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Folic acid-conjugated chitosan oligosaccharide-magnetic halloysite nanotubes as a delivery system for camptothecin

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ABSTRACT

In this research, to achieve enhanced intracellular uptake of anticancer drug carriers for efficient chemotherapy, folic acid conjugated chitosan oligosaccharides assembled magnetic halloysite nanotubes (FA-COS/MHNTs) have been tailored as multitask drug delivery system towards camptothecin (CPT). Besides magnetic targeting, the nanocomposites have been reacted with folate complex in order to selectively target cancer cells over expressing the folic acid receptor. HNTs showed to have a high storage capacity of CPT. In vitro, the release results indicated that CPT outflow from the nanocarriers at pH 5 was much greater than that at both pH 6.8 and 7.4. MTT assays showed that the CPT-loaded nanocarriers exhibited stronger cell growth inhibitory against colon cancer cell. Furthermore, nanocarriers gained specificity to target cancer cells because of the enhanced cell uptake mediated by FA moiety and presence of COS. Therefore, the rational designed HNTs nanocarrier for chemotherapy drug showed great potential as tumor-targeted drug delivery carrier.

1. Introduction

Camptothecin (CPT) is considered to be among the mainly potent anticancer agent of the 21st century that shows excellent antitumor activity over wide spectrum human cancers (Fang, Hung, Hua, & Hwang, 2009). Regrettably, CPT presents some major drawbacks with regards to therapeutic application, like poor water solubility, besides that at physiological pH the lactone ring of CPT is hydrolyzed resulting to the inactive carboxylate form (Luo, Yang, Xu, Chen, & Zhang, 2014). CPT could gained a new life through structural analogues and nanomedicine strategies that have been developed for efficient CPT delivery to target cells (Botella and Rivero-Buceta, 2017). Few CPT derivatives have been designed and used in clinical trials such as CPT-11 (also known as Irinotecan) (Bleiberg, 1999), Topotecan (Creemers et al., 1996) and Rubitecan (Pantazis et al., 1993). However, none of these derivatives surpasses camptothecin in efficiency (Minelli et al., 2012). As consequence, designing new carriers for delivery of CPT is highly needed than administrating conventional "free" CPT. To improve CPT pharmacokinetics (Botella and Rivero-Buceta, 2017), several drug carriers of CPT have been developed (Alibolandi et al., 2017; Botella et al., 2011; Galbiati et al., 2011; Hsiao, Tung, Hsiao, & Liu, 2012). The base work of nanotherapy for treatment of cancer is searching effective carriers for therapeutic agents. Inorganic nanoparticles such as GO (Sahoo et al., 2011; Zhang, Xia, Zhao, Liu, & Zhang, 2010), Fe_3O_4 nanoparticles (Zhu, Lei, & Tian, 2014), and mesoporous silica (Lu, Liong, Zink, & Tamanoi, 2007; Ma et al., 2012) are among the nanocarriers that have been widely developed for this purpose. High drug-loading capacity, low cytotoxicity toward normal cells, selectivity toward target cell, good hemocompatibility, and low cost are the most important criteria that must be provided for an ideal drug nanocarrier (Butler et al., 2016).

Nanoclays are natural materials with the nanoscale organization and show many promising proprieties for several applications (Rawtani and Agrawal, 2012). Clay minerals have a layered structure of tetrahedral silica oxide and octahedral Al, Fe, or Mg oxide (Lvov, Wang, Zhang, & Fakhrullin, 2016). As unique tubular nanoclays, halloysite nanotubes (HNTs) are structurally and chemically similar to those of kaolinite and has a molecular formula of $Al_2Si_2O_5(OH)_4{\cdot}nH_2O$. This

