL, 2 mg/mL). The tumor of each mouse in the treatment group was then exposed to 808 nm laser light at a power density of 2.0 W/cm² for 5 min. Three other groups, including untreated mice (control, n = 5), mice exposed to the 808 nm light (laser irradiation only, n = 5), and mice injected with PEGylated BP nanoparticles without laser irradiation (BP nanoparticles only, n = 5) were used as control. The tumor sizes of mice from the treated and control groups were measured every 2 days after treatment. It is remarkable that for the mice treated with both PEGylated BP nanoparticles and laser irradiation, their solid tumors shrank gradually and were scabbed over after 3 days of treatment (Fig. 5c–d). In contrast, the tumors of mice

![Fig. 5.](image-url)
3.6. Histological analysis

To further evaluate the therapy efficacy and the in-vivo toxicity of PEGylated BP nanoparticles, major organs of the mice were sliced and stained by hematoxylin and eosin (H&E) for histology analysis (Fig. 6). From Fig. 6, we can see that the organs of the mice in the control group have aggressive metastases, especially in the liver and lung, compared with the healthy mice. In contrast, the treated mice that were sacrificed 42 days after photothermal therapy with BP nanoparticles exhibited no significant damage to their normal tissues, including the heart, liver, spleen, lung, and kidney, indicating that PTT treatment had no influence on the normal tissues.

4. Conclusion

In summary, water-soluble and biocompatible PEGylated BP nanoparticles were prepared with a high yield by means of the solventless high energy mechanical milling (HEMM) technique. The resultant PEGylated BP nanoparticles have a uniform size and exhibit excellent biocompatibility, photostability, and capability of converting near infrared (NIR) light into heat, which make them suitable as a novel nanoantheranotic agent for PA imaging and photothermal therapy of cancer. Both in-vitro and in-vivo results demonstrate their great potential in these aspects. They can be accumulated in tumors through the enhanced permeability retention (EPR) effect for PA imaging and are easily excreted from the liver and kidney. The tumor-bearing mice completely recovered after photothermal treatment using PEGylated BP nanoparticles as a heat mediator. Our research highlights the great potential of PEGylated BP nanoparticles in nano-biomedicine.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81471657, 81527901, 20504026), Jiangsu Provincial Key Laboratory of Radiation Medicine and Protection, A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), Shanghai Natural Science Foundation (No.13ZR1411900), the Shanghai Leading Academic Discipline Program (B502), the Shanghai Key Laboratory Project (08DZ2230500). Z. Li acknowledges the support from the program of Jiangsu Specially Appointed Professorship. The authors would like to thank Dr. Tania Silver for critical reading of the manuscript.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2016.03.022.

References
