Carbohydrate Polymers 185 (2018) 85–95

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

# Targeted delivery of SNX-2112 by polysaccharide-modified graphene oxide nanocomposites for treatment of lung cancer

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ARTICLE INFO

*Keywords:*
Graphene oxide
Polysaccharide
Lung cancer
Drug delivery
Biocompatibility

ABSTRACT

Graphene oxide (GO) is a promising material for biomedical applications, particularly in drug delivery, due to its exceptional chemical and physical properties. In this work, an innovative GO-based carrier was developed by modifying GO with chitosan (CHI) to improve the biocompatibility, and followed by the conjugation of hyaluronic acid (HA), the target ligand for CD44, to realize the specific recognition of tumor cells and improve the efficiency of anti-tumor drug delivery. The resulting product GO-CHI-HA was loaded with an anti-cancer drug SNX-2112, which is the Hsp90 inhibitor. The total release amount and release rate of SNX-2112 were significantly higher in acidic condition than in physiological condition. GO-CHI-HA with a low concentration had little impact on the lysis of red blood cells (RBCs) and blood coagulation and showed low toxicity in A549 cells and NHBE cells. The GO-CHI-HA/SNX-2112 proved to be effective in inhibiting and killing A549 cells while having lower cytotoxicity against normal human bronchial epithelial cells (NHBE cells). Furthermore, *in vivo* toxicity of the materials towards vital organs in SD rats were also studied through histological examinations and blood property analyses, the results of which showed that although inflammatory response was developed in the short-term, GO-CHI-HA/SNX-2112 caused no severe long-term injury. Therefore, this drug delivery system showed great potential as an effective and safe drug delivery system with little adverse side effects for cancer therapy.

## 1. Introduction

Chemotherapy is commonly used in cancer therapy, however, low targeting efficiency at tumor sites, insufficient cell uptake and non-specific accumulation in normal tissues decrease the therapeutic efficacy of anti-tumor drug and may lead to serious side effects (Yang et al., 2011). Considerable researches using nanoparticles as drug carriers to improve drug availability were reported, which based on the enhanced permeation and retention (EPR) effects, on account of the differences in microenvironment between cancerous and normal tissues (Mohanty, Das, Kanwar, & Sahoo, 2010).

Graphene oxide (GO) has shown great potential as a drug carrier, due to its high drug-loading capacity, and plasma membrane traversing capability (Sun et al., 2008). With abundant hydrophilic groups, such as hydroxyl, epoxide and carboxylic groups, GO can be well-dispersed in water (Yasoda et al., 2016). The large surface area on both sides of the sheet and the interactions through π-π stacking, hydrophobic interaction or hydrogen bonding (Guo et al., 2010; Liu, Sun, Nakayama-Ratchford, & Dai, 2007; Xie et al., 2012) between GO and various drugs provides GO with a high drug-loading capacity.

However, GO is prone to aggregate in salted or protein rich environments (Liu, Robinson, Sun, & Dai, 2008), and is cytotoxic at high concentration, which greatly hamper its applications. Many researches have been done in an effort to keep the stability and improve the biocompatibility of GO-based materials. The most efficient way involves surface functionalization of GO *via* either covalent or noncovalent conjugation. Poly (ethylene glycol) (PEG) (Li et al., 2016), poly (acrylic acid) (PAA) (Xu et al., 2016) and dextran (DEX) (Alibolandi, Mohammadi, Taghdisi, Ramezani, & Abnous, 2017) are the three most biocompatible surface coatings for GO. Besides, other coatings such as bovine serum albumin (BSA) (Cheon, Bae, & Chung, 2016), poly (amido amine) (PAMAM) dendrimer (Xiao, Yan, Zeng, & Liu, 2016), and Pluronic F127 (Hong, Compton, An, Eryazici, & Nguyen, 2012) have also been used. Furthermore, there are studies linking targeting