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tubular nonmaterial have recently attracted a growing scientific interest. Typically, 10–15 alumosilicate layers are rolled into a hollow cylinder having multilayer walls with a periodicity of 0.72 nm in dry form (Lvov, Aerov, & Fakhrullin, 2014). The length of halloysites is within a micrometer range (0.4–1 μm), inner lumen diameter is 10–70 nm and outer (overall) diameter is 20–200 nm (Lvov et al., 2014). Compared to carbon nanotubes, and regardless to the low cost of HNTs, aluminosilicate chemistry is not toxic and durable with high mechanical strength. In addition, halloysite is possesses a one-dimensional tubular porous structure on the mesoporous and macroporous scale (Churchman, Davy, Aylmore, Gilkes, & Self, 1995). Furthermore, the empty lumen of HNTs is a good nanocontainer for loading active chemical agents ranging from biomolecules and polymers to drugs and anti-corrosion agents. Presently, scientists and engineers have developed several new applications for this distinctive, cheap, robust and plentifully available naturally occurring clay with nanoscale lumens. Some interesting applications of HNTs were mainly focused on controlled/sustained release of drugs or bioactive molecules, medical implants (Liu, Du, Zhao, & Tian, 2015), cancer cell isolation (Hughes and King, 2010) and tissue engineering scaffolds (Fakhrullin and Lvov, 2016). The suitable size of these nanotubes, numerous hydrophilic hydroxyl groups for functionalization and good stability in biological liquids made HNTs suitable nanocarrier for drug delivery carrier applications. In addition, HNTs can entrap molecules via adsorption to the external and internal walls of the tubes or loading the drugs into the lumen and intercalation of substances between layers. In order to increase the dispersion ability in body fluids, HNTs can be selectively functionalized on the inner or outer surfaces. Mingxian Liu modified the surface groups of HNTs–COOH via grafting with biocompatible chitosan (CS), then used it as a carrier of curcumin delivery (Liu et al., 2016). However, the surfaces of HNTs were shielded by covalent modification of CS, which leads to decreased surface areas and declined drug-loading ability. To solve this drawbacks, novel carrier based chitosan oligosaccharide-grafted HNTs (HNTs-g-COS) for delivery of DOX was designed by Yang et al. (2016). COS was selected in this research instead of chitosan due to its relatively low molecular weight compared to chitosan and also for enhancing DOX antitumor efficacy by a dual-targeted strategy of mitochondria and nuclei. The designed carrier exhibit low hemolysis ratio, favorable biocompatibility, and appropriate drug releasing in vitro. Chitosan oligosaccharide (COS) is an oligomer of β-(1 → 4)-linked D-glucosamine. It has been prepared by the enzyme hydrolysis of chitosan (with MW equal or less than10 kDa) (Xu, Wang, Yang, Du, & Song, 2017). COS is water soluble, non-cytotoxic, willingly absorbed through the intestine and mostly excreted in the urine (Muanprasat & Chatsudthipong, 2017). Several studies proved that COS treatment can interrupt cancer progression at multiple steps including growth, invasion, and metastasis (Park, Chung, Choi, & Park, 2011). Due to these exciting proprieties, COS (Liu, Xia, Jiang, Yu, & Yue, 2018) and COS/HNTs (Sandri et al., 2017) have recently attracted more and more scientific attention specially over the last 5 years in the biomedical field where chitosan oligosaccharide has been introduced to various types of nanoparticles to improve their colloidal stability and in vivo blood circulation (Bae et al., 2012). Consequently, a new nanoformulation based COS is highly needed for other anticancer drugs.

