Black phosphorus: A novel nanoplatform with potential in the field of bio-photonic nanomedicine

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Single- or few-layer black phosphorus (FLBP) has attracted great attentions in scientific community with its excellent properties, including biodegradability, unique puckered lattice configuration, attractive electrical properties and direct and tunable band gap. In recent years, FLBP has been widely studied in bio-photonic fields such as photothermal and photodynamic therapy, drug delivery, bioimaging and biosensor, showing attractive clinical potential. Because of the marked advantages of FLBP nanomaterials in bio-photonic fields, this review article reviews the latest advances of biomaterials based on FLBP in biomedical applications, ranging from biocompatibility, medical diagnosis to treatment.

Keywords: Black phosphorus; biosensing; drug delivery; biocompatibility; photothermal and photodynamic therapies.

1. Introduction

Two-dimensional (2D) materials, such as graphene,1–9 transition metal dichalcogenides (TMDs, e.g., MoS2,10–19 WS2,20–25 TiS2) and black phosphorus (BP), have been applied in various fields because of their distinctive physicochemical properties,26–39 including easy surface modification, electrical conductivity and strong light response.40–50 Especially, 2D materials own more advantages in bio-photonic applications such as biosensing,51–61 cancer imaging,62–68

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and drug delivery platform.\textsuperscript{69–73} However, each 2D material has its own shortcoming which hinders its clinical applications.\textsuperscript{74–77} Graphene displays an extremely high carrier mobility, but it has no bandgap,\textsuperscript{78–88} which limits its applications in biosensing and bioimaging. In spite of possessing a finite bandgap,\textsuperscript{17,89–96} the low carrier mobility of TMDs prevents their practical applications.\textsuperscript{97–107} Taken together, it is important to find a new 2D material with a well-balanced performance.

BP is a novel nanoplatform with potential applications in such as energy storage,\textsuperscript{35} sensor\textsuperscript{108} and bio-photonic because of its excellent properties.\textsuperscript{109–120} The bandgap of BP is tunable in a large range (from 0.3 eV to 2 eV) under different thicknesses.\textsuperscript{121–124} As the result of the direct and tunable bandgap, few-layer black phosphorus (FLBP) processes a widely light absorption indicating a great potential in photothermal and photodynamic therapy. FLBP also shows high carrier mobility\textsuperscript{25–130} while preserving large ON/OFF ratio.\textsuperscript{131–137} Due to the balance of these features, BP is often occurred in biosensing and bioimaging field. FLBP, with the unique puckered lattice configuration, possesses much larger surface area-to-volume ratio than other 2D materials, which results in extremely high drug loading capacity.\textsuperscript{138} Furthermore, FLBP displays negligible toxicity and outstanding biodegradability in the biological environment which distinguishes it from other common 2D materials. FLBP is completely degradable in vivo, and the products, such as phosphate ions, phosphate ions and other P\textsubscript{2}O\textsubscript{5}, are nontoxic and can be easily exhibited a desire renal filtration. In addition, FLBP possesses attractive electrical characteristics, unique band structure and natural biocompatibility, indicating that FLBP has a great potential for biomedical field.\textsuperscript{37}

BP has aroused more and more research interest on its biomedicine applications with its unique physical and chemical properties. However, there is still a lot of problems needs to be solved to achieve the requirements of clinical applications, such as stability,\textsuperscript{139–144} in vivo toxicity,\textsuperscript{145} biodegradation,\textsuperscript{146} excretion,\textsuperscript{147} etc. In order to meet the urgent demand for the novel 2D materials used in biomedical field, it is necessary to summarize the latest achievement of BP in bio-photonic fields. In this paper, we mainly review the biomedical research of BP in biological diagnosis and therapy.

