International Journal of Biological Macromolecules 93 (2016) 582–590

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Sodium alginate conjugated graphene oxide as a new carrier for drug delivery system

Lihong Fan [a], Hongyu Ge [a], Shengqiong Zou [a], Yao Xiao [a], Huigao Wen [a], Ya Li [a], Han Feng [a], Min Nie [b,*]

[a] College of Chemical Engineering, Wuhan University of Technology, Wuhan 430070, China
[b] The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) & Key Laboratory of Oral Biomedicine Ministry of Education, School & Hospital of Stomatology, Wuhan University, Wuhan 430079, China

ARTICLE INFO

Article history:
Received 25 June 2016
Received in revised form 5 September 2016
Accepted 7 September 2016
Available online 9 September 2016

Keywords:
Graphene oxide
Sodium alginate
Doxorubicin hydrochloride
Drug delivery
Controlled release

ABSTRACT

The biomedical applications of graphene-based materials, including drug delivery, have grown rapidly in the past few years. The aim of this present study is to enhance the efficiency and specificity of anticancer drug delivery and realize intelligently controlled release and targeted delivery. Graphene oxide (GO) was first prepared from purified natural graphite according to a modified Hummers' method. Then GO was functionalized with adipic acid dihydrazide to introduce amine groups, and sodium alginate (SA) was covalently conjugated to GO by the formation of amide bonds. The resulting GO–SA conjugate was characterized and used as a carrier to encapsulate the anticancer drug doxorubicin hydrochloride (DOX·HCl) to study in vitro release behavior. The maximum loading capacity of DOX on GO–SA was 1.843 mg/mg and the drug release rate under tumor cell microenvironment of pH 5.0 was significantly higher than that under physiological conditions of pH 6.5 and 7.4. Methylthiazol tetrazolium (MTT) assay was applied to evaluate the Hela cells and NIH-3T3 cells cytotoxicity of GO–SA. Results showed that GO–SA had no obvious toxicity and GO–SA/DOX exhibits notable cytotoxicity to Hela cells. Cell uptake studies indicated that GO–SA could specifically transport the DOX into Hela cells over-expressing CD_{44} receptors and showed enhanced toxicity.

1. Introduction

In recent years, design and development of powerful new drug delivery systems has been relentless, with ever more attention devoted to developing new methods for realizing controlled drug release [1,2]. In conventional drug delivery, the drug concentration in the blood rises quickly, and then declines [3,4]. Each drug has a plasma level above which it is toxic and below which it is ineffective [5]. The main aim of an ideal drug delivery system (DDS) is to maintain the drug within a desired therapeutic range after a single dose, and/or target the drug to a specific region while simultaneously lowering the systemic levels of the drug [6–9]. Biopolymers have frequently been used as raw materials for the design of drug delivery formulations owing to their excellent properties [10], such as non-toxicity, biocompatibility, biodegradability and environmental sensitivity, etc [11–13].

Graphene, a two-dimensional nanomaterial reported for the first time in 2004, has been widely investigated for its novel physical properties and potential applications in nanoelectronic devices, transparent conductors, and nanocomposite materials [14,15]. As a graphene derivative, graphene oxide (GO) has been widely explored in the last several years for drug delivery applications by many other research groups [16,17]. GO can be readily exfoliated into monolayer sheets to yield stable suspensions in water because of the hydrophilic oxygenated functional groups on its basal planes and edges [18–20]. These groups enable GO to be functionalized through covalent and noncovalent approaches [21–23], hence making it a building block for synthesizing versatile functional materials [24,25]. Further, the large two-dimensional plane of GO sheets provides large specific surface area to carry drugs via surface adsorption, hydrogen bonding, and other types of interactions [26]. Meanwhile, the excellent biocompatibility and nontoxicity of GO makes it a promising material for drug carrier substances [27–29].

Sodium alginate (SA) is a natural hydrophilic polysaccharide derived from seaweed [30,31]. SA is a water soluble salt of alginic acid, a naturally occurring non-toxic polysaccharide found in all

* Corresponding author.
E-mail address: niemin@whu.edu.cn (M. Nie).

http://dx.doi.org/10.1016/j.ijbiomac.2016.09.026
0141-8130/

species of brown algae [32]. And SA is a biodegradable polymer used extensively in food processing, medical and pharmaceutical industries such as drug carrier [33,34]. In addition, earlier literature cites many applications of SA in drug delivery [35,36], and it has been used to prepare the sustained release particulate systems for a variety of drugs, proteins, and cells [37,38].

In this study, a new anticancer drug carrier system with the abilities of controlled and targeted release was developed. GO was firstly prepared via an improved Hummers' method and characterized by FTIR, XRD, Raman, TGA, TEM [39]. To integrate the advantages of GO and SA, GO was functionalized with adipic acid dihydrazide (ADH). And then the introduced amino groups were used to conjugate SA. The resulting GO–SA conjugate was characterized by FTIR, TGA, TEM. The in vitro toxicity studies of the GO–SA were carried out by conducting MTT assays. As an anti-tumor drug, DOX was then loaded onto the surface of this conjugate via π-π stacking and hydrogen-bonding interaction [40], and in vitro release behavior at different pH conditions was monitored via UV–vis spectrometry. The in vitro selective targeting and cytotoxic effect of the DOX–loaded GO–SA (GO–SA/DOX) to HeLa cells over-expressing CD_{44} receptors and NIH-3T3 cells low-expressing CD_{44} receptors were examined.

