Journal of Colloid and Interface Science 516 (2018) 332–341

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

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

Regular Article

Design and evaluation of galactosylated chitosan/graphene oxide nanoparticles as a drug delivery system

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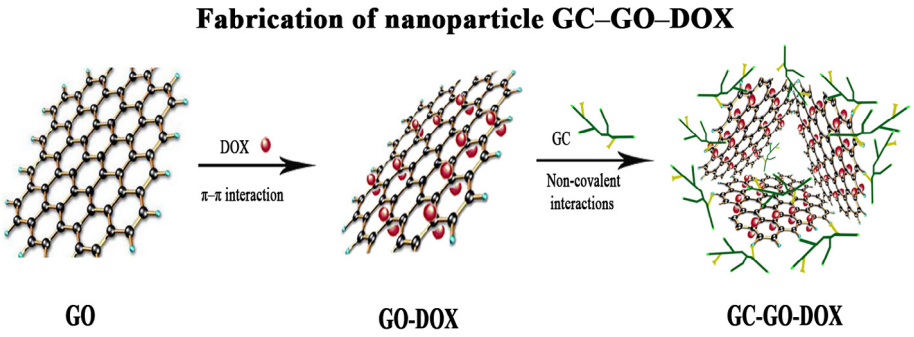
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 November 2017
Revised 18 January 2018
Accepted 19 January 2018

Keywords:
Graphene oxide
Cyclic RGD
pH-responsive
Controlled release
Anti-tumor effect
Cancer cells targeting

ABSTRACT

We investigated a novel drug delivery system comprising nanoparticles based on galactosylated chitosan/graphene oxide/doxorubicin (GC–GO–DOX) for the therapeutic treatment of cancer. The drug delivery system was synthesized by loading a drug sample with galactosylated chitosan (GC) on a graphene oxide (GO) carrier. The results showed that the drug loading capacity was as high as 1.08 mg/mg (drug per polymer). The nanoparticles remained stable under physiological conditions, and the drug was released in a low pH environment (i.e., a tumor environment) and was pH-responsive. Cell uptake experiments and a cell proliferation analysis demonstrated that the nanoparticles had higher cytotoxicity for HepG2 and SMMC-7721 cells than chitosan/graphene oxide/doxorubicin (CS–GO–DOX) nanoparticles. Compared with CS–GO–DOX nanoparticles, the GC–GO–DOX nanoparticles exhibited a higher fluorescence intensity in tumor cells. *In vivo* anti-tumor experiments demonstrated that the GC–GO–DOX nanoparticles inhibit tumors better than the CS–GO–DOX nanoparticles. Nude mouse weight, tumor weight and tumor volume data indicated that the GC–GO–DOX tumor inhibition effect was better than that of the control group and the blank group. In summary, the nanoparticle investigated in this article is significant for tumor therapy.

1. Introduction

Recent studies have indicated that the treatment of diseases caused by biological pathogens [1,2], infectious diseases [3,4], and non-communicable diseases [5,6] has been greatly improved, but cancer treatments remain relatively underdeveloped [7,8]. The World Cancer Report released by the World Health Organization in 2014 indicated that fourteen million people worldwide were diagnosed with cancer in 2012, and that the number would increase to twenty-four million by 2035. The worldwide death rate

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https://doi.org/10.1016/j.jcis.2018.01.073
0021-9797/

for liver cancer is the second highest after that of lung cancer [9,10]. Research on and the development of anti-cancer drugs plays a vital role in cancer therapy. However, due to the side effects of anti-cancer drugs on normal tissues and cells, the development of anticancer drugs has been extremely limited. Many drugs failed to significantly exhibit a healing efficacy at tumor sites. A new drug delivery system with efficient drug loading is therefore essential for targeting tumor cells and releasing drugs into the tumor environment [11–15].

A large number of novel drug carriers have recently been developed to reduce the side effects of anti-cancer drugs and to improve the therapeutic effect as well as bioimaging. Carbon nanomaterials such as carbon dots have attracted a great deal of attention for bioimaging and drug loading [16–19]. However, the enhanced permeability and retention (EPR) effect of tumor tissues requires a larger particle size, and the carbon dots are too small to passively target tumor tissues via EPR [20]. Mesoporous silica with high a surface area has been shown to load drugs such as doxorubicin (DOX) efficiently and has obvious sustained drug release characteristics [21,22]. However, some types of mesoporous silica can cause severe hemolysis, which limits their use *in vivo* [23]. GO is a vehicle with good biocompatibility that exhibits high drug loading among drug carrier groups.

GO has been believed to be a carrier on which a large amount of a drug could be loaded. Research on GO has become increasingly extensive and has gradually implicated GO as a potential drug carrier [24–26]. GO is a type of two-dimensional monatomic carbon allotrope [27]. GO contains a large number of functional groups such as epoxy groups, hydroxyl groups and carboxyl groups [28,29]. GO has a large specific surface area and a π-conjugated structure. Interactions such as π–π stacking and van de Waals interactions can be employed to load drugs onto GO [30–33]. GO has been widely studied as a drug carrier that benefits from all the properties listed above.

