Original Article

Toxicity of Graphene Quantum Dots in Zebrafish Embryo*

WANG Zhen Guo[1,a], ZHOU Rong[2,3,4,a], JIANG Dan[1], SONG Jing E[1], XU Qian[1], SI Jing[2,3,4], CHEN Yun Ping[1], ZHOU Xin[2,3,4], GAN Lu[2,3,4], LI Jian Zhen[5], ZHANG Hong[2,3,4,#], and LIU Bin[1,#]

1. School/Hospital of Stomatology, Lanzhou University, Lanzhou 730000, Gansu, China; 2. Department of Heavy Ion Irradiation Medicine, Institute of Modern Physics, Chinese Academy of Sciences, Lanzhou 730000, Gansu, China; 3. Key Laboratory of Heavy Ion Radiation Biology and Medicine of Chinese Academy of Sciences, Lanzhou 730000, Gansu, China; 4. Key Laboratory of Heavy Ion Radiation Medicine of Gansu Province, Lanzhou 730000, Gansu, China; 5. College of Life Sciences, Northwest Normal University, Lanzhou 730070, Gansu, China

Abstract

Objective To evaluate the bio-safety of graphene quantum dots (GQDs), we studied its effects on the embryonic development of zebrafish.

Methods *In vivo*, biodistribution and the developmental toxicity of GQDs were investigated in embryonic zebrafish at exposure concentrations ranging from 12.5-200 μg/mL for 4-96 h post-fertilization (hpf). The mortality, hatch rate, malformation, heart rate, GQDs uptake, spontaneous movement, and larval behavior were examined.

Results The fluorescence of GQDs was mainly localized in the intestines and heart. As the exposure concentration increased, the hatch and heart rate decreased, accompanied by an increase in mortality. Exposure to a high level of GQDs (200 μg/mL) resulted in various embryonic malformations including pericardial edema, vitelline cyst, bent spine, and bent tail. The spontaneous movement significantly decreased after exposure to GQDs at concentrations of 50, 100, and 200 μg/mL. The larval behavior testing (visible light test) showed that the total swimming distance and speed decreased dose-dependently. Embryos exposed to 12.5 μg/mL showed hyperactivity while exposure to higher concentrations (25, 50, 100, and 200 μg/mL) caused remarkable hypoactivity in the light-dark test.

Conclusion Low concentrations of GQDs were relatively non-toxic. However, GQDs disrupt the progression of embryonic development at concentrations exceeding 50 μg/mL.

Key words: Graphene quantum dots; Zebrafish; Embryo; Developmental toxicity

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INTRODUCTION

Graphene and its derivatives have attracted tremendous research interest because of their unique composition and physicochemical properties[1-6]. Graphene, which is composed of sp^2 hybridization carbon atoms of two-dimensional (2D) single-atom-thick materials, possesses features such as large surface area, favorable mechanical property, and a superior

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[a]These authors contributed equally to this work.

[#]Correspondence should be addressed to ZHANG Hong, Tel: 86-931-4969344, Fax: 86-931-8272100, E-mail: zhangh@impcas.ac.cn; LIU Bin, E-mail: liubkq@lzu.edu.cn

Biographical notes of the first authors: WANG Zhen Guo, male, born in 1987, postgraduate, major in stomatology; ZHOU Rong, female, born in 1984, postdoctor, major in radiobiology.

thermal and chemical stability[1,7]. These features are useful for various applications including in optoelectronic devices, energy storage media, and drug-delivery systems[1,7]. The new graphene quantum dots (GQDs) have found extensive application for use in biosensors as well as drug and gene delivery[8-11] because of their chemical stability, electronic properties, and photoluminescence (PL)[12-16]. However, the potential biological toxicity of GQDs has become a health risk because of their inherent chemical composition and nanoscale properties[17-18]. Further research such as *in vitro* and *in vivo* imaging studies of the toxic effects of GQDs, is greatly required to ensure their safety in bio-applications. Several cell lines such as neurosphere cells, pancreas progenitor cells, cardiac progenitor cells, human osteosarcoma (MG-63) cells, MC3T3 cells, two different human breast cancer cell lines MDA-MB-231 and T47D, as well as HeLa cells have been used to evaluate the cytotoxicity of GQDs using the methylthiazolyldiphenyl-tetrazolium bromide assay. The results of these studies suggested that GQDs have low cytotoxicity and were promising for bio-application such as *in vitro* and *in vivo* imaging studies[14,19-23]. Although the assessment of GQDs' toxicity in *in vitro* cell culture is fairly simple and effective, it is difficult to obtain correlative effects in *in vivo* systems. Therefore, there is very limited research focusing on the *in vivo* toxicity study of GQDs. Nurunnabi et al.[24] assessed the *in vivo* toxic effect of GQDs using a long-term *in vivo* study, and the results indicated that the GQDs did not cause significant toxicity in the treated animals. However, there is a considerable lack of *in vivo* data evaluating the developmental toxicity of GQDs.