2. Biocompatibility of BP

Latiff et al.\textsuperscript{145} established human lung carcinoma cancer epithelial cells (A549) model to study the toxicity of FLBP. To ensure the credibility of the results, graphene and MoS\textsubscript{2} are used as comparison and two similar methods based on similar principles were used to assess cell viabilities. In this work, the toxicity of FLBP increases with increasing concentration, when the concentration of FLBP is below 50 ppm. They found that toxicity of FLBP is lower than that of graphene but higher than that of MoS\textsubscript{2}. Due to the limitations of the test method and the size of FLBP used in the paper, there are still a lot of toxicity assessments to do before the clinical applications of FLBP. Zhang et al.\textsuperscript{148} studied the dependence of FLBP toxicity on size, concentration, exposure time and cell type, as presented in Fig. 1. FLBP was fabricated in oxygen-free Millipore water, and centrifugation at different speeds was used to obtain FLBP with three different sizes, named BP-1, BP-2 and BP-3, from large to small. The results of this paper indicate that FLBP with the BP-3 dimensions is appropriate for biomedical applications. They proposed and verified two possible mechanisms of the cytotoxicity of FLBP: (1) FLBP produces reactive oxygen species (ROS) to kill cells and (2) FLBP destroys cell membrane integrity. The cytotoxicity of BP-1 is much higher than that of BP-3. Fortunately, the size of BP-3 happens to be the most widely used size of FLBP nanosheets in the biomedical field. The results of this paper indicate that FLBP is appropriate for biomedical applications. Mu et al.\textsuperscript{149} explored toxicological studies on BP quantum dots (BPDQDs), which underwent faster renal filtration than BP nanosheets, systemically with cell and animal model. Comparing the cells viability and the endocellular ROS levels in experimental group with different concentrations of BPDQDs and the control group, they found that the cytotoxicity of BPDQDs is mainly derived from ROS produced by itself. The results of in vivo assays indicated that catalase activity in liver will reduce 24 h after injection, but there was no obvious adverse effect after a week without recurrence after a month. Song et al.\textsuperscript{150} studied the dependence of FLBP cytotoxicity on dose and time. When the concentration exceeded 4 ppm, FLBP showed significant cytotoxicity, which conflicts with other works probably for the reason of the difference in the size of FLBP.
The percentage of live cells was detected within 12 h at a dose of 10 ppm and was significantly reduced after 6 h, indicating the need for effective modification before BP achieves clinical applications.

3. Medical Diagnosis

3.1. Biomolecular biosensors

With a high carrier mobility and large switching ratios, FLBP has great applications potential on highly sensitive and selective biosensors.\textsuperscript{151–160} Chen et al.\textsuperscript{161} investigated FLBP for human immunoglobulin G (IgG) detection. FLBP was fabricated by a mechanical exfoliation method and passivated with Al\textsubscript{2}O\textsubscript{3}. The FLBP-based device showed rapid response performances and excellent sensitivity (\textasciitilde10 ng mL\textsuperscript{−1}) to human IgG. Furthermore, the FLBP-based device exhibited good stability without obvious changes in performance. Mayorga-Martinez et al.\textsuperscript{162} obtained FLBP with the size of 40 \textasciitilde 200 nm by electrochemical exfoliation. The as-fabricated FLBP showed active electrocatalytic performances for the hydrogen evolution.
reaction and IgG detection. In addition, FLBP with poly-L-lysine (PLL) displays a great potential in label-free detection of myoglobin (Mb), which is an important signal of cardiovascular events. FLBP was created by liquid-phase exfoliation (LPE) with an aqueous surfactant solution in argon atmosphere, and PLL was used to functionalize FLBP in order to accelerate binding with anti-Mb DNA aptamers. The PLL-BP dispersed stability in aqueous medium and showed a stable detection performance in phosphate-buffered saline (PBS) and serum samples, indicating successful surface modification. The FLBP-based device possesses a high sensitivity (36 \mu A pg^{-1} mL^{-2}) and a low detection limit (0.524 pg mL^{-1}), with a widely dynamic response range (1 pg mL^{-1} to 16 \mu g mL^{-1}).

In addition to excellent electrical properties, the unique fluorescence properties of FLBP were also applied to biological detection. Gu et al. investigated BPQDs with a sonication-assisted solvothermal method for the applications of acetylcholinesterase activity sensing probes. The BPQDs showed strong green fluorescence at 497 nm and an extremely high quantum yield (8.4%). Furthermore, pH relevant fluorescence property with reliable photostability had been observed in this work. Lee et al. prepared BPQDs with a strong visible blue-emitting performance by LPE in various organic solvents. They used catechol-grafted poly(ethylene glycol) (CA-PEG) to functionalize BPQDs in basic buffer to achieve a stable dispersible in water and an extremely low cytotoxicity. The photoluminescence emission centered of PEG-BPQDs is 428 nm and the photoluminescence quantum yield is \approx 5\% when the excitation wavelength is 365 nm. Yew et al. demonstrated FLBP as a platform in fluorescence-based DNA biosensors. BP crystals transformed from red phosphorus allotrope in the high pressure, and FLBP was synthesized by share force milling at 17,000 rpm. FLBP showed an obvious photoluminescence emission centered at 527 nm with an excitation wavelength of 200 nm, indicating potential applications as fluorescent sensing platform. This biosensor shows a low detection limit (5.9 pM) and quantification limit (19.7 pM). In addition, an excellent linearity (r = 0.91) and a widely dynamic response range (4–4000 pM) were observed in this work.

