Colloids and Surfaces B: Biointerfaces 113 (2014) 223–229

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Controlled release of anticancer drug using graphene oxide as a drug-binding effector in konjac glucomannan/sodium alginate hydrogels

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ARTICLE INFO

Article history:
Received 28 April 2013
Received in revised form 31 August 2013
Accepted 3 September 2013
Available online 12 September 2013

Keywords:
pH sensitive
Drug-binding effector
Graphene oxide
Konjac glucomannan

ABSTRACT

In order to find new composite materials for the controlled release of drugs, a series of novel pH sensitive konjac glucomannan/sodium alginate (KGM/SA) and KGM/SA/graphene oxide (KGM/SA/GO) hydrogels were prepared, using GO as a drug-binding effector for anticancer drug loading and release. The hydrogels were characterized using Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy (SEM). The effects of component ratio and pH on the swelling properties of hydrogels were studied. The release amount of 5-fluorouracil (5-FU) incorporated into KGM/SA/GO-3 hydrogels was about 38.02% at pH 1.2 and 84.19% at pH 6.8 after 6 h and 12 h, respectively. Therefore, the release rate of 5-FU from the functionalized KGM/SA using GO could be effectively controlled, Go has a great potential to be a promising drug-binding effector for hydrogel functionalization in drug delivery.

1. Introduction

Cancer remains a challenging health problem to human beings. To overcome this problem, two major strategies have been extensively studied. The first strategy involves synthesizing new anticancer drugs. The second strategy involves developing novel anticancer drug delivery systems [1–3] allowing for a more effective use of drugs to fight against tumors.

During the recent decades, natural biopolymers have frequently been used as raw materials for the design of drug delivery formulations owing to their excellent properties, such as non-toxicity, biocompatibility, renewability, biodegradability and environmental sensitivity. Starch [4], guar gum [5], chitosan [6], konjac glucomannan (KGM) [7], and sodium alginate (SA) [8] have been used for controlled drug delivery. However, the disadvantages such as weak mechanical properties and burst release of drugs are hard to avoid when pristine biopolymers are used as drug carriers. The aforementioned disadvantages are mainly due to the weak interaction between the biopolymers and the drugs and the quick disintegration of the biopolymer carries during the release process. Therefore, many methods such as blending with other polymers and grafting with monomers have been used to improve the properties of the biopolymer vehicles [9,10].

Recently, the preparation and application of novel biopolymer/nanomaterial composites [11–13] as controlled drug delivery vehicles have attracted much attention owing to their unique structure and properties. Graphene oxide (GO) is an ideal material for the preparation of drug scaffolds because of its one-atom thickness and oxygenated defects, which are rather suitable for covalent and non-covalent functionalization owing to its excellent surface activity and solution processability [14,15]. GO sheets are enriched with oxygen-containing functional groups such as hydroxyl and epoxide on the basal planes and carbonyl and carboxylic groups at the sheet edges. 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 [16]. Thus, the excellent biocompatibility and nontoxicity of GO makes it a promising material for drug carrier substances [17]. Moreover, GO could be crosslinked by divalent and multivalent cations in aqueous solution [18], similar to SA. However, the use of GO in drug delivery formulations has rarely been studied.

5-Fluorouracil (5-FU), one of the major antimetabolites, has been the most widely used chemotherapeutic agent [19] against colorectal cancer for many decades. However, intravenous administration of 5-FU produces severe systemic toxic effects due to its cytotoxic nature toward rapidly dividing normal cells, thus limiting its clinical application. Therefore, the rate controlled target delivery of 5-FU is expected to reduce systemic side effects and provide an effective therapy involving reduced dose and treatment period for colorectal cancer. Attempts are being made to develop methods for the controlled release of 5-FU using different drug delivery systems.

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http://dx.doi.org/10.1016/j.colsurfb.2013.09.009

Table 1
Feed composition for the preparation of KGM, KGM/SA, and KGM/SA/GO hydrogels and drug loading (*EEs*).

Sample	KGM (g)	SA (g)	GO (g)	H_2O (ml)	$Ca(OH)_2$ (ml)	*EEs* (%)
KGM	1.5	0	0	150	10	10.29
KGM/SA-1	1.125	0.375	0	150	10	13.32
KGM/SA-2	1	0.5	0	150	10	15.50
KGM/SA-3	0.75	0.75	0	150	10	17.49
KGM/SA/GO-1	0.75	0.75	0.15	150	10	22.73
KGM/SA/GO-2	0.75	0.75	0.30	150	10	27.09
KGM/SA/GO-3	0.75	0.75	0.45	150	10	32.04

Jin et al. reported the pH-controlled release of 5-FU from 5-FU/β-cyclodextrin complex intercalated in double layered hydroxide [20]. Jin demonstrated a proof-of-concept approach for constructing a novel micelle-drug conjugate system for the photo-triggered release of 5-FU under physiological conditions [21]. However, the degradation of carriers produced unwanted toxic effects [22].

