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Full Length Article

Surface modification of graphene oxide nanosheets by protamine sulfate/sodium alginate for anti-cancer drug delivery application

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ABSTRACT

In order to improve the efficiency of anticancer drug delivery, a graphene oxide (GO) based drug delivery system modificated by natural peptide protamine sulfate (PRM) and sodium alginate (SA) was established via electrostatic attraction at each step of adsorption based on layer-by-layer self-assembly. The nanocomposites were then loaded with anticancer drug doxorubicin hydrochloride (DOX) to estimate the feasibility as drug carriers. The nanocomposites loaded with DOX revealed a remarkable pH-sensitive drug release property. The modification with protamine sulfate and sodium alginate could not only impart the nanocomposites an improved dispersibility and stability under physiological pH, but also suppress the protein adhesion. Due to the high water dispersibility and the small particle size, GO-PRM/SA nanocomposites were able to be uptaken by MCF-7 cells. It was found that GO-PRM/SA nanocomposites exhibited no obvious cytotoxicity towards MCF-7 cells, while GO-PRM/SA-DOX exhibited better cytotoxicity than GO-DOX. Therefore, the GO-PRM/SA nanocomposites were feasible as drug delivery vehicles.

1. Introduction

Cancer has been recognized as one of the major malignant diseases concerns worldwide. The conventional cancer treatment is mainly surgery, chemotherapy, and radiotherapy. Although chemotherapy has been widely used in clinical treatment, most chemotherapeutic drugs are lowly specific and highly toxic, leading to side effects and systemic toxicity [1,2]. In order to decrease the adverse effects and to improve the therapeutic efficiency of chemotherapeutic drugs, numerous drug delivery systems have been developed, such as liposomes [3,4], polymeric micelles [5–9], silica nanoparticles [10–14], carbon based materials [15–17], metal–organic frameworks [18–20], and so on.

The nanomaterial-mediated drug delivery systems have generated great interest because of their notable superiority in enhancing antitumor efficiency and decreasing systemic toxicity in cancer therapy [1,2]. Among the various nanomaterials of different sizes and shapes, graphene oxide (GO) has attracted considerable attention in drug delivery owing to its superior biocompatibility and high surface area practicable for drug loading [21–23]. In recent years, an amount of studies have been reported for modificated GO as drug carrier. For example, Nasrollahi et al. synthesized Transferrin/Poly (allylamine hydrochloride)-functionalized graphene oxide for targeted delivery of docetaxel [24]. Wang et al. Prepared thermo-sensitive graphene oxide–polymer nanoparticle hybrids as a carrier for drug delivery [25]. Cao et al. developed folic acid-conjugated GO as a transporter of chemotherapeutic drug and siRNA for reversal of cancer drug resistance [26]. These developments suggest that it is possible to construct GO based drug delivery system for drug delivery.

Despite of various advantages, GO is subjected to aggregation in physiological conditions, which severely limited its application in drug loading and biomedical application. Further functionalization of GO is therefore needed [27,28]. The method of layer-by-layer (LbL) technique has emerged as a novel approach for surface modification [29,30]. The technique involves alternative immobilization of polymers with opposite charges on the surface of the bases via electrostatic attractions to form functional multilayers. For example, Ramasamy et al. developed lipid-polymer hybrid nanoparticles via LbL coating for drug

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delivery [31]. Haidar et al. prepared alginate and chitosan modified liposomes for delivery of biomacromolecules [32]. In addition, polyethylenimine /poly(acrylic acid) coated rGO sheets were also prepared as reactive precursors [33]. We previously prepared nanocomposites of graphene oxide with chitosan/ dextran or chitosan/sodium alginate as drug carriers and the functionalization significantly improved the stability of the drug loaded system [34,35].

Although these literatures have been reported before, seldom attention was paid on the LbL immobilization of natural peptides or proteins on nano-sized GO surfaces for drug delivery applications. In this work, a natural peptide protamine sulfate (PRM) was selected and to prepare multi-layered nanocomposites with oppositely charged sodium alginate (SA) by layer-by-layer deposition on GO nanosheets (Scheme 1). Protamine sulfate is a biodegradable cationic peptide derived from fish sperm and it is positively charged owing to their high arginine content [36,37]. It occurs naturally in sperm and has been sometimes applied for surface coating [38]. More importantly, protamine contains arginine-rich nuclear localization signal (NLS), which can specifically direct the particles to the nucleus, thus has an application in gene delivery [39,40]. Sodium alginate (SA), a linear anionic natural polysaccharide, which is biocompatible, and biodegradable under normal physiological conditions, has been widely used in biomedical applications [41,42]. GO surfaces were negatively charged, which was available for the immobilization of cationic protamine sulfate and anionic sodium alginate via electrostatic interactions. The modification was demonstrated via AFM and zeta potential measurements. The resultant GO-PRM/SA nanocomposites were applied to deliver doxorubicin (DOX), an effective anticancer drug in physiological environments. The drug release behaviors at different pH values have been evaluated. Finally, the cellular uptake of fluorescent nanocomposites and the cytotoxicity of the DOX loaded ones were investigated by CLSM and MTT assay respectively to evaluate their potential for anti-cancer drug delivery.