3.2. Tumor imaging

Cancer is a malignant disease that kills millions of people every year. With the enhanced permeability and retention (EPR) effect, FLBP can be passively enriched to the tumor site, so the tumor imaging based on FLBP has received extensive attention. Shao et al. investigated that BPQDs, which manufactured by the simple liquid exfoliation method, with poly lactic-co-glycolic acid (PLGA) were used to modify BPQDs in order to enhance the stability and solubility of BPQDs in water. As-fabricated BP-PLGA can effectively passively accumulate to the tumor region with tail vein injection because of the EPR effect. Figure 2(a) demonstrates the infrared thermographic images of the mice irradiated by near-infrared (NIR) laser 24 h after injection. Drawing a comparison between the tumor temperature of the test groups and of the control groups under the same power irradiation, it is obvious that the tumor temperature of the test groups (26.3°C of BP-PLGA) increased much more than that of the control groups (6.2°C, 7.8°C and 10.8°C of PBS, PLGA and bare BPQDs, respectively), indicating the excellent photothermal performance of BP-PLGA. In addition, many other researchers also have achieved extremely good photothermal imaging by different modifications of FLBP.

Sun et al. investigated BPQDs with excellent photostability for the applications of photoacoustic (PA) imaging, as shown in Fig. 2(b). The PEGylated BPQDs with a uniform size were fabricated by a simple high energy mechanical milling method. When the concentration of the PEGylated BPQDs is in the range of 0–250 \mu g mL^{-1}, the intensity of the PA signal rises linearly as the concentration increases. Furthermore, the PA signal intensity in tumor was still higher than that of liver and kidney, 24 h after injection, representing a long retention time and EPR effect. Sun et al. reported BPQDs loaded with titanium ligand (TiL4) as PA imaging agent. Dispersibility and stability of TiL4@BPQDs in water are much better than those of bare BPQDs. With increasing wavelength of the irradiation, the intensity of the PA signal decreased because the optical absorption of wavelength range from 680 nm to 808 nm reduces.

Yang et al. prepared FLBP coated with Au nanoparticles for surface-enhanced Raman scattering (SERS) imaging. BP–Au NPs were fabricated with a facile reflux method, and mPEG-SH was added to improve the dispersion and stability in water. The molecular mechanism of photothermal therapy (PTT) was investigated by SERS analysis.
The SERS analysis illustrates that PTT damages the membrane microstructure in tumor, and some BP–Au NSs appear in the nucleus region.

4. Medical Therapy

4.1. Phototherapy

Phototherapy, mainly including PTT\textsuperscript{194,195} and photodynamic therapeutic (PDT),\textsuperscript{196,197} has become a potential alternative to traditional cancer therapy with its advantages of little double consequences,\textsuperscript{198–205} excellent targeting\textsuperscript{206–215} and intelligent controllability.\textsuperscript{216–222} The principle of PTT is based on the heat energy generated by the photothermal agent under irradiation to achieve the therapeutic effect. PDT agent can generate ROS to kill cancer cells under laser irradiation. FLBP has broadband absorption characteristics because of its tunable direct band gap, indicating great potential for the applications of cancer phototherapy.

4.1.1. PTT

Sun \textit{et al.}\textsuperscript{223} fabricated BPQDs by liquid exfoliation method as photothermal agent. BPQDs were functionalized with PEG to prevent BPQDs from aggregating in PBS. As-fabricated PEG-BPQDs possessed a large extinction coefficient (14.8 L g\textsuperscript{-1} cm\textsuperscript{-1} at 808 nm), which is much more larger than that of Au nanorods. In addition, PEG-BPQDs showed a high photothermal conversion efficiency (28.4\%) and favorable photostability. The aqueous solutions of PEG-BPQDs rise 31.5 °C in 10 min at the concentration of 50 ppm when the power density of NIR laser irradiation is 1.0 W cm\textsuperscript{-2}, indicating an excellent photothermal performance. The percentage of live cells had no significant decrease even with a high incubation concentration of PEG-BPQDs (200 ppm) without NIR laser. The PEG-BPQDs killed the most of the cancer cells with a low concentration after the NIR irradiation for 10 min, as shown in Fig. 3. The results of this work illustrate the good biocompatibility and photothermal performance of BPQDs.