Zebrafish (*Danio rerio)* is one of the most promising *in vivo* model systems for toxicity studies[25]. Zebrafish is gaining popularity as the reliable toxicity model of choice because of its usability, inexpensiveness, optical transparency, and high homology to the human genome[26-27]. Recently, zebrafish have been widely used to evaluate the toxicity of various nanomaterials such as silica dioxide nanoparticles, nanosilver, and Cadmium Telluride (CdTe) QDs[28-33]. It has been widely proven that zebrafish is an inexpensive and facile model for the rapid evaluation of the potential toxicity and biodistribution of nanomaterials[34].

In this study, zebrafish were used to study the developmental toxicities associated with exposure to low concentrations of GQDs. To investigate the effects of GQDs on zebrafish embryonic development, a series of assessments including embryonic mortality, hatch rate, malformation, body length, heartbeat, swimming behavior, and GQDs uptake were performed. We selected embryonic toxicity and larval behavior as co-indicators for evaluating the GQDs' toxicity because they are more beneficial and comprehensive parameters in safety evaluation for biomedical application.

MATERIALS AND METHODS

GQDs Preparation and Characterization

The graphene oxide (GO) was prepared from natural graphite powder (325 mesh) in accordance with the modified Hummers method[35]. For the typical preparation of the GQDs as previously performed by Zhu[36], 10 mL of GO (about 2 mg/mL) was mixed with 1 mL of ammonia solution (28 wt. % in water) and 6 mL of hydrogen peroxide (H_2O_2 30 wt. % in water). Then the mixture was transferred to a Teflon-lined stainless-steel autoclave and heated at 180 °C for 8 h. The reaction mixture was cooled to room temperature, and then filtered through a 220 nm microporous membrane (retained molecular weight, 1000 Da). The prepared solution was dialyzed in a dialysis bag for one day, and the water was frequently renewed.

A transmission electron microscope (TEM, JEOL, JEM-2010, Japan) was used to observe the size of the GQDs and their size distribution was measured using the Image software (National Institutes of Health, USA). The Fourier transform infrared (FTIR) spectrum was observed using a FTIR spectrometer (Nexus 870, Nicolet, USA). The PL intensity of the GQDs was determined using a fluorescence spectrophotometer (Cary Eclipse, Varian). All other chemicals used in the study were analytical reagents.

Zebrafish Husbandry and Embryos Collection

Adult zebrafish of the wild-type strain (AB) were raised and maintained at 28±1 °C with a 14 h light/10 h dark photoperiod (lights on at 8: 00) in a recirculation system. The fish water supplied to the system was filtered by reverse osmosis (pH 6.5-7.5), and instant ocean salt was added to the water, to raise the conductivity to 450-500 μs/cm. The zebrafish were fed twice daily with decapsulated, freshly hatched brine shrimps (Brine Shrimp Direct, USA) according to the description of Zhou[37].

The zebrafish embryos were obtained from

spawning adults in tanks overnight with a sex ratio of 1:1. The embryos were collected within 1 h after the light was switched on and washed using standard zebrafish E3 culture medium (5 mmol/L NaCl, 0.33 mmol/L $CaCl_2$, 0.33 mmol/L $MgSO_4 \cdot 7H_2O$, and 0.17 mmol/L KCl). The zebrafish use and handling protocol conformed to the Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Use and Care Committee (IAUCC) of the Lanzhou University. At 4 h post-fertilization (hpf), the embryos were examined under a dissecting light microscope (Nanjing Jiangnan Novel Optics, China), and the specimens that had developed normally were selected for the further experiments according to the description of Kimmel[38].

Zebrafish Toxicity Test

The zebrafish embryos were exposed to the GQDs (0, 12.5, 25, 50, 100, and 200 μg/mL) for 4-96 hpf and then assessed for toxicity. The exposure concentrations and period, as well as the toxicological endpoints for the zebrafish used for each experiment are listed in Table 1. The toxicological endpoints were determined based on previous reports in the literature[28-31,38]. Each group consisted of 90 embryos randomly divided into three replicate groups. The embryos were kept in sterile 96-well plates with one embryo per well containing 200 μL of the solution. The plates were covered with sealing film to prevent evaporation. The GQDs solutions were renewed every 24 h, and the mortality of the zebrafish was recorded at 120 hpf.

Normal embryos were exposed to control vehicle and GQDs (12.5, 25, 50, 100, and 200 μg/mL) from 4-24 hpf. Ten embryos were selected randomly from control and experimental groups. The zebrafish embryonic spontaneous movement (5 min) was recorded using a stereoscopic dissecting microscope (Motic, SMZ-161, Motic China Group CO., LT, China) and Media Cruiser recording software (Canopus Corporation, Kobe, Japan). Data were analyzed using the EthoVision XT 10.0 software (Noldus Information Technology, Wageningen, Netherlands).

After exposure of the normal embryos to the control vehicle and GQDs (12.5, 25, 50, 100, and 200 μg/mL) from 4-48 hpf, the heart beats of the zebrafish were determined at 48 hpf. The zebrafish larvae were anesthetized using 0.01% MS-222 (Sigma, USA) and the heartbeats (1 min) were measured using a stereoscopic dissecting microscope and Media Cruiser recording software. The data was analyzed using the EthoVision Heartbeat Detector software (Noldus Information Technology, Wageningen, Netherlands).

