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Controlled drug release improves the therapeutic efficacy and reduces toxicity during cancer treatment. There are no therapeutic effects if the drug fails to be released rapidly and reaches the minimum therapeutic concentration when the nanomedicine carriers reach the tumor sites, and in fact, such failure can increase the drug resistance of the cancer cells. Therefore, much effort has been devoted to the establishment of smart drug delivery systems, in which the drug release can be controlled by light, temperature, ultrasound, pH, mechanical stress, or biological molecules. Among the various stimuli, near-infrared (NIR) light has received increasing interest as it is non-invasive and has high spatial resolution with a range of adjustable parameters (e.g., wavelength, duration, intensity) and a good tissue penetrating ability.

Besides controlling the rate of drug release, conversion of NIR light into hyperthermia mediated by nano-photothermal conversion materials such as gold nanoparticles and two-dimensional (2D) nanomaterials produces the well-known photothermal effect. For example, mesoporous silica-coated graphene nanosheets enable combined therapy with a much higher rate of death of glioma cells compared to either single chemotherapy or photothermal therapy. The multifunctional nanoplatform composed of MoS2 nanosheets and Cu11.8S nanoparticles integrates the imaging diagnosis and chemo-photothermal therapy of tumors. However, most of these photothermal conversion materials are not biocompatible in vivo and their degradation products are toxic, thereby hampering their clinical application as drug release systems.

As a new type of 2D material, black phosphorus (BP) with good biodegradability has large potential in biomedical areas such as photothermal therapy, photodynamic therapy, drug delivery, imaging diagnosis, antibacterial activity, and so on. Compared with other nano-photothermal conversion materials, BP is particularly attractive due to its excellent biodegradability since phosphorus is an essential element in the human body and the degradation products are nontoxic phosphate and phosphonate. The photothermal effect of BP nanosheets has been studied and BP nanosheets loaded with doxorubicin (DOX) are used in the multimodal therapy of cancer. Compared to BP nanosheets, BP quantum dots (BPQDs) are more attractive for the combined therapeutic system due to their smaller size. However, a drug release system based on BPQDs has not been reported yet.

In this study, a BP–liposome composite is designed and fabricated by incorporating BPQDs into liposomal bilayers (BPQDs@Lipo). Liposomes consisting of a lipid bilayer surrounding an aqueous phase are the most extensively investigated nanocarriers for transportation of anticancer drugs. Embedding biocompatible materials which are responsive to certain stimuli in the bilayer can achieve a controlled release of drugs from liposomes. Herein, BPQDs are embedded in the hydrophobic bilayer and thus the aqueous core of the liposome can be used to load therapeutic drugs. The photothermal conversion of BPQDs and NIR-light-controlled drug release from liposomes are evaluated and the

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**Synthesis of lipid–black phosphorus quantum dot bilayer vesicles for near-infrared-controlled drug release**

Shengyong Geng, Lie Wu, Haodong Cui, Wenyong Tan, Tianfeng Chen, Paul K. Chu and Xue-Feng Yu

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The effects of the combined chemo-photothermal therapy on cancer cells are investigated.

The BP–liposome composite (BPQDs@Lipo) was prepared by thin lipid film hydration as shown in Fig. 1a. Ultrasmall BPQDs are synthesized in N-methyl-2-pyrrolidone (NMP) by liquid exfoliation and the titanium sulfonate ligand (TiL4) is synthesized to coordinate the surfaces of the BPQDs to improve the stability in water.

The transmission electron microscopy (TEM) image in Fig. 1b reveals good dispersibility of the TiL4 modified BPQDs and the average size is 3.2 nm according to the statistical analysis of 100 BPQDs. The high-resolution TEM image in Fig. 1c shows lattice fringes of 0.34 nm corresponding to the (021) plane of the BP crystal. The 1H NMR spectrum of the BPQDs reveals a peak at 2.3 ppm (–CH3 group) and two peaks at 7.2 and 7.5 ppm (benzyl group), indicating successful coordination of TiL4 on the BPQDs (Fig. S1, ESI†). High-resolution X-ray photoelectron spectroscopy (HR-XPS) was performed to assess the chemical quality of the BPQDs. As shown in Fig. 1d, the binding energy of P 2p (131.7 eV) shows Ti–P coordination and the peaks at 464.2 and 458.4 eV correspond to Ti 2p1/2 and Ti 2p3/2, respectively. The results confirm the fabrication of BPQDs with TiL4 coordination and they are designated as TiL4 modified BPQDs.