Shao \textit{et al.}\textsuperscript{147} demonstrated BPQDs coated with PLGA for PTT. PLGA enhanced the stability of BPQDs and adjusted the degradation rate of nanoparticles by adjusting the chemical composition. PLGA-BPQDs maintained stable photothermal properties within 8 days, and it degraded by almost 80\% after 8 weeks, indicating a balance of...
therapeutic and biodegradable. In vitro experiments showed that PLGA-BPQDs killed the most of cancer cells at an extremely low concentration (10 ppm). What’s more, the final degradation products of PLGA-BPQDs are carbon dioxide, water, phosphate and phosphonate, which normally exist both in vivo and in vitro and result in little side effects. Fu et al.224 prepared three types of FLBP with average size of 394 ± 75 nm, 118 ± 22 nm and 4.5 ± 0.6 nm by LPE method, named as L-BP, M-BP and S-BP. The temperature of L-BP solution with a concentration of 25 ppm was able to rise by 24°C under NIR laser irradiation for 10 min, whereas temperatures of M-BP and S-BP solutions with the equal concentration could only rise by 21.8°C and 19.2°C, indicating that L-BP has the best photothermal performance. In addition, there are some studies dedicated to improving the photothermal stability of FLBP.225-227 Some researchers explored new applications for the thermal performance of BP, such as post-surgical treatment of cancer, 3D-printed scaffolds and neuroprotective nanomedicine.189,228,229

Fig. 3. The dependence of cell viability on concentration of BPQDs and cell lines. The irradiation time is 10 min and power density of 808 nm laser is 1.0 W cm⁻². (a) Fluorescence images of cancer cells incubated with BPQDs after irradiation of 808 nm laser. (b) C6 cells viability after treatments with different concentrations of BPQDs. (c) MCF7 cells viability after treatments with different concentrations of BPQDs.
4.1.2. **PDT**

FLBP for PDT was fabricated by LPE in an aqueous solvent. At a wavelength of 530 nm, the quantum yield of FLBP producing singlet oxygen is very high (0.91). *In vitro* and *in vivo* FLBP experiments showed good PDT effects, as shown in Fig. 4. BPQDs were synthesized by LPE in N-methyl pyrrolidone and coated with PEG to achieve enhanced stability in water. Guo *et al.* used BP dispersion to incubate cancer cells under 670 nm laser irradiation and researched the dependence of cell survival rate on BPQD concentrations, illumination time and laser intensity. The ROS generated by FLBP was able to kill the cancer cells efficiently at very low concentration (1.6 ppm) and at very weak laser power (160 mW cm$^{-2}$). Furthermore, 65% of BPQDs was found excreted with urine within 8 h, probably for the reason of the ultra-small hydrodynamic size of BPQDs, indicating that BPQDs have a good biocompatibility. Chen *et al.* found that the bleaching signal of BPQDs is built up rapidly (<2 ns) and lasts a long time (100 μs).

![Fig. 4](image)

Fig. 4. *In vivo* PDT. (a) Tumor volume of different time post-injections. (b) Tumor pictures of experimental group and control group after treatments. (c) PCNA and TUNEL analysis of tumor tissues.
The triplet generation attributed to intersystem crossing was observed and may be the reason of the highly efficient singlet oxygen generation of BPQDs. Tan et al. achieved in situ disinfection with PDT based on FLBP. FLBP was modified with poly(4-pyridonemethylstylene) endoperoxide (PPMS-EPO). PPMS not only enhances the stability of FLBP, but also stores singlet oxygen. The PPMS-EPO/BPS showed good photodynamic performance even in the absence of light, indicating a good clinical potential.

