



Synthesis of polymer-functionalized nanoscale graphene oxide with different surface charge and its cellular uptake, biosafety and immune responses in Raw264.7 macrophages[☆]

Bing Wang^{a,*}, Xiaopeng Su^a, Junlong Liang^a, Lifeng Yang^a, Qinli Hu^a, Xinyi Shan^b, Junmin Wan^a, Zhiwen Hu^b

^a Department of Polymer Materials, Zhejiang Sci-Tech University, Hangzhou 310018, China

^b Key Laboratory of Advanced Textile Materials and Manufacturing Technology, Ministry of Education, Zhejiang Sci-Tech University, Hangzhou 310018, China

ARTICLE INFO

Keywords:

GO-PEI
GO-PEG
Raw264.7 cell
Cellular uptake
Cytotoxicity
Immune response

ABSTRACT

Polymer-functionalized graphene oxide (GO) has superior properties such as large surface area, extraordinary mechanical strength, high carrier mobility, good stability in physiological media and low cytotoxicity, making it an attractive material for drug and gene delivery. Herein, we successfully synthesized GO with an average size of 168.3 nm by a modified Hummers' method. Branched polyethylenimine (PEI) and 6-armed polyethylene glycol (PEG) functionalized GO complexes (GO-PEI and GO-PEG) with different zeta potentials of 47.2 mV and −43.0 mV, respectively, were successfully synthesized through amide linkages between the COOH groups of GO and the NH₂ groups of PEI and PEG. Then, the interactions between GO-PEI and GO-PEG complexes and Raw264.7 mouse monocyte-macrophage cells were investigated. The GO-PEI and GO-PEG complexes could both be internalized by Raw264.7 cells. However, compared with the GO-PEG complex, the GO-PEI complex showed higher intracellular delivery efficiency in Raw264.7 cells. Moreover, it was found that the GO-PEI complex not only gathered in endosomes but also in the cytoplasm, whereas GO-PEG gathered in endosomes only. The MTT tests showed that both GO-PEI and GO-PEG complexes exhibited very low cytotoxicity towards Raw264.7 cells when at a low concentration. The cellular immune response test demonstrated the GO-PEG complex enhanced the secretion of IL-6, illustrating it was more stimulus towards macrophage cells. The above results indicated that the GO-PEI complex, with a positive surface charge, demonstrated better potential to be used in effective drug and gene delivery.

1. Introduction

Therapeutic drug and gene delivery to cure diseases has gained significant public attention [1–4]. Nanocarriers such as microcapsules, inorganic nanoparticles, polymeric nanoparticles, nanotubes, etc. have been widely used to deliver therapeutic drugs and genes [5–8]. Recently, graphene-based nanocarriers have shown promise in biomedical applications due to their superior properties such as large surface area, porosity, low toxicity, and chemical stability [9–12]. Currently, graphene has become one of the most widely used materials in the fields of drug delivery, biosensing probes, bioimaging and photothermal agents [13–16]. Various types of graphene and graphene-based nanomaterials such as graphene oxide, graphene nanoribbons, and graphene–metal nanocomposites have been synthesized [17–19]. In particular, researchers have found that nanoscale graphene oxide (GO) exhibits

lower cytotoxicity and higher cellular uptake amount than random larger-scale graphene oxide [20].

Even though GO has shown promise in drug and gene delivery, it has been functionalized with hydrophilic and biocompatible polymers to enhance its stability in physiological environments and further reduce its cytotoxicity. Sun et al. found polyethylene glycol-functionalized GO to be a promising material for cellular imaging and drug delivery; compared to bare GO, GO-PEG was stable in cellular media and exhibited low cytotoxicity [21]. Shan et al. functionalized graphene sheets with biocompatible poly-L-lysine (PLL) and found PLL-functionalized graphene to be water-soluble and biocompatible. Graphene sheets played an important role as connectors to assemble the active amino groups of PLL, which provides a highly biocompatible environment for further functionalization [22]. Zhang et al. functionalized GO with polyethylenimine via electrostatic interactions and found that GO-

[☆] The authors declare no competing financial interest.

* Corresponding author.

E-mail address: wbing388@163.com (B. Wang).

PEI complexes showed higher gene transfection efficiencies compared to bare PEI [23]. Liu et al. studied the *in vivo* behaviors of nanographene sheets (NGSs) with a PEG coating by a fluorescent labeling method. Their work suggested that the NGS-PEG nanomaterial could be efficient in *in vivo* photothermal therapy by intravenous administration [24]. These polymer-functionalized graphene nanomaterials exhibited different morphologies and physical and chemical properties, depending on the synthesis method and modifying polymer materials.

These functionalized graphene nanomaterials are of key significance for studying the influences of graphene-based nanomaterials on cells and human bodies. Researchers in recent years have begun to focus on the interactions between cell functions and graphene-derived agents [25–29]. Several groups have found that nanomaterial toxicity mainly arises from two aspects: first, from the size of the material [30], and second, from the surface chemical modification, which is likely to undermine the cells in many biochemical processes. [31] Some researchers have further studied the interactions between the immune system and graphene-based nanomaterials [32–36]. However, the surface charges of cell cytomembrane and most drugs are widely known to be negative [37]. Therefore, the study of polymer–graphene nanomaterials with different surface charges and their interactions with immune cells (such as macrophage cells) still requires in-depth study.

