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Synthesis of a biocompatible gelatin functionalized graphene nanosheets and its application for drug delivery

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ABSTRACT

A simple and environmentally friendly synthetic route for the preparation of gelatin functionalized graphene nanosheets (gelatin-GNS) was reported by using exfoliated graphene oxide as a precursor, in which gelatin acted as not only a reducing reagent but also a functionalization reagent to guarantee good dispersibility and stability of the GNS in distilled water and various physiological solutions. The obtained biocompatible gelatin-GNS attaching methotrexate (MTX) via strong π-π stacking interaction, exhibited a high drug loading capacity of MTX and excellent ability for controlled drug release. The pH-dependent release behavior of MTX from MTX@gelatin-GNS showed that the release amount under acid conditions is much higher than that under neutral conditions, which experienced a gelatin-mediated sustained release process. From the cytotoxicity assay, we can see that the MTX@gelatin-GNS showed remarkable toxicity while the gelatin-GNS showed nontoxic at appropriate concentration, both of them might be taken up by A549 cells through a nonspecific endocytosis process. The prepared nanohybrids system offers a novel formulation that combines the unique properties of a biodegradable material, gelatin, and graphene for biomedical applications. Therefore, the gelatin-GNS with good stability and biocompatibility can be selected as an ideal drug carrier to be applied in biomedicine studies.

1. Introduction

Since its discovery by K. S. Novoselov and A. K. Geim in 2004 [1], graphene, a one-atom-thick planar sheet of sp^2-bonded carbon atoms densely packed in a honeycomb crystal lattice [2], has become a rapidly rising star among carbon materials, triggering a gold rush to exploit its possible applications both in academics and industry. Graphene is considered as the mother element of some carbon allotropes, which is a basic building block for graphitic materials of all other dimensionalities, and can be converted into fullerenes, carbon nanotube (CNT), or 3D graphite via wrapping, rolling, or stacking, respectively [3]. Because of its unique nanostructure, graphene has many novel properties, such as high surface area, excellent electrical conductivity and electron mobility at room temperature, and has unique thermal and mechanical properties [4]. These characteristics drive the dreams of applying graphene in various areas, such as nanoelectronic devices [5], transparent conductors [6], sensors [7], capacitors [8], and nanocomposite materials [9]. At present, great efforts have been made for the preparation of graphene nanosheets (GNS), such as micromechanical exfoliation of graphite [1], chemical vapor deposition [10], epitaxial growth on electrically insulating surfaces [11], and solution-based reduction of exfoliated graphene oxide (EGO) [12]. Among these methods, the chemical reduction of EGO is the most commonly used approach because it has advantages of low-cost and bulk-scale production. However, the GNS is hydrophobic and tends to form agglomerates or even re-graphitized to graphite due to the strong π-π stacking and Van der Waals interactions which may limit its further biological applications [13]. Therefore, the prevention for aggregation by functionalizing GNS is of vital importance because most of its unique properties are only associated with individual sheets. This obstacle can be overcome through covalent modification or noncovalent functionalization, of which noncovalent strategies, particularly using polymers as functional agent, are more favorable than the covalent ones [14]. The most commonly used reducing agents are hydrazine monohydrate, sodium borohydrydride (NaBH4), p-phenylene diamine, hydroquinone, and sodium hydrosulfite [15–19]. However, these chemicals are hazardous to human health and the environment. Additionally, in the most successful cases, the chemical reduction of the EGO was conducted using hydrazine or hydrazine hydrate as the reducing agent. However, because hydrazine and hydrazine hydrate are highly poisonous and explosive, precautions must be taken

Abbreviations: gelatin-GNS, Gelatin functionalized graphene nanosheets; GNS, Graphene nanosheets; GO, Graphene oxide; EGO, Exfoliated graphene oxide; MTX, Methotrexate; MTX@gelatin-GNS, Methotrexate attached on gelatin functionalized graphene nanosheets; R6G, Rhodamine 6G.

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when large quantities of hydrazine or hydrazine hydrate are used [19]. Consequently, a new approach for effectively converting EGO into stable graphene sheets under mild conditions needs to be explored. Zhang et al. have suggested that the reducing ability of L-ascorbic acid is very close to that of hydrazine monohydrate [20]. Tuan et al. have developed a green and facile approach to produce graphene by using an environmentally friendly reagent, namely, l-glutathione as a reducing agent [21]. Dextran has also been used as an effective, multifunctional reducing agent [22]. Salas et al. have shown that GO can be reduced via bacterial respiration [23]. Very recently, Wang et al. have demonstrated a simple synthesis of soluble graphene via a green reduction of GO in a tea solution [24]. Up till now, biological reduction of GO is still a current topic in graphene research.

Gelatine is a linear polypeptide that consists of different amounts of 18 amino acids which is generally prepared by the partial hydrolysis of collagen, the chief protein component in skin, bones and white connective tissues [25]. It is extremely heterogeneous, composed of polypeptides of various sizes and it possesses a molecular weight distribution in the range of 15,000 to 250,000 [26]. Gelatin has a triple-helical structure and offers distinctive advantages, such as excellent membrane-forming ability, good adhesion, biocompatibility, biodegradability and non-toxicity, non-immunogenic, therefore, it has been widely utilized in the food and pharmaceutical industries [27,28]. It is a good forming material for film and particle with uses in medicine such as plasma expander, wound dressing, adhesive and controlled drug delivery [29]. Moreover, gelatin is known to be an efficient dispersing agent for many colloids and it is not surprising to find that it gives a stable dispersion of CNTs in water [30]. That is because gelatin can stabilize surfaces by the formation of a steric barrier [31]. More significantly, gelatin backbone has abundant amino side chain, which could be oxidized to nitrite, therefore, it could be naturally employed as a reductant due to its mild reductive ability in the synthesis of nanomaterials [32]. Furthermore, the non-polar amino acid chain of the gelatin could immobilize on the surface of graphene through hydrophobic–hydrophobic interactions, which leads to the formation of stable dispersion of graphene [33]. Considering such advantageous properties, herein, we developed a simple approach for the production of GNS by chemical reduction of GO using gelatin as both reducing agent and stabilizing agent in an aqueous solution under mild condition, which open new opportunities for using graphene in a wide range of potential applications.