4.2. Therapeutic agent delivery

Chemotherapy is an effective cancer treatment, but the high toxicity, low targeting, and drug resistance limit its therapeutic effect. In order to solve the aforementioned problems, researchers load therapeutic agents onto drug delivery systems (DDSs) to enhance chemotherapeutic effect. With large surface area-to-volume ratio, unique pleated structure and excellent light response characteristics, FLBP has great potential for DDSs. Tao et al. first applied FLBP to DDSs, as shown in Fig. 5. PEG-FA/Cy7-functionalized FLBP exhibited good biocompatibility, obvious tumor targeting and strong fluorescence signal and was loaded with doxorubicin (DOX) via electrostatic adsorption. In addition, the endocytosis pathway of the FLBP nanoparticle had also been screened. Chen et al. prepared a drug loading platform with a pH/photo response based on FLBP. Their results showed that FLBP has a very high DOX loading (980%) due to its puckered lattice configuration, large interlay distance 0.524 nm and negative charge. They used the therapeutic system to achieve synergistic treatment of PTT/PDT/chemotherapy and achieved good anti-tumor effects. Wang et al. used one-pot method to prepare HSA-modified FLBP, which can effectively load paclitaxel by hydrophobic interactions. Qiu et al. fabricated a BP@hydrogel-based DDS. The DDSs enable intelligent light-controlled drug release and degradation, and the degradation products are completely nontoxic and easily metabolized. Yin et al. fabricated FLBP loaded with interfering RNA as the applications of gene delivery systems. Compared with the commercial delivery reagents, the BPQDs exhibited a higher transfection efficiency and low toxicity.

5. Summary

This paper summarizes the latest progress in FLBP research from three aspects, including biocompatibility of BP, medical diagnosis and medical therapeutic based on BP. Various factors such as size, concentration and test cell line can affect the toxicity of FLBP, but overall, FLBP shows relatively low toxic at effective dosing dose. Due to its attractive electrical properties, fluorescence characteristics and large surface area-to-volume ratio.
ratio, FLBP shows a great potential for biosensors and imaging. In terms of biological therapy, FLBP mainly achieves therapeutic effects through its excellent optical response characteristics and as a drug delivery platform.

Although studies on the biomedical applications of FLBP have made a lot of progress, there are still some problems that need to be solved before its clinical translation. First of all, because the size of the FLBP has a great impact on toxicity and treatment effects, it is very important to find a novel method to fabricate FLBP with uniform size and high production. Secondly, other treatments such as chemotherapy, immunotherapy, etc. could be combined with BP to achieve synergistic effect. Finally, the targeted treatments based on FLBP should be developed for specific diseases to achieve smaller side effects and better therapeutic effects. This requires multidisciplinary researchers to work together. FLBP has great potential in biomedicine. To promote the clinical applications of FLBP, we summarized the progress of FLBP in biomedical field.

Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>FLBP</td>
<td>few-layer black phosphorus</td>
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<td>2D</td>
<td>two-dimensional</td>
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<td>BP</td>
<td>black phosphorus</td>
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<td>TMDs</td>
<td>transition metal dichalcogenides</td>
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<td>ROS</td>
<td>reactive oxygen species</td>
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<td>QDs</td>
<td>quantum dots</td>
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<td>IgG</td>
<td>immunoglobulin G</td>
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<td>PLL</td>
<td>poly-L-lysine</td>
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<td>Mb</td>
<td>myoglobin</td>
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<td>PBS</td>
<td>Phosphate-buffered saline</td>
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<td>CA-PEG</td>
<td>catechol-grafted poly (ethylene glycol)</td>
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<td>EPR</td>
<td>enhanced permeability and retention</td>
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<td>PLGA</td>
<td>poly lactide-co-glycolic acid</td>
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<td>NIR</td>
<td>near-infrared</td>
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<td>PA</td>
<td>Photoacoustic</td>
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<td>HEMM</td>
<td>high energy mechanical milling</td>
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<td>TiL4</td>
<td>titanium ligand</td>
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<td>SERS</td>
<td>surface-enhanced Raman scattering</td>
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<td>PTT</td>
<td>photothermal therapy</td>
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<td>PDT</td>
<td>photodynamic therapeutic</td>
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<tr>
<td>PEG</td>
<td>poly (ethylene glycol)</td>
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<td>NMP</td>
<td>N-methyl pyrrolidone</td>
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<tr>
<td>PPMS-EPO</td>
<td>poly(4-pyridonemethylstyrene)</td>
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<td>drug delivery systems</td>
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<td>DOX</td>
<td>Doxorubicin</td>
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