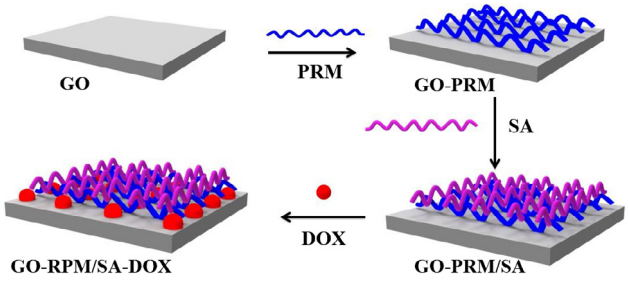
2. Experimental section

2.1. Materials

Protamine sulfate salt was purchased from Sigma-Aldrich (Steinheim, Germany). Sodium alginate was purchased from Sinopharm Chemical Reagent Company (Shanghai, China). Doxorubicin hydrochloride (DOX) was purchased from HVSF United Chemical materials Co. Ltd. (Beijing, China).

2.2. Production of GO-PRM/SA

The GO-PRM/SA composites were fabricated by LbL technology. The preparation of GO nanosheets has been previously reported in our works [34,35]. Nano-sized GO sheets were obtained via sonication using an ultra-sonic cell disrupter system for 1 h. Protamine modified GO was prepared with 1:4 weight ratio of GO and protamine. Typically, protamine sulfate was dissolved deionized water (1 mg/mL). Then, GO suspension (0.2 mg/mL) was slowly added to the solution with stirring for 20 min. After that, the suspension was filtered via a membrane (0.22 μm). The obtained solid was redistributed in purified water and filtered three times to remove the residue protamine, and then dispersed in pure water to obtain protamine functionalized GO suspension. The GO-PRM/SA complex was synthesized via the same procedure. The weight ratio of protamine sulfate and sodium alginate was at 1:1.



Scheme 1. Illustration showing the synthesis of GO-PRM/SA and DOX loading.

2.3. Characterization

Fourier transform infrared (FT-IR) spectra were registered with a Nicolet Model Nexus spectrometer to identify the functional groups of the composites. The zeta potential analysis was performed on the Malvern ZEN3600 equipment. AFM images were carried out by a Multimode 8 atomic force microscopy system (Bruker, USA) to investigate the morphology of the samples.

2.4. Preparation of fluorescent nanocomposites

Fluorescein isothiocyanate (FITC) was conjugated to protamine sulfate via covalent bond to prepare protamine-FITC for imaging. Firstly, 100 mg protamine sulfate was dissolved in 10 mL carbonate buffer (pH 9.0). Then, 0.1 mL FITC solution (in ethanol, 10 mg mL^{-1}) was added to the solution and was stirred for 12 h in the dark. After that, unreacted FITC was removed by dialysis in purified water in the dark for 24 h. Then, protamine-FITC and sodium alginate were deposited on the surface of GO to form GO-PRM-SA fluorescent nanocomposites by the same procedure as described above.

2.5. Non-specific protein adsorption

By using Bovine Serum Albumin (BSA) as a model protein, we investigated the protein attachment on GO-PRM/SA nanocomposites. Generally, BSA was dispersed with PBS buffer (pH 7.4) to a concentration of 1 mg mL^{-1}. 2 mL of the formed BSA solution was mixed with 5 mL GO or GO-PRM/SA suspension (at a GO concentration of 0.2 mg mL^{-1}) and stirred for 24 h. Following this, the unbounded BSA molecules in the mixture was separated with the aid of centrifugation. Finally, the measurement of the content of protein absorbed was obtained via UV–vis spectroscopy at 280 nm based on the BSA in feed subtracting the unbounded BSA molecules.

2.6. Drug loading studies

To prepare the drug loaded nanocomposites, 2 mL PBS solution of DOX (0.5 mg mL^{-1}, pH 7.4) was directly added to the suspensions of GO, GO-PRM and GO-PRM/SA (2.5 mL, at a GO concentration of 0.2 mg mL^{-1}) respectively, and stirred for 24 h. The unbound DOX in the supernatant was removed by extensive centrifugation (13,000 rpm, 30 min). The amount of DOX in the supernatant was determined with a UV spectrophotometer at the wavelength of 480 nm. The extent of DOX loading could be evaluated by subtracting the amount of drug in the supernatant from the original DOX solution.