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TOXICITY ASSESSMENT OF BLACK PHOSPHORUS QUANTUM DOTS IN NEMATODE *Caenorhabditis elegans*

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Abstract: Black phosphorus quantum dots (BPQDs) are very promising for many applications in biomedical area including photothermal therapy of cancer, due to their unique properties. However, the toxic effects of BPQDs and the potential risk of BPQDs on environment are still not fully understood. In the present study, we performed the *in vivo* toxicity assessment of BPQDs using *Caenorhabditis elegans* assay system. Our data show that BPQDs exposure induced toxicity on reproduction, development, locomotion behavior, learning ability and lifespan of *C.elegans* at the concentrations which was thought to be within the safe range. Moreover, we found that toxic effects of BPQDs could be transferred from exposed nematodes to their progeny, implying that BPQDs may have long-term influences on both organisms and environment. Therefore, our data will be useful for the better understanding of the toxicity of BPQDs, which is important for their safe application and utilization in future.

Keywords: Black phosphorus quantum dots; toxicity; *Caenorhabditis elegans*; environmental risk.

1. Introduction

As a newly emerged 2D material, Black phosphorus (BP) has attracted broad attentions in many fields such as photodetectors, transistors and optoelectronics. More importantly, BPQDs exhibit great potential in biomedical application areas including photothermal therapy of cancer, due to their distinctive optical and electrical properties[1]. Thus, a fully understanding of potential toxicity of BP and BPQDs is of utmost importance.

The toxic effects of BP and BPQDs have been investigated though both *in vitro* and *in vivo* studies in recent years[2]. However, there is some controversy about the safe concentration of BP, which may be due to their size effect[3]. In addition, little is known about the potential risk of BP and BPQDs on environment.

Nematode *Caenorhabditis elegans* (*C.elegans*) is an alternative animal model for toxicity

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testing[4]. Many useful lethal and sub-lethal endpoints for toxicity assessment have been established successfully such as lethality, locomotion behavior, brood size, body length and lifespan. Many studies have been conducted to assess the toxicity effects of nanomaterials using *C.elegans* assay system in recent years[5]. Importantly, *C.elegans* is useful for the study of potential ecotoxicological effects of toxicants, considering that nematodes are among the most abundant invertebrates in the soil[6].

In the present study, we performed the *in vivo* toxicity assessment of BPQDs using *C.elegans* assay system. Moreover, we investigated the transgenerational toxic effects of BPQDs for the assessment of their long-term ecotoxicological effects. Our results will be useful for the better understanding of toxic effects of BPQDs.

2. Experimental

2.1. Preparation of BPQDs, strain preparation and BPQDs exposure

20 mg bulk BP powders were dispersed in 2 mL of NMP, ultrasonic for 20 min. Then 2 mL of the dispersion was taken, 18 mL NMP and 200 mg NaOH was added, then stirred to obtain the second dispersion. Taking the dispersion to the high pressure reaction vessel, heated at 140 ^{0}C for 18 h, centrifuged at 7000 r/min for 20 min, the supernatant was collected to obtain BPQDs. BPQDs working solution was prepared by diluting the stock solution with K medium.

Wild-type N2 nematodes were maintained on nematode growth medium (NGM) plates with *E.coli* OP50 as food at 20 ^{0}C. Age sunchronous populations of nematodes were obtained as described previously[7]. BPQDs exposures were performed from L1-larvae for 48-hr in K-medium with OP50.

2.2. Lethality, reproduction, development and locomotion behavior

Lethality was evaluated by the percentage of survival nematodes. After exposure, nematodes were examined under dissecting microscopy and judged as dead when they did not respond to stimulus using a small, metal wire. Approximately 90 nematodes were used for each assy. Reproduction was assessed by the brood size and generation time of nematodes. Brood size was determined as the number of offspring. Generation time was determined as the time interval from the oviposition of the P0 adults to the oviposition of their F1 generation adults.

Development was assessed by the body length, which was determined by measuring flat surface area of nematodes using Image-Pro® Express software. Locomotion behavior assay was performed as described[7]. 8 replicates were performed.

2.3. Learning ability, lifespan and statistical analysis

Learning ability assay was performed basically as described[8]. Lifespan assay was performed basically as described[9]. The result of lifespan is representative of three trials. All data were expressed as mean ± S.D. and analyzed by Microsoft Excel software.