The TEM image of BPQDs@Lipo in Fig. 1e discloses a uniform morphology with small unilamellar vesicles and the BPQDs are distributed in the liposomes. The HR-TEM image clearly reveals that the BPQDs are incorporated into the lipid bilayers via hydrophobic self-assembly with the lipid molecules (Fig. 1f). The mean particle size of the liposomes is 105.6 ± 6.8 nm according to dynamic light scattering (DLS) as shown in Fig. 1g, and the incorporation of BPQDs has no influence on the size of the liposomes (Table S1, ESI†). The zeta potential of BPQDs@Lipo changes from −5.6 ± 0.3 to 0.5 ± 0.1 mV after the incorporation of the BPQDs (Table S1, ESI†). Moreover, even after storing at 4 °C for two weeks, the size and structure of BPQDs@Lipo do not change (Fig. S2, ESI†), suggesting its good stability. The structural characterization studies by cryo-TEM and atomic force microscopy (AFM) of the QD–liposome vesicles reveal nonuniform distributions of QDs in the liposomes.

In our study, the BPQDs are clearly observed and distributed uniformly in the liposomes since the ultrasmall BPQDs facilitate incorporation into the lipid bilayers. In this way, the internal water phase of BPQDs@Lipo can be used to load anticancer drugs.

The photothermal properties of BPQDs@Lipo are determined by 808 nm laser irradiation at different power densities. An infrared thermal imaging camera is used to monitor the temperature rise of the sample. As shown in Fig. 2a, the solution temperature increases by 9.9, 13.0, and 13.4 °C after irradiation for 10 min at power densities of 0.5, 1.0, and 1.5 W cm−2, respectively. In contrast, the temperature of the liposome solution without BPQDs increases by only 3.3 °C, indicating that the BPQDs in the liposomal bilayers can rapidly and efficiently convert NIR light into thermal energy.

A common anti-cancer drug, DOX, is encapsulated into BPQDs@Lipo using the transmembrane pH gradient-driven encapsulation technique to investigate the NIR-light-controlled drug release behavior, and the drug encapsulation efficiency is 89.6%. The concentration of the released drug in the phosphate buffered saline solution (PBS, pH 7.4) is monitored by fluorescence spectrometry. As shown in Fig. 2b, DOX is released rapidly from liposomes upon light irradiation. The release profile...
exhibits burst release in the first 5 min and sustained release afterwards. After irradiation for 5 min at power densities of 0.5, 1.0, and 1.5 W cm$^{-2}$, the drug release percentages are 20.0%, 42.6%, and 48.1%, respectively, indicating that the released rate can be adjusted by laser power. Furthermore, the percent release profile of the drug in the alternating presence and absence of NIR light is obtained (Fig. S4, ESI†). The drug release ceases when the light is off and it restarts when the light is back on. These results demonstrate that the amount of the drug released can be well controlled by the NIR light.

The BPQDs@Lipo samples after the NIR-controlled drug release are examined by TEM to make clear the releasing mechanism. After 30 min irradiation using an 808 nm laser with different power densities, most of the vesicles maintain their intact spherical structures, while some fragments of the vesicles are observed (Fig. S5, ESI†). Under 808 nm laser irradiation, the BPQDs located in the bilayers of liposomes convert light into thermal energy, thus increasing the fluidity of the lipid membranes or even destroying the structure of the liposome, leading to the release of encapsulated drugs from the liposomes. The versatility of the liposomal structure lies in its capacity to cargo drugs or biological molecules that are either hydrophobic for incorporation into the lipid bilayer or hydrophilic for encapsulation into the inner aqueous core. Encapsulation of nanoparticles in the liposomes can enhance their stability in blood plasma.21 Here, a BP-based drug delivery system boosting NIR-light-controlled drug release is prepared by the incorporation of BPQDs into liposomal bilayers and the incorporation of BPQDs does not affect the stability of the liposomes (Fig. S3, ESI†).