Nanoscaled drug carriers have emerged as a bridge linking nanotechnology and advanced drug delivery, involving nanoscaled materials such as liposomes [34], nanoparticles [35], carbon nanohorns [36], CNTs [37] and nanoscaled graphene oxide (NGO) [38]. The medicine loaded on these nanoscaled materials by many kinds of mechanisms, such as embedding, surface absorption, hydrogen bonding, and other types of interactions [34,37–39]. At present, some efforts have been performed to explore EGO in drug delivery system. Dai's group initially developed polyethylene glycol functionalized NGO as a nanocarrier to load anticancer drugs via noncovalent physisorption and evaluated its in-vitro cellular uptake capacity [38]. Yang and colleagues investigated the loading and release behaviors of doxorubicin hydrochloride (DXR) on GO, and they found that the weight ratio of the loaded drug to the GO carrier could reach 200% [40]. Inspired by these findings, Zhang et al. functionalized NGO with sulfonic acid and folic acid (FA) groups, which rendered it physiologically stable and provided a specific cell-targeting ability. Furthermore, the controlled loading of two anticancer drugs, doxorubicin (DOX) and camptothecin (CPT), onto the FA-conjugated NGO (FA-NGO) via π-π stacking and hydrophobic interactions was studied [41]. However, until now, reports on application of graphene for drug delivery are relatively rare, due to its poor solubility and stability in physiological environments. On the basis of these observations, a new approach is essential to develop novel biocompatible graphene with high solubility and stability for the application of drug delivery.

Considering the above outlined advantages of graphene and gelatin, we report here the synthesis of gelatin functionalized GNS (gelatin-GNS) for high drug loading capacity of methotrexate (MTX) and excellent ability for controlled drug release behavior with good biocompatibility and physiological stability. The simple and environmentally friendly approach is expected to have a significant impact on nanopharmaceutical products development, including cancer therapy field, as the distribution of drug-loaded nanocarrier can be visualized in vitro and in vivo.

In our research, we focused on a gelatin-GNS mediated drug delivery system and described the chemical synthesis of the nanocarrier, which exhibited excellent biocompatibility and physiological stability, controlled drug loading and release ability and anticancer activity in vitro. Chemical characterization of gelatin-GNS and the loading process of MTX was confirmed through detailed investigation by Fourier transform infrared spectroscopy (FTIR) spectra, Raman spectra, fluorescent spectra, ultraviolet–visible (UV–vis), X-ray powder diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM). The potential viability of the system was proven by investigating the drug delivery response in a simulated environment and the cellular cytotoxicity assay of gelatin-GNS and MTX@gelatin-GNS to the human lung cancer A549 cells was conducted simultaneously.

2. Experimental section

2.1. Materials

Graphite powder (KS-10) and rhodamine 6G (R6G) were from Sigma. MTX (100138) was purchased from the National Institute of China for the Control of Pharmaceutical and Biological Products. Gelatin (99%), hydrazine hydrate (98%), $K_2S_2O_8$ (99%), P_2O_5 (99%), H_2O_2 (30%) and concentrated sulfuric acid (AR) were from Tianjin Guangfu Fine Chemical Industry Research Institute. (Tianjin, China). Dulbecco's modified eagle's medium (DMEM)/high glucose was from Thermo Fisher Biochemical Products Co., Ltd. (Bejing, China). Fetal bovine serum (FBS) was from Shanghai Shangbao Biological Technology Co. Ltd. (Shanghai, China). Trypsin and 3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenylte-trazolium bromide (MTT) were from Amresco Company (America). All aqueous solutions were prepared using ultrapure water.

2.2. Apparatus

Infrared spectra were obtained on Nicolet NEXUS 670 (America) Fourier transform infrared spectrometer. UV–vis spectra were collected from Perkin-Elmer Lambda 25 (America) UV–vis Spectrophotometers. TEM was performed on a JEOL JEM-1230 (Japan) TEM. SEM was conducted on a JEOL JSM-6380 LV (Japan) SEM using an accelerating voltage of 30 kV. AFM was carried out on an Agilent 5500 atomic force microscope operated in tapping mode with sample on silicon wafer (America). XRD was performed on a Rigakn D/Max-2400 (Rigakn). Raman spectra were conducted on a Renishaw Raman microscope (Britain). Fluorescence spectra were obtained from a RF-5301PC spectro flurophotometer (Shimadzu). Confocal laser scanning microscope (CLSM) image was obtained using a TCS SP5 (Leica) CLSM. Absorbance in MTT assay was recorded at 570 nm using a Bio-Rad 680 microplate reader (USA).

2.3. Preparation of gelatin reduced GNS

Graphite oxide containing some oxygen functional groups were synthesized from natural graphite by modified Hummers method [42]. Briefly, native graphite powder (1 g) was mixed with concentrated H_2SO_4 (12 mL), $K_2S_2O_8$ (2.5 g), and P_2O_5 (2.5 g), and then incubated at 80 °C for 4 h to pre-oxidize the graphite. The product was then dried at 60 °C overnight, after washing with distilled