2. Experimental

2.1. Materials

Graphite power (D50 < 600 nm) and adipic acid dihydrazide (ADH) were purchased from Aladdin. *N*-hydroxy sulfosuccinimide (NHS) and 1-ethyl-(dimethylaminopropyl) carbodiimide (EDC) were purchased from Huashun Biological Technology Co. Ltd., Wuhan, China. Sodium alginate (SA) and other reagent used in this article were of analytical grade and without further purification. They were purchased from Sinopharm Group Chemical Reagent Corp.

2.2. Synthesis of the GO–SA conjugate

Graphene oxide (GO) was prepared from normal graphite powder by a modified Hummers' method [39]. To obtain the uniform ultrasmall GO nanosheets, the as-prepared random distributed sized GO nanosheets should be further oxidized twice. Firstly, natural graphite (2 g), $K_2S_2O_8$ (1 g), and P_2O_5 (1 g) were put into concentrated H_2SO_4 (20 mL). The mixture was strongly magnetic stirred at 80 °C for 5 h, then filtered and washed several times with distilled water, dried in the vacuum oven at 40 °C for 12 h. The resultant mixture was slowly cooled to room temperature over a period of about 6 h. Then filtered and washed several times with distilled water, dried in the vacuum oven at 40 °C for 12 h. Secondly, the preoxidized graphite powder and $NaNO_3$ (2 g) were slowly dispersed into concentrated H_2SO_4 (100 mL) in an ice bath with stirring. Meanwhile, $KMnO_4$ (6 g) was gradually added. The dispersion was kept at 35 °C for 5 h and then distilled water (500 mL) and 30% H_2O_2 (10 mL) were added gradually to terminate the reaction, after which the color of the mixture changed to bright yellow. The mixture was filtered and washed with 1:10 HCl solution (1000 mL) in order to remove metal ions. The solution was filtered and washed until the filtrate became pH neutral. Finally, the filter cake was sonicated in deionized water for 2–3 h, the single layer GO dispersion was obtained after dialysis and lyophilization.

GO (50 mg) was sonicated and dispersed in distilled water (40 mL), and then *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC, 50 mg) and *N*-hydroxysuccinimide (NHS, 150 mg) were dissolved in distilled water (10 mL), and they were introduced to activate the carboxylic acid groups of GO for 30 min [41]. ADH (100 mg) was added to the activated GO solution, followed by 24 h of stirring. The produced GO–ADH was separated by centrifugation and rinsed repeatedly with distilled water. Sodium alginate (15 mg), EDC (30 mg) and NHS (50 mg) were dissolved in distilled water (15 mL), then the above-mentioned GO–ADH was introduced, and the mixture was stirred at room temperature for another 24 h. The resulting GO–SA was collected by centrifugation, washed repeatedly with distilled water, and then obtained after lyophilization. An overview of the preparation of the conjugated GO–SA anticancer drug-carrier with targeting function and pH-sensitivity was shown in Fig. 1.

2.3. Characterization

Transmission electron microscopy (TEM) images were obtained on a JEOL JEM-2100F (Japan) transmission electron microscope. Fourier transform infrared (FT-IR) spectra were recorded with a Nicolet 170SX Fourier transform infrared spectrophotometer (USA) in the wavenumber ranging from 400 to 4000 cm^{-1}. All the test samples were prepared by the KBr disk method. Raman spectroscopy was collected with a RENISHAW INVIA Raman Spectrometer at room temperature with an excitation laser source of 532 nm. Spectra were recorded from 300 to 3300 cm^{-1}. X-ray diffraction (XRD) patterns were obtained on a Rigaku DMAX 2000 diffractometer using Cu-Ka radiation (k = 0.15405 nm) (40 kV, 40 mA). Ultraviolet–visible (UV–vis) absorption spectra were obtained with a BeckMan coulter DU 730 spectrophotometer. Thermogravimetric analysis (TGA) was performed using a TG 209F1 (Netzsch Instruments) thermogravimetric analyzer with a heating rate of 20 °C/min and a temperature range of 30–600 °C in nitrogen.

2.4. Loading of DOX onto GO–SA

GO–SA (50 mg) was first dispersed in PBS (pH = 7.4, 50 mL) and then sonicated for a few minutes. The different concentration of DOX·HCl (1 mL) were added into the sonicated GO–SA, respectively. Loading DOX onto GO–SA was performed by stirring for 20 h under dark conditions at room temperature [42]. The DOX drug-loaded GO–SA were collected by centrifugation and dried under vacuum for 24 h, then washed with ionized water three times to remove the excess drug. The loading ratios of DOX were estimated by UV–vis spectroscopy at 490 nm.

2.5. Release of DOX from GO–SA/DOX complex

To study the release behavior of DOX from GO–SA, the release behavior of DOX from GO–SA/DOX was investigated at 37 °C at three different pH, using a dialysis bag with 5000 Da molecular cutoff [43]. The GO–SA/DOX complex (5 mg) was suspended in a PBS buffer (10 mL, pH 7.4, 6.5 and 5.0), and the dialysis bags were incubated in PBS buffer (50 mL) of the same pH value at 37 °C under shaking (200 rpm min^{-1}). A known quantity (3 mL) of solution from the container was removed after every time step, making sure to replace it with the same amount of fresh PBS solution. The amount of the released DOX was measured at different time points from 0 h to 96 h by a UV–vis spectrophotometer (490 nm).

2.6. Cytotoxicity measurement

The in vitro cytotoxicity investigation was conducted using MTT assays [27]. HeLa cell lines and NIH-3T3 cell lines were used to evaluate the in vitro cytotoxicity of the materials [44]. The cells were employed and cultured in DMEM medium supplemented with penicillin (100 units/mL), streptomycin (100 μg/mL), and 10% (v/v) heat-inactivated FBS. Hela cells and NIH-3T3 cells were seeded into 96-well plates at a density of 4×10^3 cells/well in 100 μL DMEM,