Relevant research has indicated that many ligand modified polymers effectively target liver cancer cells, for example, mannose modified cyclodextrin [34], lactose functionalized magnetoliposomes [35], folate modified nanomaterials [36,37], etc. Galactose is widely studied for its ability to target hepatocellular carcinoma cells through the asialoglycoprotein receptor (ASGP-R). A large amount of materials modified with galactose (i.e., galactosylated chitosan, galactosylated PEG, galactose modified polyethylene acetate and galactose modified polycaprolactone) have been used as a drug delivery system for the hepatic carcinoma therapy [38–41]. Galactose-modified chitosan has biocompatibility characteristics that make it an admirable drug delivery carrier [42,43]. However, the drug loading capacity of galactose-modified materials is not high enough for cancer therapy, which limits the efficacy of such drugs. Our strategy was to coat GO with a membrane (GC) for targeting hepatocellular carcinoma, allowing for efficient drug loading and controlled drug release. The stability of the nanocomposites was very important for this drug delivery system in biological media [44]. Nanocomposites including GO can aggregate in biological media [45–47]. CS has been reported to improve the stability of nanocarriers [48,49]. The purpose of CS in the present study was not only to increase the biocompatibility of the drug carrier for sustained and controlled drug release but also to improve the stability of the nanocomposite in biological media.

This research developed an innovative multifunctional drug delivery system with cancer cell targeting properties, controlled drug release and efficient loading, due to the advantages of graphene oxide and galactosylated chitosan. The model drug DOX was loaded onto GO via π–π stacking, hydrogen bonding and van de Waals interactions. The resultant nanoparticle was characterized by fourier transform infrared Spectroscopy, UV–vis, atomic force microscopy and ^{1}H NMR. *In vitro* and *in vivo* studies verified the targeting and controlled release abilities of the drug delivery system, by means of cellular uptake experiments, a cell proliferation assay and anti-tumor experiments. The results showed that the nanoparticle had a high drug content, was pH-responsive (releasing the drug in a tumor cell environment at pH 5.5 and remaining stable in normal physiological environment at pH 7.4), and targeted hepatocarcinoma.

2. Material and methods

2.1. Reagents and material

Chitosan with a deacetylation degree of 75%, lactobionic acid (97.00%), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC, 99.00%), N,N,N′,N′-tetramethylethylenediamine (TEMED, 99.50%), fluoresceine isothiocyanate (FITC, 97.5%) and a cell counting kit (CCK-8) were purchased from Sigma-Aldrich. Graphene oxide (1 mg/mL) and doxorubicin hydrochloride (98.00%) were purchased from the Aladdin-reagent company. All other reagents were of analytical grade and purchased from the Sinopharm Chemical Reagent Co., Ltd.

Samples of the hepatocellular carcinoma cell lines HepG2 and SMMC-7721 were obtained as a gift from the Key Laboratory of Xiamen Medical College (Xiamen, China). The cells were cultured in an incubator with 5% CO_2 at 37 °C in MEM in 10% fetal bovine serum. Male BALB/c nude mice 4–5 weeks of age and weighing 18–22 g were supplied by the Laboratory Animal Center of Xiamen University. All animal tests were assessed and approved by the ethics committee of Xiamen University. Tumor-bearing nude mice were obtained by subcutaneously inoculating HepG2 cells at a destiny of 1×10^7 into the right flanks of the nude mice.

2.2. Synthesis of galactosylated chitosan (GC)

Chitosan was coupled with lactobionic acid via a carbodiimide reaction [50–52]. NHS (1.80 g, 15.5 mM) /EDC (3.0 g, 15.5 mM) dissolved in 20 mL of TEMED/HCl buffer solution at a pH of 4.7 activated the carboxyl group of lactobionic acid (2.80 g, 7.75 mM). Chitosan (1.25 g, 7.75 mM) was dissolved in 200 mL of a HCl (0.1 M) buffer solution. The chitosan solution and the lactobionic acid solution were mixed, and then the mixture was stirred using a thermostatic magnetic stirrer for 72 h at ambient temperature. The resultant product was dialyzed for three days in distilled water in a dialysis tube of MWCO = 3500 to purify the galactosylated chitosan. After the above processes had been carried out, the product was dried by lyophilization using a UNICRYO system (MC2L, Germany). The formation of GC was determined by ^{1}H NMR of JNM-ECZ400S JEOL (Japan) and FT-IR with a Thermo Fisher Nicolet iS50 (Japan).

Prior to synthesizing GC, we labeled the CS with FITC for a further co-focusing fluorescence experiment. FITC was dissolved in methanol (2 mg/ml), then the CS (1.0 g), dehydrated methanol (200 mL) and FITC (100 mL) were sequentially added into 200 mL of 0.1 M acetic acid. The mixture was stirred for 4 h in the dark at ambient temperature in order to allow the reaction to proceed to completion. Then NaOH (0.5 M) was added until the pH of the mixture was 9.0 to precipitate CS labeled with FITC. Impurities were removed by washing and centrifuging three times. Finally, CS labeled with FITC was obtained by dialysis for 3 days using deionized water, followed by lyophilization.

2.3. Characterization of GO–DOX nanoparticle

GO was dispersed in ultrapure water and the solution was homogenized by sonication for 60 min (Sonics VCX750, USA). To load the DOX onto the GO, 200 mL of a phosphate buffered saline