When normal embryos were exposed to the control vehicle and GQDs (12.5, 25, 50, 100, and 200 μg/mL) from 4-72 hpf, the hatch rate was measured at 72 hpf. The normal embryos (4 hpf) were exposed to the control vehicle and GQDs (200 μg/mL) for 96 hpf, and then the malformation of the zebrafish were observed using a stereoscopic dissecting microscope. The normal embryos (4 hpf) were exposed to the control vehicle and GQDs (12.5, 25, 50, 100, 200 μg/mL) for 120 hpf, and then the body length of the zebrafish were measured.

Larval Behavioral Assay

The larval behavioral testing was performed at 144 hpf between 13:30 and 16:00. The larvae were cultured in a 96-well plate at a density of one embryo per well. For testing, the larvae were placed in fresh E3 culture medium without the test substances. The visible light test allowed larvae to first acclimate to the light conditions in the well for 20 min, and then locomotor activities were recorded for the ensuing 5 min using a Noldus tracking device (Noldus Information Technology, Wageningen, Netherlands) and Media Cruiser recording software. Videos of the locomotor activities of the larvae were assessed to calculate the total swimming distance (mm/5 min) and speed (mm/s) using the EthoVision XT 10.0 software.

Table 1. Experimental Design of Toxicity Study

Toxicological Endpoints	Exposure Concentrations (μg/mL)	Result
Mortality (120 hpf)	0, 12.5, 25, 50, 100, and 200	Figure 2A
Hatch rate (72 hpf)	0, 12.5, 25, 50, 100, and 200	Figure 2B
Spontaneous movement (24 hpf)	0, 12.5, 25, 50, 100, and 200	Figure 3
Heart beats (48 hpf)	0, 12.5, 25, 50, 100, and 200	Figure 4
Malformation (96 hpf)	0 and 200	Figure 5
Body length (120 hpf)	0, 12.5, 25, 50, 100, and 200	Figure 6
GQDs uptake and distribution (96 hpf)	0 and 200	Figure 7
Larval behavior assay (144 hpf)	0, 12.5, 25, 50, 100, and 200	Figures 8 and 9

The light-dark test was conducted as previously described in the literature[39]. Briefly, the larvae were allowed to acclimate to the light for 10 min, and then locomotor activities during the 25-min light-to-dark (5 min for each period) transitional stimulation. The data of the locomotor activities of the larvae were assessed using the EthoVision XT 10.0 software to calculate the total swimming distance (mm).

GQDs Uptake and Distribution Assessment

Normal embryos (4 hpf) were exposed to the control vehicle and GQDs (200 μg/mL) for 96 hpf. The embryo were washed with the E3 culture medium, anesthetized using 0.01% MS-222 (Sigma, USA) at 96 hpf, and then the zebrafish larvae were observed under a fluorescence microscope (Olympus BX53, Japan), for GQDs uptake. The larval fluorescence was measured and quantified using the Image-Pro Plus software (Media Cybernetics, Inc., Silver Spring, MD, USA).

Statistical Analysis

Each experiment was replicated thrice. All results were expressed as mean±standard error (SE). The one-way analysis of variance (ANOVA) was used to detect the significant differences between the control and exposure groups (P<0.05). The figures were plotted using the Origin 8.0 (OriginLab, USA).

RESULTS

Characterization of GQDs

Figure 1A shows the TEM image of the GQDs, which reveals that they are planar nanocrystals. The size and morphological analysis indicated that the GQDs are uniform with a diameter of about 2-5 nm (Figure 1B). Figure 1C shows that the PL intensity of the GQDs occurred at an emission wavelength of 490 nm. Figure 1D presents the FTIR results for the GQDs, which demonstrates the existence of -OH, C-OH, and C-O groups at about 3407, 1384, and 1110 cm^{-1}, respectively. The presence of the oxygen-functionalized groups renders the GQDs water-soluble. Furthermore, the peak at about 3190 cm^{-1} can be attributed to the stretching vibrations of N-H and the peak at 1358 cm^{-1} are attributed to the vibration of C-N. These results indicate that nitrogen

