Original Article

Toxicity of Multi-Walled Carbon Nanotubes, Graphene Oxide, and Reduced Graphene Oxide to Zebrafish Embryos*

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Abstract

Objective This study was aimed to investigate the toxic effects of 3 nanomaterials, i.e. multi-walled carbon nanotubes (MWCNTs), graphene oxide (GO), and reduced graphene oxide (RGO), on zebrafish embryos.

Methods The 2-h post-fertilization (hpf) zebrafish embryos were exposed to MWCNTs, GO, and RGO at different concentrations (1, 5, 10, 50, 100 mg/L) for 96 h. Afterwards, the effects of the 3 nanomateria on spontaneous movement, heart rate, hatching rate, length of larvae, mortality, and malformations ls were evaluated.

Results Statistical analysis indicated that RGO significantly inhibited the hatching of zebrafish embryos. Furthermore, RGO and MWCNTs decreased the length of the hatched larvae at 96 hpf. No obvious morphological malformation or mortality was observed in the zebrafish embryos after exposure to the three nanomaterials.

Conclusion MWCNTs, GO, and RGO were all toxic to zebrafish embryos to influence embryos hatching and larvae length. Although no obvious morphological malformation and mortality were observed in exposed zebrafish embryos, further studies on the toxicity of the three nanomaterials are still needed.

Key words: Zebrafish Embryos; Toxicity; Multi-Walled Carbon Nanotubes; Graphene Oxide; Reduced Graphene Oxide

Biomed Environ Sci, 2014; 27(9): 676-683 *doi: 10.3967/bes2014.103* *ISSN: 0895-3988*

www.besjournal.com (full text) *CN: 11-2816/Q*

INTRODUCTION

Recently, carbon nanomaterials have attracted considerable attention in different areas of nanotechnology research. And more studies have been conducted on graphene, a one-atom-thick monolayer of sp2-bonded carbon atoms arranged in a two-dimensional honeycomb structure[1], due to its unique optical, electrical, mechanical, and thermal properties. One of the major methods of preparing large amounts of graphene is reducing graphene oxide, and graphene prepared by this method is called reduced graphene oxide (RGO)[2]. Carbon nanotubes (CNTs), a cylinder made of graphene[3], have many properties similar to those of graphene. Graphene oxide (GO), the product of chemical exfoliation of graphite, is the most important

*This work was supported by the Specialized Research Fund for the Doctoral Program of Higher Education (200800191013) and the Fundamental Research Funds for the Central Universities.

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derivative of graphene with the presence of oxygen functional groups such as carboxylates, epoxides, and hydroxyls at the basal planes and edges of graphene sheets[4]. Because of their unique and desirable characteristics, the application of nanomaterials, such as RGO, CNTs, and GO, have significantly increased in the fields of field-effect transistors, chemical sensors and biosensors, organic solar cells, and flexible displays[5-7]. With more application of nanomaterials in the production of goods used in our daily life, such as pharmaceutical, biomedical, cosmetic, and sporting products, it is almost unavoidable for them to release into the environment through air, water, and soil. So, besides the benefit from the use of nanomaterials products, it is very important to improve the people's awareness about toxicity of these nanomaterials to prevent their harmful environmental effects[8-9]. In addition to immediate effects, the potential toxicity to the environment after exposure to nanomaterials remains uncertain and close attention should be paid to it. Therefore, the study to determine nanotoxicity has great importance and high scientific, social, and economic value[10]. In recent years, some studies focused on the toxicity of metallic nanoparticles, semiconductor quantum dots, carbon materials and others[11-14]. However, the environmental risks of the novel carbon nanomaterials still remain unclear. Surface characteristics, different structural features, and nanoparticle aggregation in actual environment may change their toxicity[15]. In the recent years, a few studies reported the environmental effects of carbon nanomaterials. Zhang et al. reported some studies on the toxicological responses of carbon nanomaterials to different cell types and mice, and discovered that multi-walled carbon nanotubes (MWCNTs) was very important factor in cytotoxicit due to its surface hydrophilicity[16-22]. Zhu et al.[23] demonstrated that aggregation of buckminster- fullerene (nC_{60}) decreased the survival and hatching rates of zebrafish. Zhang et al.[24] and Liu et al.[25] reported the respective effect of graphene and GO on human health. Akhavan et al.[26] investigated the toxicity of graphene and GO against bacteria. In addition, the effect of graphene on terrestrial plants was also reported[27]. These carbon nanomaterials have a certain effect on human health, plants, animals, and so on. However, the toxicity of RGO, GO, and MWCNTs in aquatic environment remains unclear. As these nanomaterials have been widely studied and applied, their effect and the potential future impact on aquatic environment should not be ignored and the knowledge about their fundamental toxicity is needed. So, the present study aiming to evaluate developmental toxicity of the three carbon nanomaterials including MWCNTs, GO, and RGO in aquatic environment will provide useful information about the toxicity of these nanomaterials for the practical and safe applications in future.

Zebrafish, an aquatic vertebrate species, is used as a basic model organism for the assessment of toxicity in aquatic environment according to the reports of the National Institute of Environmental Health Sciences (NIEHS) and the Institute for Environment and Sustainability (IES)[28-29]. Zabrafish embryos is an alternative model for the test for evaluating developmental toxicity of chemicals during early life stage with the characteristics of small-scale, high throughput, and easy observations[30].

In this study, an embryo-larval test was performed by using 2 hpf embryos of zebrafish to investigate the toxicity of MWCNTs, GO, and RGO on early stages of development. The tests' concentrations ranged from 1 mg/L to 100 mg/L. And major endpoints such as spontaneous movement in 20 s, heart rate, hatching rate, length of larvae, mortality, and malformation were examined.

MATERIALS AND METHODS

Nanomaterials: MWCNTs, GO, and RGO

MWCNTs (outer diameter, 10-20 nm; length, 10-30 μm) were purchased from Chengdu Organic Chemicals Co. Ltd., Chinese Academy of Sciences. GO was synthesized from natural graphite using a modified Hummer's method[31]. Briefly, potassium permanganate ($KMnO_4$) (5 g) was slowly added to a suspension of graphite (1 g) and potassium nitrate (KNO_3) (5 g) in 98% sulfuric acid (H_2SO_4, 150 mL) while keeping the temperature under 10 °C. The mixture was heated to 40 °C in an oil bath for 10 h and then stirred at 90 °C for 2 h. Then, a 30% hydrogen peroxide (H_2O_2) solution was added dropwise to the mixture until gas evolution (bubbling) ceased. The mixture was heated to 70 °C in the oil bath for 2 h and then allowed to cool to room temperature. For purification, the resulting mixture was washed and centrifuged for several times, first with 1:1 (v/v) mixture of 20% H_2O_2 and 10% H_2SO_4 and then with deionized water. GO was