Magnetically controlled drug targeting is one of the different possibilities of drug targeting. The use of magnetic nanoparticles as a drug delivering agent system under the influence of external magnetic field has attracted significant research attentions, based on their simplicity, ease of preparation, and ability to modify their properties for particular biological applications (Mody et al., 2014). To attain the active targeting of the clay based nanocarrier, much research efforts such as conjugation of targeting ligands or incorporation of magnetic nanoparticles (MNPs) have been performed. Conjugation of specific affinity ligand such as folic acid provides selective delivery of drugs to target cells via receptor-mediated endocytosis (Guo et al., 2012). Nanosystem containing MNP can be used in the magnet-guided drug delivery. Use of magnetic field to augment MNPs accumulation in target site has been realized both in vitro (Kim et al., 2017) and in vivo (Alexiou et al., 2006). However, the field of magnetic drug delivery is still at infancy, and synthesis of better magnetic drug delivery system and integration of multifunctional ligands are being continuously investigated so as to carry it from the bench-top to the clinic (Mody et al., 2014). Therefore, multi-targeting system which can be achieved by combining the advantages of biopolymer COS, HNTs, ligand conjugation and magnet guiding would synergistically enhance the therapeutic efficiency of nanoclays vehicles.

In this study, to provide the multi-targeted drug carriers, we pursued to prepare ligand conjugated COS assembled MHNTs for the delivery of CPT. Firstly, Magnetic HNTs was prepared by simple co precipitation method for magnetic targeting purposes. Secondly, COS was assembled to MHNTs by a simple solid-liquid interaction. To enhance the therapeutic efficiency of the prepared nanocarrier, ligand conjugation and magnet targeting were combined. Folic acid (FA) was conjugated on the surface of COS/MHNTs via *N*-(3-dimethylaminopropyl)-*N*/-ethylcarbodiimide/N-hydroxysuccinimide (EDC/NHS) coupling, yielding the multi-targeted drug carrier based magnetic halloysite nanotubes. The properties of the generated nanocomposites were characterized by fourier transform infrared (FTIR), thermogravimetric analysis (TGA), X-ray photoelectron spectroscopy (XPS), zeta potential, dynamic light scattering (DLS), transmission electron microscopy (TEM), and UV–vis spectroscopy. Afterwards, CPT was loaded into the lumen of FA-COS/MHNTs through adsorption inside the pores of HNTs particles (intraparticular) or aggregates (interparticular). The loading capacity conditions of CPT were optimized using the response surface methodology. Drug release profile in different simulated pH was also studied. Finally, biocompatibility of prepared nanocomposites and the efficiency of anticancer drug-loaded FA-COS/MHNTs was measured using MTT assay.

2. Experimental

2.1. Material and reagents

Halloysite clay was supplied from DanjiangKou, China. Chitosan oligosaccharides were purchased from Wuhan Yuancheng Technology Development Co., Ltd (average molar mass 3000 Da, with > 95% deacetylation degree). Folic acid was purchased from Sigma − Aldrich and used as received. 1-ethyl-3-(3-(dimethylamino) propyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) and drug Camptothecin (CPT) were obtained from Aladdin reagent. All chemical agents used in these experiments were of analytical grade and used directly without further purification.

2.2. Preparation of chitosan oligosaccharide/MHNTs nanocomposites

Magnetic halloysite nanotubes were prepared according to our previous method (Fizir et al., 2017). Briefly, 2.5 g of halloysite powder was suspended in 150 mL of deionized water by sonication for 15 min, and then 5.8 g of $FeCl_3{\cdot}6H_2O$ and 4.8 g of $FeSO_4{\cdot}7H_2O$ were added. The mixture was stirred for 10 min at 60 °C in N_2 atmosphere. Subsequently, 50 mL (25%) of ammonia solution was added dropwise into the mixture solution. Then, the resulting reaction mixture was aged for 4 h at 70 °C. The MHNTs were separated by an external magnetic field and washed for several times sequentially with water and ethanol till pH = 7. Finally, the MHNTs were dried in vacuum at 60 °C. Then, Chitosan oligosaccharide/MHNTs nanocomposites were self-assembled by simple solid-liquid interactions. Magnetic HNTs (1 g) was dispersed in 50 mL chitosan oligosaccharide aqueous solution (1% w/w) then stirred at 150 rpm for 24 h in an oil bath (at room temperature). The solid phases were subsequently recovered by centrifugation at 11,000 rpm for 30 min, frozen at 20 °C for 24 h and freeze-dried for 24 h (Sandri et al., 2017).