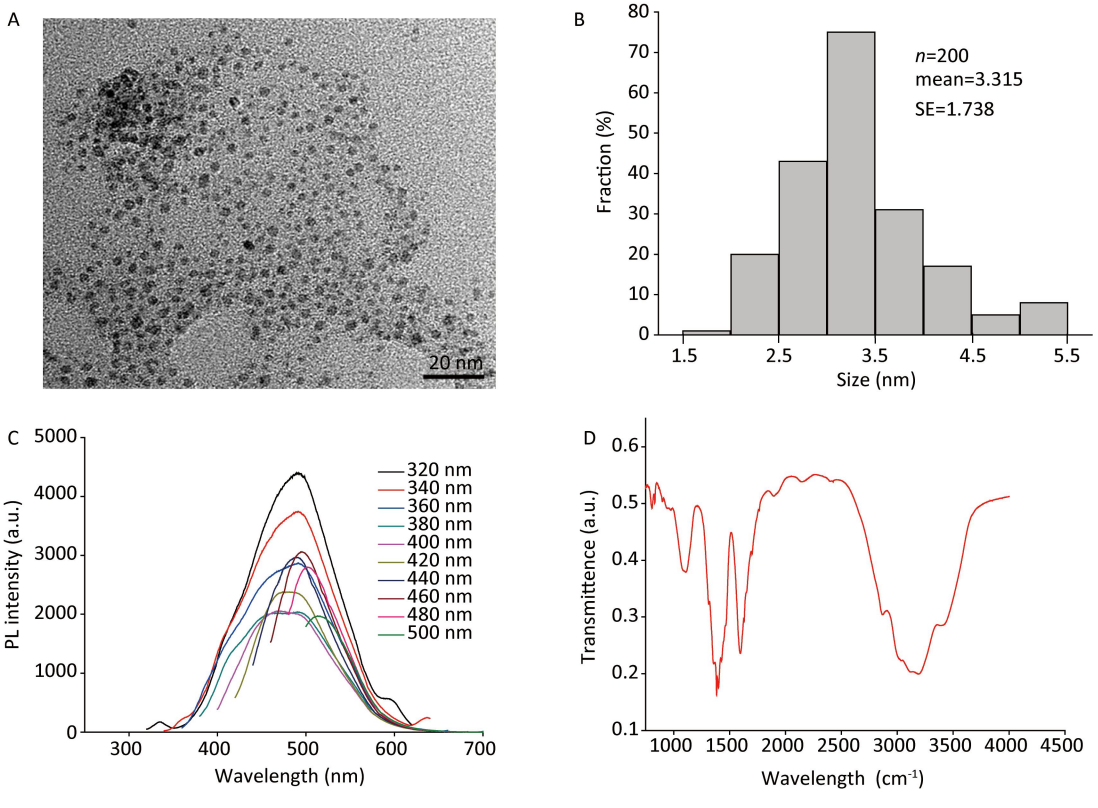

Figure 1. Characterization of graphene quantum dots (GQDs). (A) TEM image of GQDs (scale bar=20 nm); (B) Size distribution of GQDs; (C) PL intensity of GQDs; (D) FTIR spectra of GQDs. TEM, transmission electron microscopy; PL, photoluminescence; FTIR, Fourier transform infrared.

is present on the surface of the GQDs. All of these functional groups are significant to the water-solubility of the GQDs.

GQDs-induced Mortality and Hatchability of Zebrafish

To evaluate the possible toxicity of the GQDs (12.5, 25, 50, 100, and 200 μg/mL) to zebrafish embryos, the hatchability and mortality were measured at 72 and 120 hpf, respectively. As shown in Figure 2A, there was no significant difference in the mortality at the low concentration (12.5 and 25 μg/mL). The mortality of the 50, 100, and 200 μg/mL-treated groups increased significantly compared to that of the control group. The normal embryos had a hatching period from 48-72 hpf. Figure 2B shows that the hatching rate of the 12.5, 25, 50, and 100 μg/mL-treated groups was not significantly different compared to the controls during the 72 h exposure period. The hatchability of the 200 μg/mL-treated group (73.33%) was significantly lower than that of the control was (87.67%). Our data showed that exposure to the GQDs caused a dose-dependent embryonic toxicity.

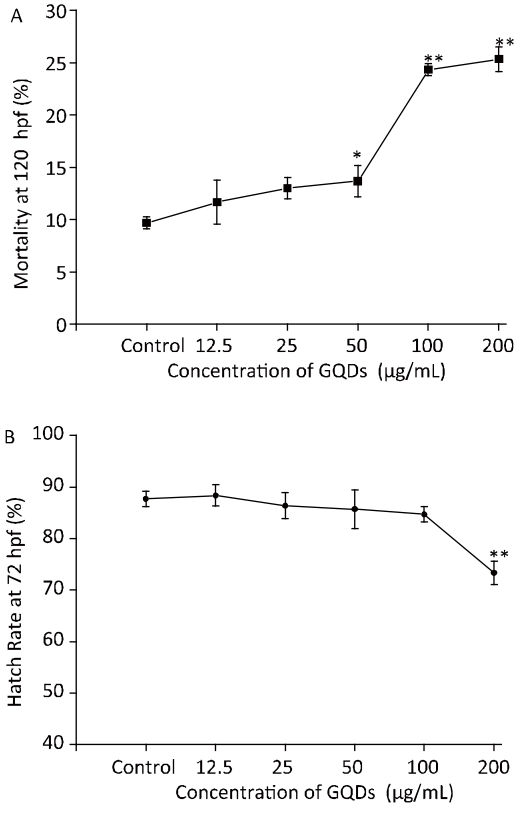

Figure 2. Effects of graphene quantum dots (GQDs) on zebrafish (A) mortality at 120 hpf and (B) hatch rate at 72 hpf (n=30); $^{*}P$<0.05 and $^{**}P$<0.01 compared to control. Values represent the mean±SE of three replicates.

