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Oxidative stress and immunotoxicity induced by graphene oxide in zebrafish

Minjie Chen [a], Junfa Yin [b], Yong Liang [b,c], Shaopeng Yuan [d], Fengbang Wang [b], Maoyong Song [b,c,*], Hailin Wang [a]

[a] School of Medicine, Jianghan University, Wuhan 430056, PR China
[b] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, PR China
[c] Institute of Environment and Health, Jianghan University, Wuhan 430056, PR China
[d] Department of Pharmacology, Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

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ABSTRACT

Graphene oxide (GO) has been extensively explored as a promising nanomaterial for applications in biology because of its unique properties. Therefore, systematic investigation of GO toxicity is essential to determine its fate in the environment and potential adverse effects. In this study, acute toxicity, oxidative stress and immunotoxicity of GO were investigated in zebrafish. No obvious acute toxicity was observed when zebrafish were exposed to 1, 5, 10 or 50 mg/L GO for 14 days. However, a number of cellular alterations were detected by histological analysis of the liver and intestine, including vacuolation, loose arrangement of cells, histolysis and disintegration of cell boundaries. As evidence for oxidative stress, malondialdehyde levels and superoxide dismutase and catalase activities were increased and glutathione content was decreased in the liver after treatment with GO. GO treatment induced an immune response in zebrafish, as demonstrated by increased expression of tumor necrosis factor α, interleukin-1 β, and interleukin-6 in the spleen. Our findings demonstrated that GO administration in an aquatic system can cause oxidative stress and immune toxicity in adult zebrafish. To our knowledge, this is the first report of immune toxicity of GO in zebrafish.

1. Introduction

Graphene is a single-atom-thick, two-dimensional sheet of hexagonally arranged carbon atoms isolated from its three-dimensional parent material, graphite (Geim and Novoselov, 2007; Kopelevich and Esquinazi, 2007). Because of their unique physicochemical properties, graphene and its derivatives have attracted tremendous research interest (Geim 2009; Allen et al., 2010). They have a great range of potential applications, including hydrogen storage (Wang et al., 2009), catalysis (Scheuermann et al., 2009) and as electrodes (Eda et al., 2008). Applications also exist in biomedical fields, such as cellular imaging (Wang et al., 2010), drug delivery (Sun et al., 2008), biosensing (Zhou et al., 2009; Akhavan et al., 2012), and photothermal therapy (Zhang et al., 2011a). Because of risk factors associated with the manufacture and use of these materials, the potential toxicity of graphene in biological systems is of significant concern (Sanchez et al., 2012; Seabra et al., 2014).

Graphene oxide (GO) is one of the most important graphene derivatives. By formation of hydrogen bonds between polar functional groups on the GO surface and water molecules, a stable GO colloidal suspension is attainable (Shih et al., 2012), suggesting that GO would have advantages over other carbon-based materials for biomedical applications (Bitounis et al., 2013). However, because of its greater solubility and stability in the environment or in serum—properties that prevent its aggregation—GO might have greater uptake when used in medical applications, making its toxicity a very important consideration.

Though studies have shown that nanomaterials might have deleterious side effects (Nel et al., 2006; Song et al., 2012; Song et al., 2013), few reports on GO toxicity have been published so far. Akhavan and Ghaderi (2010) investigated toxicity of GO against Gram-negative (such as *Escherichia coli*), and Gram-positive (such as *Staphylococcus aureus*) bacteria and found that it was effective for use in antibacterial materials. Bacterial cells lost membrane

* Corresponding author at: State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, PR China. Fax: +86 10 62849178.
E-mail address: smsong@rcees.ac.cn (M. Song).

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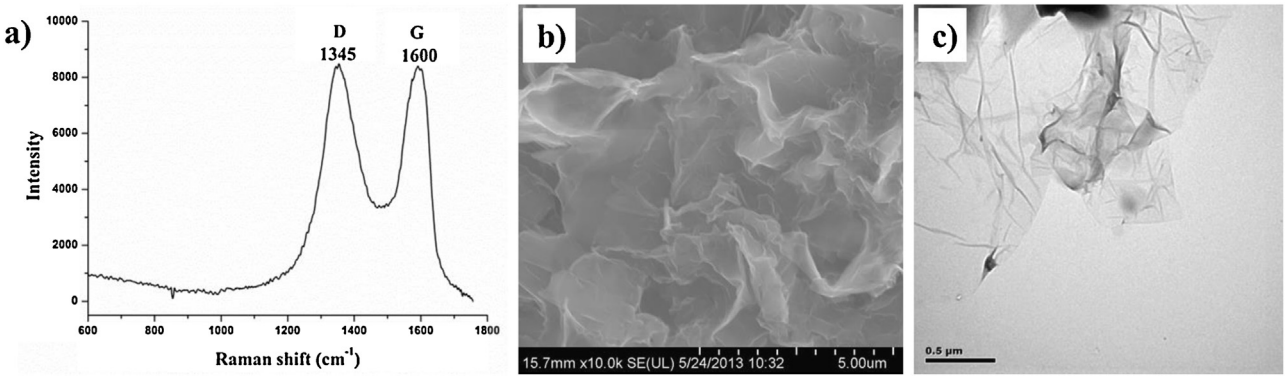

Fig. 1. Characterization of GO: Raman spectra (a), SEM image (b), and TEM images (c).