In this study, KGM was used as the matrix to prepare more stable intelligent hydrogels, using SA as the pH sensitive agent and GO as drug binding effector. Furthermore, the release profiles of a model drug, 5-FU, from the test hydrogels were studied in simulated gastric and intestinal pH media. Moreover, the interaction between 5-FU and GO has been investigated by UV–vis spectroscopy.

2. Materials and methods

2.1. Materials

KGM (99%, viscosity: 1% solution, 25 °C, ≥24,000 MPa s) was obtained from Engineering Center of Southwest University of Konjac Resources Research (Chongqing, China). SA (chemical grade, viscosity: 1% solution, 25 °C, 200 ± 20 MPa s) was purchased from Taixing Chemical Co. Ltd. (Chongqing, China) and used without further purification. Graphite powder was obtained from Shanghai Huayi Huayuan Chemical Co. Ltd. (Shanghai, China). 5-FU was provided by Hengshuo Chemical Co. Ltd. (Wuhan, China). Other analytical grade reagents were used as received.

2.2. Preparation of GO

GO was prepared by the oxidation of natural graphite powder according to the modified Hummers' method [23]. Graphite powder (3 g) and concentrated sulfuric acid (120 mL) were mixed in an ice bath followed by the slow addition of $KMnO_4$ (15 g) with stirring. The rate of addition was carefully controlled to prevent the temperature of the suspension from exceeding 20 °C, ice-bath was then removed and the temperature of the suspension brought to 35 °C and maintained for 2 h. Deionized water (250 mL) was slowly added to the reaction mixture and the temperature was controlled below 50 °C for another 2 h. Subsequently, deionized water (700 mL) and 30% H_2O_2 (20 mL) were added separately, resulting in a yellow brown mixture. Finally, the mixture was centrifuged and washed with aqueous HCl solution (10:1, v/v) and then with deionized water until the pH of the upper layer of suspension was near 7. Dry Graphene oxide powder was obtained by drying at 50 °C for 48 h under vacuum. The GO so obtained was able to form stable aqueous dispersion by ultra-sonication owing to its strong hydrophilicity.

2.3. Preparation of KGM/SA hydrogels

For the thorough intermixing of the polysaccharide systems, KGM and SA were dissolved in an aqueous NaOH solution (pH 8) at a total concentration of 1% (w/v) at room temperature, and then mixed by mechanical stirring (400 rpm) for 4 h. To evaluate the interaction between KGM and SA, several weight ratios of KGM and SA (1:0, 3:1, 2:1 and 1:1) were prepared while keeping the total polysaccharide concentration at 1% (w/v). Then, a $Ca(OH)_2$ suspension (10 mL) was added into the beaker and the speed of mechanical was increased to 800 rpm. After 10 min, the beaker was transferred to an oil bath and the mixture was heated at 90 °C for 6 h for deacetylation and crosslinking. The obtained hydrogels were washed five times with aqueous HCl solution (10:1, v/v) followed by five washings with deionized water to remove small molecules and residual base, and then dried to a constant weight at 40 °C and stored until further used. The codes of all hydrogels are listed in Table 1.

2.4. Preparation of KGM/SA/GO nanocomposite hydrogels

The KGM/SA/GO nanocomposite hydrogels were prepared according to the same procedure except that GO was first dissolved in aqueous NaOH solution (pH 8), and the weight ratio of KGM and SA was kept content at 1:1. For the evaluation of the interaction between GO and polysaccharide, several weight ratios of GO and polysaccharide (10%, 20% and 30%) were prepared while keeping the total polysaccharide concentration at 1% (w/v).

2.5. Drug loading and drug content determination

The hydrogels dried in oven at 40 °C for 12 h were equilibrated in 100 mL of 50 mg/L 5-FU solution for 24 h at room temperature in order to load the drug into the hydrogels. The difference in 5-FU concentrations between the original 5-FU solution and the supernatant solution after the loading were measured at 265 nm wavelength using an UV spectrophotometer (T6 New Century, China). The entrapment efficiencies (*EEs*) of the matrices for 5-FU was determined using Eq. (1) as follows:

$$EEs = \frac{w_1}{w_2} \times 100\% \quad (1)$$

where w_1 and w_2 are the actual and theoretical 5-FU contents of the hydrogels. The *EE* of all the hydrogels are listed in Table 1.

2.6. Measurement of swelling ratio

Swelling kinetics is a very important property for a drug delivery vehicle because it has a significant influence on controlled drug delivery behavior. First, dry hydrogels (50 mg) were immersed a buffer solution (25 mL) consisting of pH 1.2 hydrochloric acid buffer solution (HBS) and pH 6.8 phosphate buffer solution (PBS) in order to simulate the pH of the human stomach and colon, respectively. After a pre-determined interval, the swollen samples were separated from the unabsorbed fluids by filtering through a 100-mesh screen, blotted to remove excess fluid, and then weighed immediately. The swelling ratio of the samples at a given time (t), Q_t, can be calculated by using Eq. (2) as follows:

$$Q_t = \frac{m_t - m_0}{m_0} \quad (2)$$

where m_0 and m_t are the weights of the dry and swollen sample, respectively. Q_t is calculated as grams of water per gram of sample.