3. Results and Discussion

BPQDs were prepared basically as described previously with the similar characters. Specifically, size of most of the BPQDs in K-medium after sonication was in the range of 20–30 nm according to the TEM analysis (Fig. 1a-b). In order to assess the toxicity of BPQDs, we first investigated the effects of BPQDs on lethality of nematodes. As shown in Fig. 1c, we found that BPQDs exposure did not induce lethality of nematodes at all examined concentrations. Next, we examined the effects of BPQDs on reproduction of nematodes considering that reproduction system of *C.elegans* is an important secondary targeted organ for toxicants. Brood size of nematodes exposed to BPQDs at concentrations of 0.1-10 μg/mL was significantly reduced compared to control animals (Fig. 1d). Consistently, nematodes exposed to BPQDs at the concentrations more than 0.1 μg/mL exhibited significantly prolonged generation time (Fig. 1e). These results indicate that BPQDs have toxic effects on reproduction of *C.elegans*.

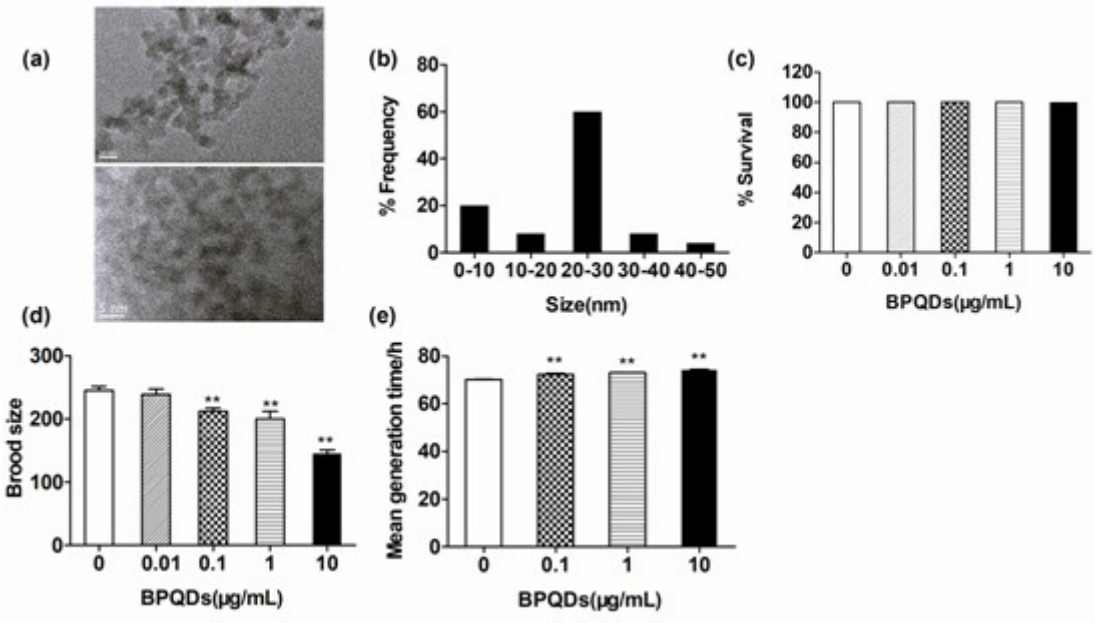

Figure 1. (a) TEM analysis of BPQDs; (b) Size distribution of BPQDs; (c) Lethality; (d) Brood size; (e) Generation time. **P<0.01.

We further investigated the effects of BPQDs on development of *C.elegans*. As shown in Fig. 2a, exposure to BPQDs at the concentrations of 0.1-10 μg/mL significantly reduced body length of nematodes. These results indicate that BPQDs have toxic effects on development of *C.elegans*. Alteration of locomotion behavior, which reflects the toxic effects of toxicants on neurons, is a sensitive endpoint for toxicity assessment of nano materials[10]. We then examined the effects of BPQDs on locomotion behavior of *C.elegans*. Exposure to BPQDs at concentrations of 0.1-10 μg/mL significantly decreased body bend and head thrash of nematodes (Fig. 2b-c), indicating that BPQDs have toxic effects on locomotion behavior of *C.elegans*. Learning ability of *C.elegans*, which is usually affected by toxicants, is another toxicity endpoint. Our results showed that although nematodes exposed to 0.1 μg/mL of BPQDs have normal learning ability, exposure to BPQDs at concentrations of 1-10 μg/mL significantly reduced the learning ability of nematodes (Fig. 2d). Lifespan of *C.elegans* is a useful endpoint which reflects the long-term effects of toxicants. Nematodes exposed to 10 μg/mL of BPQDs exhibited significantly shortened lifespan (Fig. 2e-f), implying that sublethal dose of BPQDs have long-term adverse effects on *C.elegans*.