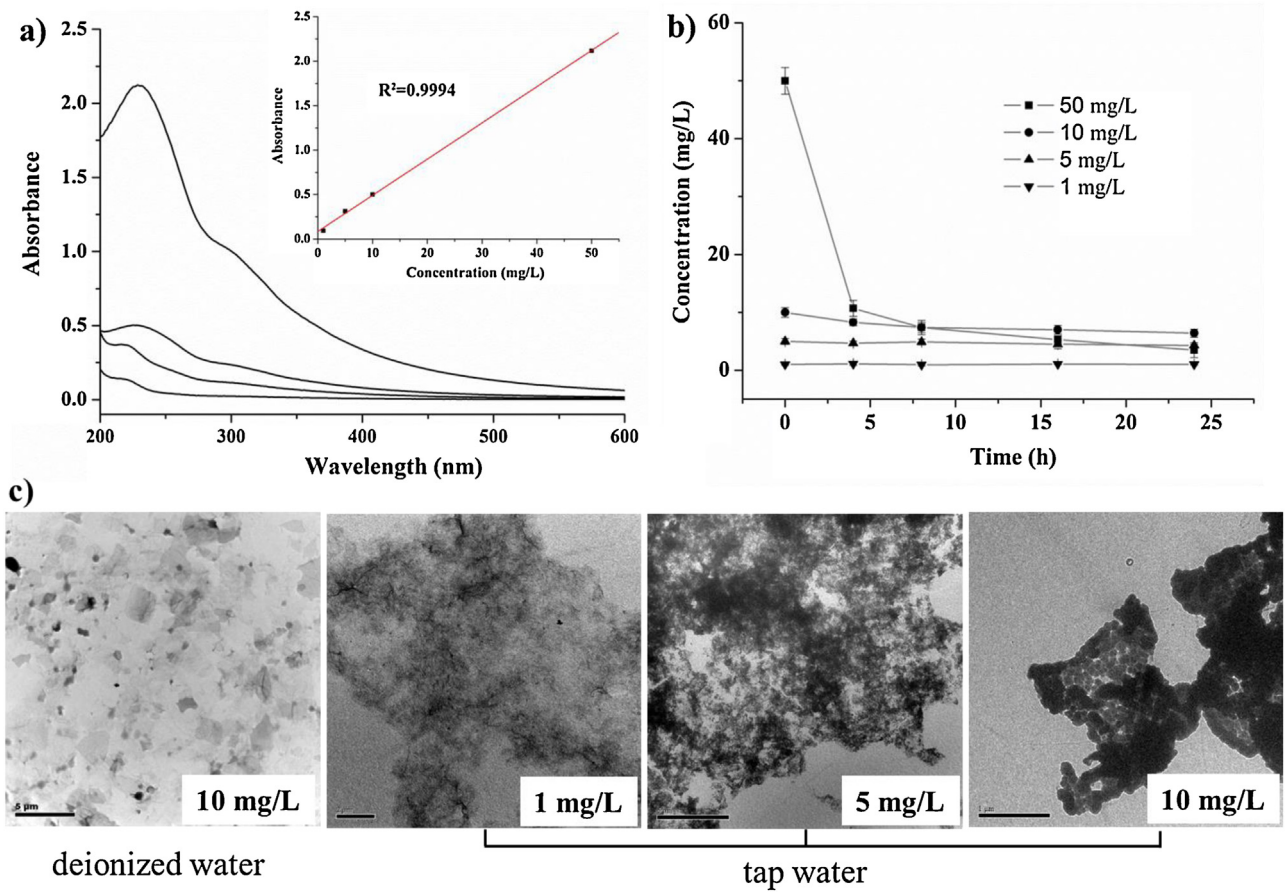

Fig. 2. (a) UV–vis spectra of GO aqueous dispersions at 1, 5, 10 and 50 mg/L (bottom to top) and the linear relationship between absorbance and concentration (insert figure). (b) The residual concentrations of GO in water during exposure. (c) TEM images of GO in deionized water and tap water. All suspensions were laid at room temperature for 24 h, and were diluted to 1 mg/L for TEM image.

integrity upon exposure to GO (Tazawa et al., 2007) based on analysis by transmission electron microscopy (TEM). Interactions between dispersed GO sheets and target cells have been studied in monolayer cultures of lung epithelial cells (Chang et al., 2011) and fibroblasts (Wang et al., 2011). Single-layer GO sheets were internalized and sequestered in cytoplasmic membrane bound vacuoles by human lung epithelial cells or fibroblasts, and induced cytotoxicity at doses above 20 μg/mL after 24 h exposure (Zhang et al., 2010). When injected intravenously into mice, GO induced granulomas in the lungs, liver, spleen and kidney, and was lethal in 4 out of 9 animals (Hu et al., 2011; Wang et al., 2011). Zhang et al. (2011b) also noted deposition and retention of GO in the lungs along with lung tissue injury, inflammation, and granuloma formation in mice receiving intravenous GO. Thus, long-term adverse health effects of GO will need to be carefully considered with respect to design of its applications as well as its release into the environment.

Zebrafish is a powerful vertebrate model for *in vivo* studies of aquatic toxicology (Sawle et al., 2010). To better understand the potential toxicity of GO in zebrafish, we examined its effects on oxidative stress responses and the innate immune system, the latter as indicated by expression of the representative immunological genes tumor necrosis factor α (TNF-α), interleukin-1 β (IL-1β) and interleukin-6 (IL-6). Knowledge about such effects of GO could help to ensure the safe application of this versatile material. Our results should offer insights into potential toxicity mechanisms of GO in aquatic ecosystems.