Effect of GQDs on Zebrafish Embryonic Spontaneous Movement at 24 hpf

The zebrafish embryonic spontaneous movement (1 min) reduced with increasing GQDs concentration at 24 hpf. As shown in Figure 3, treatment with GQDs at 12.5 and 25 μg/mL did not show toxicity compared to the control; however, the spontaneous movements of the 50, 100, 200 μg/mL-treated groups (3.11, 2.47, and 3.57, respectively) were lower than that of the control group was (8.74). This result therefore, provided the evidence to prove the high (P<0.01) embryonic toxicity of the higher doses of GQDs.

Effect of GQDs on Zebrafish Heartbeats at 48 hpf

The frequency of the heart beats of zebrafish during a 1 min period were recorded at 48 hpf after exposure to GQDs at increasing concentrations. As the exposure concentration increased, the heart beats decreased (Figure 4). At the highest concentration (200 μg/mL) the heartbeats of the embryos were lower at 116.34 min^{-1} than that of the control were at 133.08 min^{-1}. The results revealed that the GQDs exposure led to bradycardia in the embryos.

GQDs-induced Malformation of Embryos

The zebrafish were exposed to 200 μg/mL GQDs

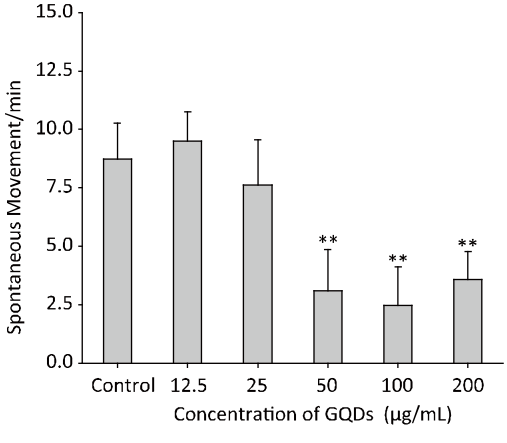

Figure 3. Effects of graphene quantum dots (GQDs) on zebrafish spontaneous movement at 24 hpf (n=10); $^{**}P$<0.01 compared with control. Values represent the mean±SE.

from 4-96 hpf and the malformation were observed at 96 hpf (Figure 5). The exposure of the zebrafish to the control solutions (E3 culture medium) did not cause toxicity (Figure 5A) while the treatment group had significantly higher malformation rates than the control group did. Several malformation patterns (including pericardial edema, vitelline cyst, bent tail, and bent spine) were observed. These observations showed that pericardial edema and vitelline cyst were the typical malformations induced in the embryos by the GQDs.

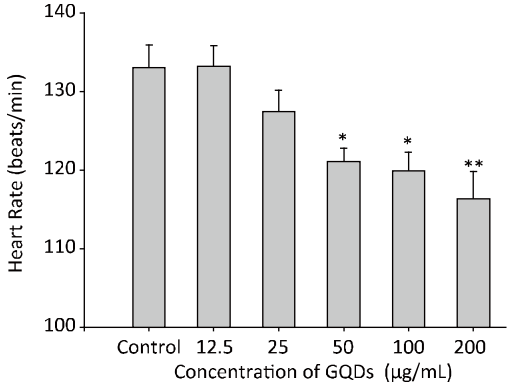

Figure 4. Effects of increasing concentrations of graphene quantum dots (GQDs) on 1 min heartbeats of zebrafish embryos at 48 hpf (n=10). $^{*}P$<0.05 and $^{**}P$<0.01 compared with control. Values represent the mean±SE.

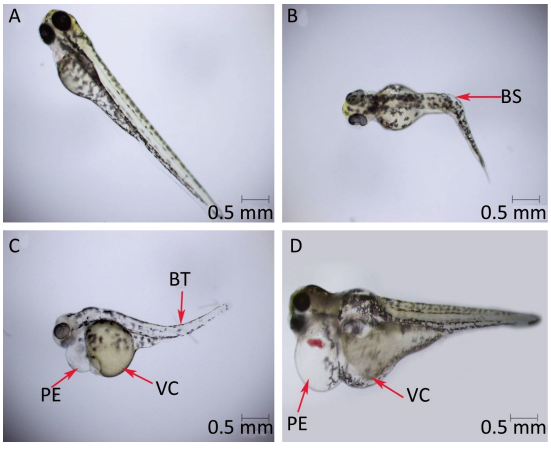

Figure 5. Malformation of zebrafish embryos exposed to 200 μg/mL graphene quantum dots (GQDs). Scale bar=0.5 mm. (A) Normal larvae and (B-D) abnormal larvae. Malformations are indicated by red arrows. PE, pericardial edema; VC, vitelline cyst; BS, bent spine; BT, bent tail.

Effects of GQDs on Body Length of Zebrafish at 120 hpf

The body length of the zebrafish reduced with increasing GQDs concentration at 120 hpf (Figure 6). Compared to the controls, treatment with GQDs at 50, 100, and 200 μg/mL but not 12.5 and 25 μg/mL GQDs showed significant toxicity to the body length of the zebrafish (P<0.05).