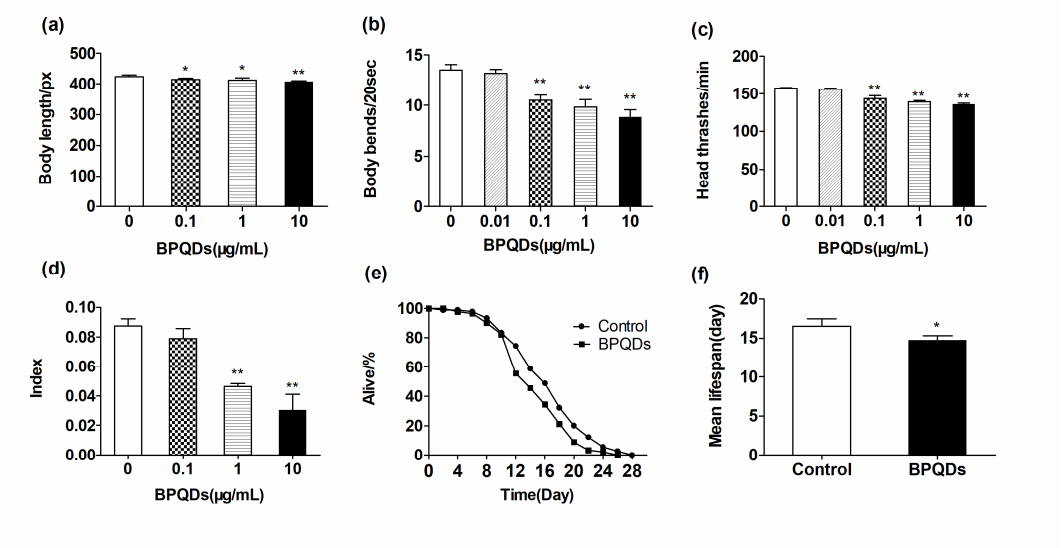

Figure. 2. (a) Body length of nematodes; (b) Body bend; (c) Head thrash; (d) Learning ability; (e) Survival curves; (f) Mean lifespans. **P<0.01.

All together, our data demonstrate that BPQDs have multi-toxic effects on *C.elegans* at the concentrations of 0.1-10 μg/mL, which was thought to be within the safe range previously. The controversy may be due to the size effect, which has been proved to have influence on toxicity for many nanomaterials. Moreover, *C.elegans* has been found to be more sensitive than other *in vitro* and *in vivo* systems in toxicity assessment[11], which may be another reason for the difference. Our results would raise attention to the toxicity and ecotoxicity of BPQDs, especially at environmental concentrations.

Adverse effects of some toxicants can be transferred from exposed nematodes to their progeny[12]. We then examined whether BPQDs have transgenerational effects on *C.elegans*. We observed the significantly reduced brood size in progeny of nematodes exposed to 10 μg/mL of BPQDs (Fig. 3a). Moreover, progeny of nematodes exposed to BPQDs at concentrations more than 1 μg/mL exhibited significant defects in locomotion behavior (Fig. 3b-c). These results imply that toxicity of BPQDs could be partially transferred from exposed animals to their progeny, which may be due to the translocation of BPQDs from parents to progeny. Therefore, the toxic effects of BPQDs on health and environment may be more serious and far-reaching than expected.

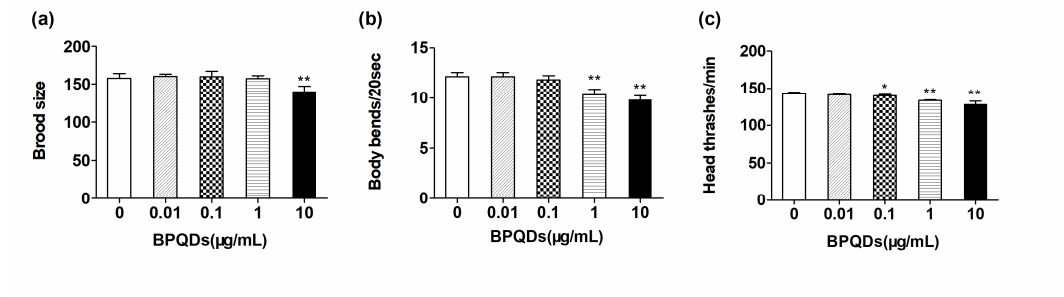

Figure. 3. Effects of BPQDs exposure on progeny of exposed nematodes: (a) brood size, (b) body bend, and (c) head thrash. **P<0.01.

4. Conclusions

Our results demonstrate that *C.elegans* assay system is useful for toxicity assessment of BPQDs. Although BPQDs did not induce lethality at all examined concentrations, we observed multi-toxic effects of BPQDs on nematodes at relatively low dose (0.1-10 μg/mL) using brood size, generation time, body length, locomotion behavior, learning ability and lifespan as endpoints. Moreover, the toxic effects of BPQDs can be transferred to progeny of exposed animals. Our data will be helpful for the better understanding of adverse effects of BPQDs and the underlying mechanism. In addition, our study provides insights on potential environmental risk of BPQDs, which should be fully considered for their safe application in future.

Acknowledgments

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Conflict of Interest

None.

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