CARBON 49 (2011) 986–995

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/carbon

# Distribution and biocompatibility studies of graphene oxide in mice after intravenous administration

*Xiaoyong Zhang* [a,b], *Jilei Yin* [a,c], *Cheng Peng* [a], *Weiqing Hu* [a], *Zhiyong Zhu* [a], *Wenxin Li* [a,*], *Chunhai Fan* [a,*], *Qing Huang* [a,*]

[a] *Laboratory of Physical Biology, Shanghai Institute of Applied Physics, Chinese Academy of Sciences, Shanghai 201800, China*
[b] *Graduate School of Chinese Academy of Sciences, Beijing 100049, China*
[c] *TCM Branch of Jiangsu Uion Technical Institute, Lianyungang 222006, China*

ARTICLE INFO

*Article history:*
Received 26 May 2010
Accepted 2 November 2010
Available online 10 November 2010

ABSTRACT

We determined the distribution and biocompatibility of graphene oxide (GO) in mice by using radiotracer technique and a series of biological assays. Results showed that GO was predominantly deposited in the lungs, where it was retained for a long time. Compared with other carbon nanomaterials, GO exhibited long blood circulation time (half-time 5.3 ± 1.2 h), and low uptake in reticuloendothelial system. No pathological changes were observed in examined organs when mice were exposed to 1 mg $kg^{-1}$ body weight of GO for 14 days. Moreover, GO showed good biocompatibility with red blood cells. These results suggested that GO might be a promising material for biomedical applications, especially for targeted drug delivery to the lung. However, due to its high accumulation and long time retention, significant pathological changes, including inflammation cell infiltration, pulmonary edema and granuloma formation were found at the dosage of 10 mg $kg^{-1}$ body weight. More attention should be paid to the toxicity of GO.



## 1. Introduction

Since its isolation in 2004 [1], graphene has attracted tremendous attention due to its unique electronic, thermal, mechanical, and optical properties. Intensive research is ongoing to investigate the quantum physics in this system and potential applications for nanoelectronic devices, transparent conductors, and composite materials [2–9]. Recent studies have showed that GO was useful for biomedical applications, such as drug/gene delivery, biosensing and bioimaging [10–19]. In particular, the potential use of GO as targeted drug delivery vehicle for cancer therapy has attracted considerable interest. Dai and colleagues first demonstrated that GO functionalized with polyethylene glycol was able to delivery aromatic, water-insoluble anticancer drugs into cells, and their intrinsic optical properties were also used for cell imaging [10,11]. Immediately after that, Chen et al. showed that doxorubicin hydrochloride (DXR) could efficiently load onto GO by a simple noncovalent method, the loading ratio of GO could reach 200%, much higher than that of other nanocarriers [12]. They also reported that GO could be modified with magnetic nanoparticle to yield GO based composite, which could move regularly in magnetic field, suggesting that GO may be useful in targeted drug delivery [13]. More recently, Zhang and coworkers reported that GO co-loaded with the two anticancer drugs (doxorubicin (DOX) and camptothecin (CPT)) showed specific targeting to MCF-7 cells, and exhibited remarkably high cytotoxicity when compared to GO loaded with either DOX or CPT only [14]. Moreover, GO was highly physiologically stable and showed excellent biocompatibility

* *Corresponding authors:* Fax: +86 21 59553021.
E-mail addresses: liwenxin@sinap.ac.cn (W. Li), fchh@sinap.ac.cn (C. Fan), huangqing@sinap.ac.cn (Q. Huang).
0008-6223/$ - see front matter 
doi:10.1016/j.carbon.2010.11.005

to various cells and bacteria [20–23]. Thus it appears that GO may be a promising candidate like fullerene ($C_{60}$), carbon nanotubes (CNT), and nanodiamonds (ND) for biomedical applications [10,11,24]. In order to investigate the usefulness of GO in biomedical fields, the experimental information about its adsorption, distribution, metabolism, and excretion (ADME) is urgently needed.

Due to the lack of suitable detection method, little is known about the biological behavior of GO *in vitro*, and no reports have focused on the distribution of GO *in vivo* thus far. Radioactive tracing technique, however, with advantages of high sensitivity, credibility and freedom from interference, has become an excellent approach to obtain information on ADME of nanomaterials *in vivo*. Since $^{99m}$Tc labeling of the fullerenol by Li et al. in 2002 [25], numerous studies have been reported on radiolabeling of a variety of carbon nanomaterials (CNM), including $C_{60}$ derivatives [26], single walled carbon nanotubes (SWCNT) [27–29], multi-walled carbon nanotubes (MWNCT) [30,31], and ND [32,33].