GQDs Uptake and Distribution

Based on the unique auto-fluorescence properties of GQDs, we measured the uptake of 200 μg/mL GQDs from the larvae at the end of the exposure period. The GQDs fluorescence was mainly localized in the heart and intestines with no distinguishable fluorescence in other organs (Figure 7A-D). We also observed that the GQDs fluorescence was located in the heart area without flowing in the blood stream. As shown in Figure 7E, the relative fluorescence intensity increased significantly following exposure. In the 200 μg/mL GQDs-treated group, the relative fluorescence intensity was 9.8- and 6.2-fold higher than that of control group was in the heart and intestines, respectively.

Alteration of Larval Locomotor Activity

The locomotor activities of the zebrafish larvae were recorded at 144 hpf, to determine whether GQDs exposure had a persistent effect on larval behavior. In the visible light test, the total swimming distances and average swimming speeds decreased concentration-dependently. Compared to the controls, GQD 50, 100, and 200 μg/mL but not 12.5 and 25 μg/mL treatments caused a significant decrease in the

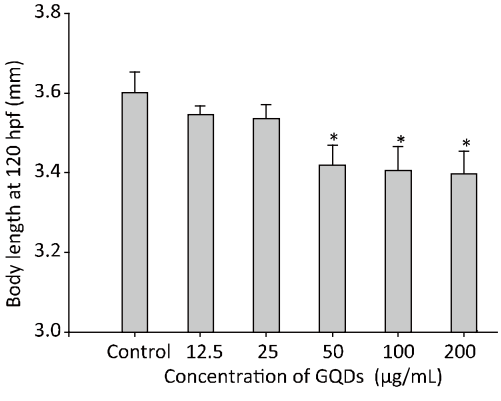

Figure 6. Effects of increasing concentrations of graphene quantum dots (GQDs) on body length of zebrafish at 120 hpf (n=30). $^{*}P$<0.05 compared with control. Values represent the mean±SE.

total swimming distance and the average swimming speed of zebrafish larvae (Figure 8).

In the light-dark test, the locomotor activities were measured using a tracking device during the alternating periods of light and dark. As shown in Figure 9, the movement of the zebrafish in this test was more active during the dark period than it was during the light period. During the dark periods, the lower exposure group (12.5 μg/mL) showed a non-significant hyperactivity compared to the control group. However, higher exposure (25, 50, 100, and 200 μg/mL) to the GQD induced significant hypoactivity.

DISCUSSION

Environmental exposure to nanomaterials is inevitable since they have become a part of our daily life. Increasing attention has been focused on nanotoxicity research. The zebrafish is a promising model for assessing biomaterial nanotoxicity[40]. The millimeter-sized zebrafish embryos allow investigators to study *in vivo* toxicity and nanomaterial uptake in the entire organism[41]. In this study, we found that exposure to GQDs (12.5, 25, 50, 100, and 200 μg/mL) during the 4-96 hpf period had persistent effects on the behavior of larval zebrafish.

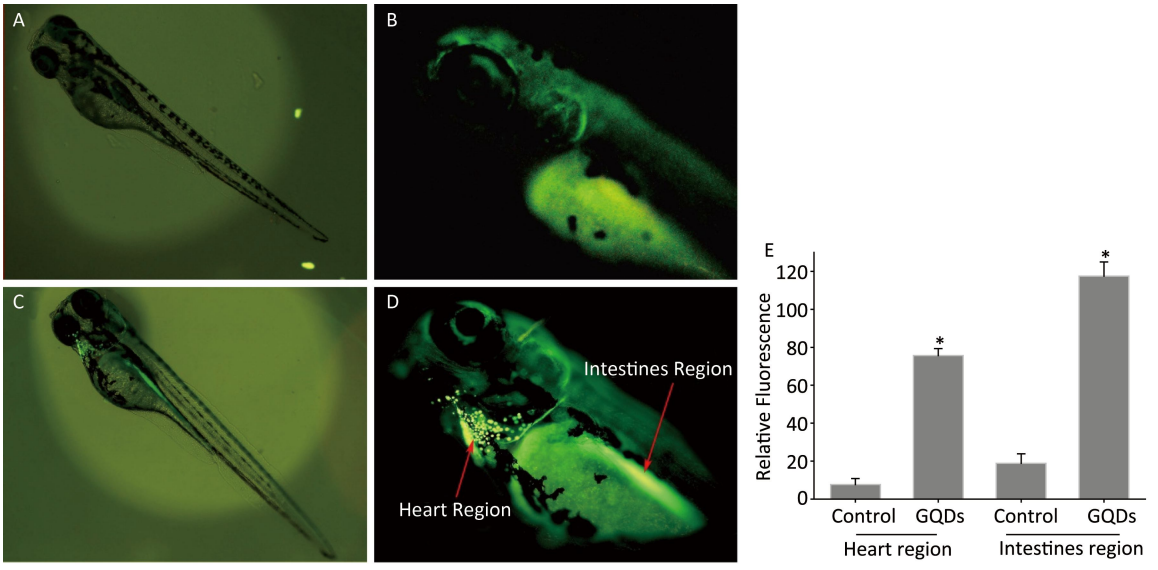

Figure 7. Graphene quantum dots (GQDs) uptake by zebrafish larvae at 120 hpf. Control groups (A and B) and GQDs fluorescence (C and D) was localized in the intestines and heart region. (E) Relative fluorescence intensity was significantly elevated compared to control group (n=10). $^{*}P$<0.05 compared with control. Values represent the mean±SE of three independent experiments.