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https://doi.org/10.1016/j.carbpol.2018.01.014
Received 26 August 2017; Received in revised form 15 December 2017; Accepted 4 January 2018
Available online 05 January 2018
0144-8617/ 

moieties to nanoparticles to realize the specific recognizing and binding to tumor cells, thereby reducing side effects of drugs, improving drug efficiency and lowering down the required drug doses. Yang et al. (2016) conjugated GO to a monoclonal antibody against follicle-stimulating hormone receptor (FSHR) which confirmed to be a highly selective tumor vasculature marker, to realise the targeting of metastatic breast cancer. Nasrollahi, Varshosaz, Khodadadi, Lim, and Jahanian-Najafabadi (2016) functionalized GO with transferrin-poly (allylamine hydrochloride), which provided targeted and specific accumulation to extracellular transferrin receptors and stabilized GO in physiological solutions.

Chitosan (CHI) is a type of polysaccharide with favourable biocompatibility, biodegradability, and non-toxicity (Jayakumar, Menon, Manzoor, Nair, & Tamura, 2010), which is often used for biocompatible modifications. Chitosan is the N-deacetylated derivative of chitin, which consists of chitobiose units (residues of 2-deoxy-2-acetamido-β-D-glucan) connected by (1–4) glikozid bonds (Muzzarelli & Muzzarelli, 2009). The amino groups provide CHI with a high density of the positive charge in acidic conditions, which contribute to the cellular uptake and endosomal escape (Zaki, Nasti, & Tirelli, 2011). CHI can assemble with negatively charged polyelectrolytes (Izumrudov, 2008), and can also act as linkers between GO sheets and bioactive molecules, which is desirable for drug delivery. Hyaluronan (HA) is also widely used in delivery system because of its excellent biocompatibility, biodegradability and non-immunogenicity (Lapcik, Lapcik, Smedt, Demeester, & Chabrecek, 1999; Necas, Bartosikova, Brauner, & Kolar, 2008). In addition, the modification of HA can result in greatly enhanced binding and endocytotic uptake (Jordan, Racine, Hennig, & Lokeshwar, 2015; Kim, Park, Shim, Choi, & Oh, 2015; Lingmei et al., 2015), because of the specific recognition of HA to the overexpressed transmembrane glycoprotein CD44 on the surfaces of various tumor cells (Lin et al., 2016; Noh et al., 2015; Yin, Lei, Yin, Zhou, & Huo, 2015). Moreover, compared to other targeting counterparts, such as peptides or antibodies, HA is more economical with better water-solubility and stability (Song, Han et al., 2014).

Heat shock protein 90 (Hsp90) is an adenosine triphosphate (ATP)-dependent molecular chaperone that promotes the maturation and conformational stabilization of a subset of cellular proteins, which regulate cell survival and proliferation and are responsible for malignant transformation. The expression levels of Hsp90 in tumor cells are two-fold to ten-fold higher than in normal cells (Brown, Zhu, Schmidt, & Tucker, 2007; Solit & Chiosis, 2008). SNX-2112 is a novel inhibitor of Hsp90, which can bind to the N-terminal ATP binding site of Hsp90 with high affinity (Liu et al., 2012). It is effective against various types of cancers, such as multiple myeloma (Okawa et al., 2008), human chronic leukaemia (Wang et al., 2013), and lung cancer (Wang et al., 2015), while having only a modest effect on normal cells. However, SNX-2112 is only slightly soluble in water and oil and is poorly soluble in other lipophilic excipients, which necessitate an effective carrier for SNX-2112 delivery.

In this study, to realize the controlled and targeted delivery of SNX-2112, an GO-based drug carrier system, denoted “GO-CHI-HA/SNX-2112”, was built. HA-CD44 interaction may be an approach to the targeted treatment of cancers, and the pharmaceutical efficacy was examined on normal and cancer cells through apoptosis detection and cell viabilities. Furthermore, the comprehensive evaluation of the *in vivo* safety of GO-CHI-HA/SNX-2112 was made. Once entering the blood circulation, the interaction between materials and numerous blood cells and plasma proteins may alter cell membrane structures and protein conformations, perturb blood functions and eventually affect the whole organism. Therefore, the hemocompatibility was firstly investigated through the lysis of human red blood cells (RBCs), along with the impact on the clotting function with thromboelastography (TEG) assays. Besides, animal experiments were conducted to assess their systemic toxicity in the short-term and long-term.