Herein, we successfully develop two nanocarriers by coating GO with branched polyethylenimine (PEI) or 6-armed polyethylene glycol (PEG). The GO-PEI complex has a positively charged surface, while GO-PEG is negatively charged. The GO-PEI and GO-PEG complexes both show good stability in water and Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS) solution. In addition, our studies focus on the interaction between GO-PEI and GO-PEG complexes and Raw264.7 mouse monocyte-macrophage cells, i.e., the regulation of cell morphology, uptake, cytotoxicity and immune response activity. This work aims to reveal new insights into the biophysical impact of surface charge differences in polymer-functionalized GOs on cell internalization, illuminating details about how a surviving cell might be affected by the surface charge. This study will provide important information towards understanding the biological effects of GO derivatives, which with appropriate surface functionalization could most likely be useful for the development of novel nanocarriers for applications in effective drug and gene delivery.

2. Materials and methods

2.1. Materials

Graphite powder, H_2SO_4 , NaNO_3 , and KMnO_4 were purchased from Merck Chemical Co. (Germany). Branched polyethylenimine (PEI) with a molecular weight of 10 kDa was purchased from Sigma-Aldrich. 6-armed polyethylene glycol (PEG) with a molecular weight of 10 kDa was purchased from Sunbio (China). Raw264.7 mouse monocyte-macrophage cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. Dulbecco's modified Eagle's medium (DMEM) was purchased from Gibco (USA). Fetal bovine serum (FBS) was purchased from Invitrogen (USA). Penicillin-streptomycin solution was purchased from Beyotime (China). All other reagents were of analytical grade and used as received. The water used in all experiments was purified by a Milli-Q water system (Millipore, USA).

2.2. Preparation of GO

GO was first prepared by a modified Hummers' method and then further treated with an ultrasonic cell disruptor. In detail, 1 g of graphite powder was mixed with 24 mL of H_2SO_4 in a flask. After having been stirred for 1 h, 0.5 g of NaNO_3 was added to the mixture, and the flask was then placed into a 0 °C ice bath. After reacting for 2 h, 4 g of KMnO_4 was gradually added into the mixture for an additional 2 h

reaction. Then, the flask was transferred into an oil bath with further stirring for 0.5 h at 38 °C, and 46 mL of H_2O was gradually added into the mixture. After stirring for 15 min at 98 °C, a mixture of 27.5 mL of H_2O and 4 mL of H_2O_2 was added to terminate the reaction. After cooled down at room temperature overnight, the GO was gathered by filtration through a 0.22 μm membrane and washed three times with 10% HCl and distilled water separately. To obtain nanoscale GO, the as-prepared GO solution was further treated with an ultrasonic cell disruptor at 630 W for 3 h.

2.3. Preparation of GO-PEI and GO-PEG complexes

For the preparation of GO-PEI, GO was first dispersed in distilled water at a concentration of 0.5 mg/mL and sonicated for 30 min. Then, 0.5 mL of PEI solution (10 mg/mL) was added into 10 mL of GO solution under sonication for 10 min. Finally, the mixture was stirred at room temperature for 12 h followed by removing redundant PEI via dialysis in a dialysis bag against distilled water for 24 h. GO-PEI was successfully obtained after freeze drying 48 h.

To obtain GO-PEG, 10 mL of GO solution (0.5 mg/mL) was sonicated for 30 min. Then, 5 mL of 6-armed amine-terminated PEG solution (1 mg/mL) was added into the GO solution with further sonication for 10 min. After removing free PEG via dialysis as with GO-PEI, GO-PEG was finally obtained.

2.4. Labeling the GO-PEI and GO-PEG complexes with Cy5.5-NHS ester

The GO-PEI and GO-PEG complexes were separately dissolved in phosphate buffered saline (PBS, 0.1 M, pH = 7.4) solution to final concentration of 0.5 mg/mL. 1 mg of Cy5.5-NHS ester was dissolved in 1 mL of DMSO solution and further diluted by PBS solution to a final concentration of 0.004 mg/mL. Then, the GO-PEI and GO-PEG complexes were each mixed with Cy5.5-NHS ester solution at a volume ratio of 1:1. The mixtures were incubated overnight at 4 °C and then centrifuged at 5000 rpm for 10 min. The precipitate was washed three times with PBS solution and centrifuged at 5000 rpm for 10 min each time. The entire treatment process was protected from light.

2.5. Characterizations of GO and the GO-PEI and GO-PEG complexes

The surface morphology of GO and the GO-PEI and GO-PEG complexes was observed using atomic force microscopy (AFM) and transmission electron microscopy (TEM). First, GO and the GO-PEI and GO-PEG complexes were diluted in ethanol, and then, a few drops of the solution were added to a silicon slice. After drying overnight, the samples were examined using AFM (XE-100E, Korea). For TEM, GO and the GO-PEI and GO-PEG complexes were diluted in ethanol; a few drops of each solution were dropped on copper grids. After drying for several hours, the samples were examined with a TEM (JEM-1230EX, Japan). For Fourier transform infrared spectroscopy (FTIR) analysis, KBr pellets were used to prepare the samples, and then the spectra were recorded using a spectrophotometer (Nicolet 5700, America). The surface compositions of GO, GO-PEI and GO-PEG complexes were investigated via X-ray photoelectron spectroscopy (XPS). The samples were measured using a spectroscope (Perkin-Elmer PHI 5900) equipped with non-monochromatic source operating at 150 W. Dynamic light scattering (DLS) was used to measure the sizes of nanomaterials in different solutions. Nanomaterials were first dispersed in PBS and DMEM containing 10% FBS and then analyzed using a sub-micron scale particle analyzer (Delsa™ Nano, Beckman Coulter, USA) at a temperature of 25 °C.

2.6. Cell culture

Raw264.7 mouse monocyte-macrophage cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences,

Download English Version:

<https://daneshyari.com/en/article/7866159>

Download Persian Version:

<https://daneshyari.com/article/7866159>

[Daneshyari.com](https://daneshyari.com)