The goal of this study was to determine the distribution and pharmacokinetic profiles of GO in mice, and evaluate its biocompatibility with target organs and red blood cells (RBC). Herein, the tissue distribution and clearance of Rhenium ($^{188}$Re)-GO in mice was determined through an effective and convenient radiotracer technique. Based on the distribution characteristics, its biocompatibility with target organs and RBC was evaluated by a serial of biological assays. The results suggested that $^{188}$Re–GO with excellent radiochemical purity and stability is highly suitable for the study of ADME behavior of GO *in vivo*. The relative long blood circulation half time of GO as well as its excellent biocompatibility with target organs and RBC could be beneficial in use of GO for biomedical applications. To the best of our knowledge, this is the first study to address this issue. And we believed this study will contribute significantly to better understanding the toxicity of GO *in vivo* and will encourage the development of GO in biomedical fields in the near future.

## 2. Materials and methods

### 2.1. Reagents and animals

$^{188}$Re was obtained from an alumina-based $^{188}$W/$^{188}$Re generator (Shanghai Ke-Xing Pharmaceutical Co.); loaded with the $^{188}$W solution supplied by the Oak Ridge National Laboratory (Oak Ridge, TN). GO was prepared by a modified Hummers method and characterized by atomic force microscopy (AFM) and Raman spectroscopy [34]. All the other chemicals used were of analytical grade, obtained from commercial sources and used without further purification.

Kun Ming mice (Sprague–Dawley rats) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd., China. The animals were housed in plastic cages, fed a commercial diet, and given water ad libitum. All animals were checked for the absence of infection for 1 week prior to experiment. Permission of the local ethics committee was obtained, and all animal experiments were performed according to Chinese law and accepted international standards in biomedical research.

### 2.2. Labeling of GO with $^{188}$Re

The radiolabeling of GO was performed by a conventional reduction method based on our previous report [33]. Briefly, 1 mL of GO (1 mg mL$^{-1}$), 50 μl ascorbic acid (40 mg mL$^{-1}$), 70 μl stannous chloride (60 mg mL$^{-1}$), and 1 mCi $Na^{188}ReO_4$ were reacted in water bath at 80 °C for 25 min. After the end of the reaction, 10 μl portions of the mixture were taken and applied at 1.5 cm from the lower end of the strips for determining the labeling yield by paper chromatograph with Whatman No. 1 (1 cm × 13 cm). The strips were developed by saline solution until the solvent reached the top portions. The strips were dried and cut into 1 cm long equal segments. The distribution of $^{188}$Re on paper chromatograph was measured with a gamma-ray counter.

The radioactivity for $^{188}$Re–GO are all at the origin on the chromatography paper developed by saline solution (retardation faction, $R_f = 0$), with $R_f$ value for free $ReO_4^-$ ions being at about 0.9–1. And the labeling yield of $^{188}$Re–GO was calculated by the following equation:

$$\text{Labeling yield} = Y\,[\text{segment0}]/Y\,[\text{segments}(0\text{–}10)] \times 100\%$$

Y [segment 0] represents the counts number of per minute (CPM) of segment 0, while Y [segments (0–10)] represents the CPM of summed over all segments.

### 2.3. Purification of $^{188}$Re–GO and examination of its stability

Before the distribution evaluation, the radiolabeling compound was washed three times with saline to remove unreacted ascorbic acid and stannous chloride. Then the $^{188}$Re–GO was dispersed in Millipore water, serum-free culture medium (RPMI-1640 only), and complete culture medium (RPMI-1640 with 10% fetal bovine serum (FBS)) at room temperature, respectively. Portions (10 μl) from each suspension were applied for determining the radiochemical purity by the manner described above. The radiochemical purity of labeled $^{188}$Re–GO obtained at various time intervals was used to examine its stability *in vitro*.

To assess the stability of $^{188}$Re–GO *in vivo*, 20 Kun Ming mice (male 20 ± 2 g, 6–8 W) were intravenously injected with 200 μl of the $^{188}$Re–GO suspension containing 50 μCi of radioactivity. The mice were then anesthetized with pentobarbital sodium at 1, 3, 6, 12, and 24 h post injection, the anticoagulant blood was collected and its radioactivity was measured with gamma-ray counter. Then the blood was centrifuged at 14,000 rpm for 5 min, the supernatant was discarded and the remaining solid was washed with deionized water. Its radioactivity was measured with gamma-ray counter for comparison with the whole blood radioactivity.

### 2.4. Distribution of GO in mice

The distribution characteristics of GO was performed with 48 Kun Ming mice (male 20 ± 2 g, 6–8 W), which were randomly divided into six groups. Mice were anesthetized with pentobarbital sodium and 20 μCi $^{188}$Re–GO was intravenously administrated to each mouse. At different time points, the mice were sacrificed by dislocation of vertebrae, and important organs