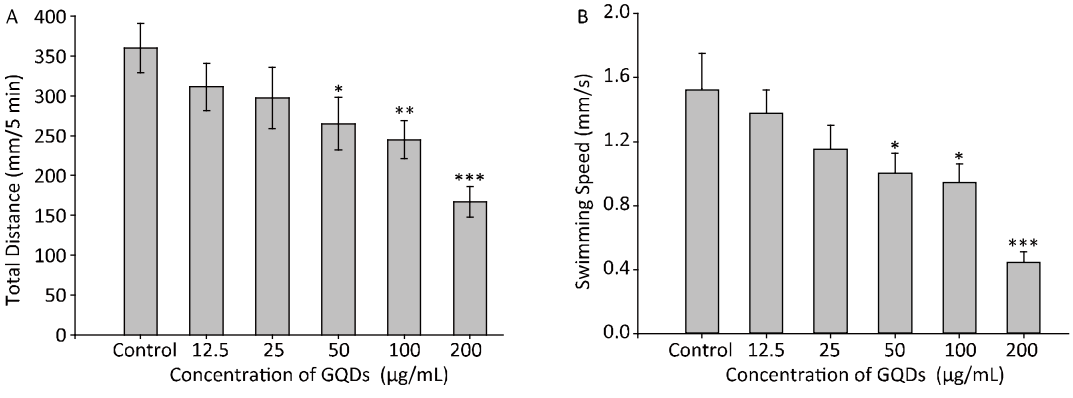

Figure 8. Effects of increasing concentrations of graphene quantum dots (GQDs) on total distance and swimming speed of zebrafish larvae at 144 hpf (n=30). $^{*}P$<0.05, $^{**}P$<0.01, and $^{***}P$<0.001 compared with control. Values represent the mean±SE.

Our findings demonstrated that exposure to GQDs led to embryonic developmental toxicity in zebrafish and affected larval locomotor activity.

In vitro experiments showed that GQDs produced low cytotoxicity[14,20-23,42]. However, the determination of the *in vivo* toxicity of the GQDs is also necessary. Zebrafish embryos are more sensitive to external substances at the earlier rather than larval or adult stages. Therefore, we selected the embryonic period (4-96 hpf) to evaluate the GQDs for potential toxicities. Duan and Zhang et al.[29,31] reported that Cadmium Telluride (CdTe) QDs induce serious malformations including pericardial edema and vitelline cyst in zebrafish embryos. Similar results were also observed in our study. Figure 5 showed various types of malformation in embryos incubated with 200 μg/mL GQDs including pericardial edema, vitelline cyst, bent tail, and bent spine. Therefore, the pericardial edema and vitelline cyst may occur as common malformations in embryos exposed to QDs. Figure 2 showed that the exposure to GQDs increased the mortality and inhibited the hatchability concentration-dependently. The GQDs decreased hatchability only at the highest concentration (200 μg/mL), and the inhibition of hatchability suggests a direct delay of embryonic development. The findings of Duan et al.[29] suggested that the CdTe QDs strongly inhibited zebrafish hatchability. The hatchability of the 20 nmol/L (0.0048 μg/mL)-treated group (16.67%) was much lower than that of the control were (95.42%)[29]. The study by Zhang et al.[31] indicated that the hatchability decreased significantly after zebrafish embryos were exposed to of 200 nmol/L (0.048 μg/mL) CdTe QDs coated with thioglycolic acid[31]. Compared to the CdTe QDs, the GQDs showed low toxicity.

To further investigate the possible mechanisms underlying the embryonic and cardiac toxicity, we measured the uptake and biodistribution of GQDs in zebrafish at the end of the exposure. The results showed that the GQDs were transferred from the solutions into the heart and intestinal region of the embryos (Figure 7). In the heart, the GQDs fluorescence did not change with the blood flow. Furthermore, the measurement of the heartbeats indicated that as the exposure concentration increased, the heart beats of the zebrafish embryos decreased (Figure 4). Therefore, we speculated that the accumulation of the GQDs in the heart might be responsible for the bradycardia observed in the embryos. However, more studies are required to