## 2. Materials and methods

### 2.1. Materials

Graphene oxide (GO), carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) were purchased from Aladdin Reagents (Shanghai, China). Chitosan (CHI, molecular weight approx. 15 kDa, determined by GPC) were purchased from Sigma-Aldrich (Shanghai, China). The degree of deacetylation (DD) was determined by FT-IR spectra according to a method previously described (El-Sherbiny, 2009), and was about 86%. HA (molecular weight approx. 10 kDa, determined by GPC) was supplied by Shandong Furuida Group Co., Ltd. (Zibo, China). 1-Ethyl-3-(3-dimethyl-aminopropyl) Dulbecco’s modified Eagle’s medium (DMEM), RPMI Medium 1640, penicillin and streptomycin, 0.25% Trypsin-EDTA, 0.25% Trypsin and Foetal bovine serum (FBS) were obtained from Gibco (Grand Island, NY, SA). The Cell Counting Kit 8 (CCK–8) was purchased from Dojindo Molecular Technologies, Inc. (Tokyo, Japan). Annexin V-FITC/PI Apoptosis Detection Kit was purchased from Keygen Biotech (Nanjing, China). Blood from healthy consenting volunteers was collected in sodium citrate tubes with a blood: anticoagulant ratio of 9:1. All other reagents used were of analytical grade.

### 2.2. Synthesis of the GO-CHI and GO-CHI-HA

CHI (60 mg) was dissolved in the aqueous solution (40 mL) containing 0.1 M NaCl and 0.02 M acetic acid. Then GO (60 mg) was added into the CHI solution, sonicated for 30 min and stirred at room temperature for 24 h. The GO-CHI was dialysed for 3 days against deionised water and lyophilised.

HA (20 mg) was activated by NHS (4 mg) and EDC·HCl (20 mg) in PBS buffer (pH 7.4) for 1 h, followed by the adding of GO-CHI (20 mg). The mixture was stirred at room temperature for 24 h, dialysed for 3 days against deionised water and lyophilised. Then the HA modified GO-CHI was collected.

### 2.3. Characterization of GO-based nanocomposites

The infrared spectra of GO, GO-CHI and GO-CHI-HA were obtained using the KBr disc technique with an FT-IR spectrometer (Vertex 70; Bruker). The spectra were obtained in the spectral region of 500–4000 $cm^{-1}$ at a resolution of 4 $cm^{-1}$ and 20 scans per sample.

Thermogravimetry analysis (TGA) measurements were performed on TG 209 F3 Tarsus (NETZSCH Corporation, Germany) thermal analysis instruments under $N_2$ purge with a heating rate of 10 °C/min.

GO, GO-CHI and GO-CHI-HA were sonicated in PBS buffer (pH 7.4) at the concentration of 0.5 mg/mL. Then the zeta potentials of the complexes were determined with a Mastersizer 2000 laser diffractometer (Malvern Instruments, Worcestershire, UK).

The morphological examination of GO, GO-CHI and GO-CHI-HA were performed by high-resolution TEM (JEM–2010HR, JEOL, Tokyo, Japan). GO, GO-CHI and GO-CHI-HA were respectively dispersed in water to the concentration of 0.2% (mass fraction). A drop of the suspension was deposited on a carbon-coated grid and dried at 37 °C.

### 2.4. Loading of SNX-2112

GO, GO-CHI and GO-CHI-HA (10 mg) were respectively sonicated with SNX-2112 in PBS buffer (pH 7.4) for 30 min and stirred at room temperature for 24 h. The products were centrifuged and washed with pH 7.4 PBS buffer several times to remove the unloaded SNX-2112. Then the SNX-2112-loaded nanocomposites were collected.

After collecting free SNX-2112 solution, the amount of free SNX-2112 unbound on nanocomposites was measured using the characteristic absorption wavelength (323 nm) of SNX-2112 with a UV–vis spectrophotometer (UV-2550, SHIMADZU, Japan).