Based on controlled release in PBS, in vitro studies are carried out to assess the intracellular drug release behavior by incubation with MCF-7 human breast cancer cells. After incubation for 2 h in the dark with DOX-loaded BPQDs@Lipo, the cells are exposed to different excitation light intensities (0.5 and 1.0 W cm$^{-2}$) with the exposure time ranging from 5 to 15 min. The confocal fluorescence images of the MCF-7 cells in Fig. 3 show weak red fluorescence without laser irradiation. After irradiation with the 808 nm laser (0.5 W cm$^{-2}$) for 5 min, the nuclei exhibit stronger red fluorescence. When either the light intensity or irradiation time increases, the red fluorescence intensity increases obviously. These results indicate that the light intensity and exposure time affect the amount of the intracellular drug released from BPQDs@Lipo. Rapid and sufficient intracellular DOX release enables reaching the required drug concentration level within the therapeutic window helping to overcome drug resistance.27

Biocompatibility is a necessary requirement for nanomaterials in a drug delivery system and the cytotoxicity of BPQDs@Lipo is evaluated for two normal cell lines L929 (mouse fibroblast cells) and QSG-7701 (normal human liver cells) and two cancer cell lines A549 (human lung carcinoma cells) and MCF-7 using a CCK-8 kit. As shown in Fig. 4a, the cell viabilities of all four cell lines are around 100% even if the concentration of BPQDs@Lipo is increased to 2.0 μmol mL$^{-1}$. That is, BPQDs@Lipo has good biosafety and is suitable for biomedical applications.

The in vitro therapeutic efficacy is investigated using the CCK-8 assay together with live (Calcein-AM, green fluorescence)/dead (propidium iodide, PI, red fluorescence) cellular staining. The blank BPQDs@Lipo or DOX-loaded BPQDs@Lipo used to
 treat with cells has 0.5 μmol mL⁻¹ lipid concentration to keep the same BPQD concentration. As shown in Fig. 4b, 1.0 W cm⁻² laser irradiation for 10 min causes only about 3% death of the MCF-7 cells, suggesting that NIR light irradiation itself has little influence on the cell viability. Regarding the cells treated with BPQDs@Lipo, 29% cells are killed after 808 nm laser irradiation for 10 min (photothermal therapy group). In the chemotherapy group, 41% cells are killed after incubation with the same concentration of DOX-loaded BPQDs@Lipo for 24 h. In comparison, more than 90% of the cells are killed when incubated with BPQDs@Lipo-DOX and exposed to 808 nm laser irradiation for 10 min. The outstanding efficiency of this chemo-photothermal therapy arises from rapid intracellular DOX release from BPQDs@Lipo and the photothermal effect of BPQDs increases the permeability of the cell membrane, thus accelerating the nucleus accumulation of the extracellular DOX. Considering the limit of the maximum permissible exposure to an 808 nm laser in in vivo photothermal therapy,²⁸ BPQDs@Lipo with the synergistic effect of the photothermal agent and the drug may help in decreasing the laser power density in the latter in vivo experiments.

In conclusion, the BP–liposome composite (BPQDs@Lipo) intended for a NIR-light-controlled drug delivery system is fabricated by the incorporation of BPQDs into liposomal bilayers. The encapsulated drug is responsively released from liposomes upon 808 nm laser irradiation due to the photothermal effect of BPQDs. The drug release rate and amount are controlled by the light intensity and exposure time. The in vitro experiments reveal that BPQDs@Lipo has good biocompatibility and intracellular drug release can also be regulated by NIR light. Owing to its excellent NIR-light-induced chemo-photothermal antitumor efficiency, the BP–liposome composite has large clinical potential in multimodal cancer therapy.

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Conflicts of interest

There are no conflicts to declare.

Notes and references