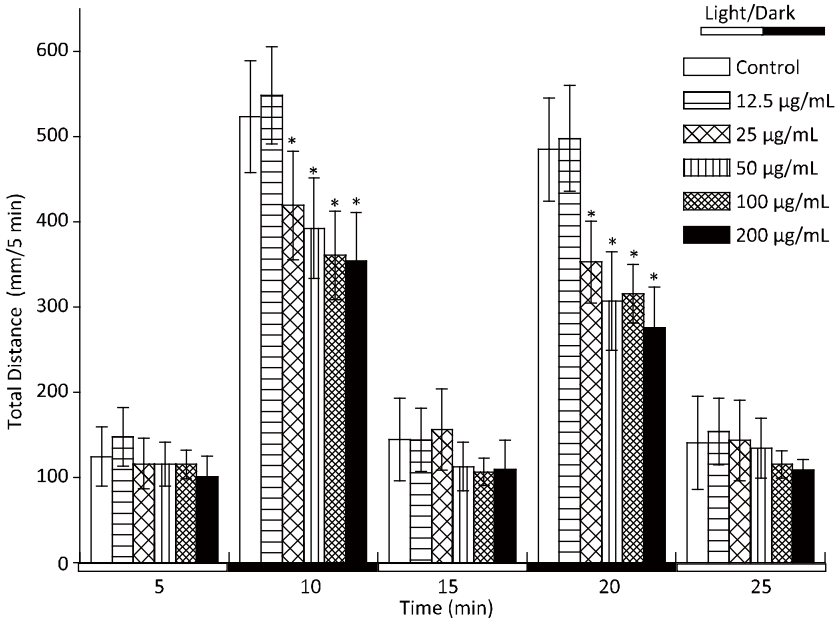

Figure 9. Effects of increasing concentrations of graphene quantum dots (GQDs) on total distance (mm/5 min) of zebrafish larvae after a 25-min light-to-dark photostimulation at 144 hpf (n=30). Light and dark periods are denoted by white and dark bars at the bottom. $^{*}P<0.05$ compared with control. Values represent the mean±SE.

clarify the mechanisms of the GQDs-induced bradycardia.

Currently, most research focused on investigating the mechanism of the toxic effects induced by acute exposure to graphene oxide and its derivatives (i.e., GQDs) is conducted in both *in vivo* and *in vitro* models [24,43-44]. While these studies are critical and provided informative data, there are other areas of potential importance regarding graphene oxide and its derivatives (i.e., GQDs). These pertinent areas of GQDs related studies that have yet to be explored include acute exposure associated with persistent effects. We observed the spontaneous movement of zebrafish embryos at 24 hpf (Figure 3). The results revealed that embryos exposed to the lower concentration (12.5 μg/mL) of GQDs showed substantial spontaneous movement. However, at higher concentrations of GQDs (50, 100, and 200 μg/mL) the embryos showed less spontaneous movement. We, therefore, suggest that the lower spontaneous movement of zebrafish embryos may be associated with developmental delay. In this study, the hatchability of the zebrafish embryos was inhibited concentration-dependently following exposure to the GQDs and the low hatchability indicated embryonic developmental delay.

Behavioral analysis often serves as a sensitive tool to detect the sub lethal effects of chemicals[45]. In addition, the larval zebrafish is emerging as a promising high-throughput model for neurobehavioral research because of its well-characterized genome, robust behavioral responses, and physiological similarity to humans[46-47]. Our data showed that the total distance (mm/5 min) and swimming speed (mm/s) were decreased concentration-dependently in the visible light test (Figure 8). As shown in Figure 9, the light-dark periods produced a consistent pattern of locomotor activity. In visible light, the movement of the larval zebrafish, and then the activity increased slowly during the 5-min period. During the dark period, the zebrafish larvae activity first increased rapidly and markedly, and then it slowly decreased with time. The larval zebrafish displayed a biorhythm during which the larvae became hyperactive following exposure to sudden darkness and then slowed down, which was consistent with the previous reports[29-31,48-50]. The above findings suggest that the GQDs disturbed the neurobehavior of the larval zebrafish. However, the physiological and biochemical mechanisms of the GQDs-induced locomotive behaviors in response to the photostimulation are still unclear. Previous studies have indicated that the involvement of motoneurons and muscle fibers was considered as a critical factor in the overall locomotive behavior[51-54]. Abramsson et al.[55] showed that the zebrafish amyloid precursor protein-b is required for motor neuron guidance and synapse formation. Moreover, other studies indicated that the whole-body cortisol level is the main mediator of physiological response to stress in zebrafish[56-57]. We speculated that the alteration in the larval locomotor activity induced by GQDs treatment might be related to changes in the amyloid precursor protein-b expression and cortisol level. Further studies are necessary to elucidate the potential mechanisms of the GQDs-induced biochemical and physiological changes and to explore the stress-related behavioral responses in the larval zebrafish.

CONCLUSION

In summary, the zebrafish is a reliable and convenient model for assessing potential nanotoxicity of caused by GQDs exposure. In this study, after zebrafish embryos were exposed to GQDs their mortality increased while their hatchability, heart rate, and spontaneous movement decreased concentration-dependently. In addition, as the concentration of GQDs increased, the locomotor activities of zebrafish larvae also changed. Our findings demonstrated that GQDs induced developmental nanotoxicity, which resulted in persistent effects on zebrafish larvae. Therefore, we speculated that the exposure to high concentrations (>50 μg/mL) of GQDs might constitute a developmental hazard to zebrafish. The developmental toxicity observed would be useful in establishing environmental quality standards to protect human health. Finally, bio-safety evaluations and the biological mechanisms of GQDs should be elucidated in further studies.

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