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pH-Controlled drug delivery with hybrid aerogel of chitosan, carboxymethyl cellulose and graphene oxide as the carrier

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ABSTRACT

Hybrid aerogels of chitosan (CS), carboxymethyl cellulose (CMC) and graphene oxide (GO) are successfully prepared by using calcium ion (Ca^{2+}) as the crosslinker. The resultant hybrid aerogels, CS/CMC/Ca^{2+}/GO, are characterized by field emission scanning electron microscopy (FESEM) and Fourier transform infrared (FT-IR) spectroscopy. Due to the pH sensitivity of CS and CMC, pH-controlled drug delivery with CS/CMC/Ca^{2+}/GO as the carrier is investigated using 5-fluorouracil (5-FU), an effective chemotherapeutic agent in the treatment of cancers, as the model drug. Finally, Higuchi model and Korsmeyer-Peppas model are used to study the release kinetics, and it reveals that the release of 5-FU from the hybrid aerogels is controlled by Fickian diffusion. Collectively, the findings demonstrate the CS/CMC/Ca^{2+}/GO would be a potential material for the construction of pH-controlled drug delivery platform.

1. Introduction

The past decades have witnessed the use of 5-fluorouracil (5-FU) as one of the effective chemotherapeutic agents in the treatment of cancers [1], including breast, stomach, intestine and colon cancers [2–4]. The current mode of treatment with infusions is inconvenient and costly, because repeated doses are essential due to the short half-life of 5-FU [5]. On the other hand, injurious bone marrow suppression or mucositis does sometimes happen with standard doses of 5-FU on infusion therapy [6]. Therefore, developing oral delivery of 5-FU in a controlled way to the cancer area would be highly beneficial [7].

Due to the fascinating features such as low cost, extensive sources, environmental friendliness and biodegradability, natural polysaccharides have been widely used in food industry, tissue engineering, bio-medicine and environmental protection [8–10]. Among the reported polysaccharides, chitosan (CS) [11], alginate [12], cellulose [13], starch [14] and β-cyclodextrin [15] have been successfully applied as the carriers for drug encapsulation and delivery. Especially, CS and carboxymethyl cellulose (CMC, a derivative of cellulose) are attractive and promising pH-sensitive drug carriers. The pH-sensitivity of CS is attributed to the pH-induced protonation/deprotonation of the amino groups (—NH_2) on CS chains [16]; the change in pH can cause the transition between the acid form (—COOH) and the base form (—COO^-) of CMC, resulting in significantly different swelling ratios of CMC [17]. However, drug burst release in controlled delivery systems based on CS or CMC can not be entirely ignored, probably due to the poor mechanical strength of CS and CMC.

Inorganic materials are widely used for the construction of biomaterials since the incorporation of rigid inorganic materials can improve the mechanical strength as well as stability. Among the commonly used inorganic materials, graphene oxide (GO) has received tremendous attention in recent years because of excellent biocompatibility, low toxicity, prominent thermal stability and mechanical properties, and it has been applied in biosensors [18], biomedicine [19] and drug delivery systems [20–23]. The two sides of GO sheet can be accessible for drug binding, which contributes greatly to the enhancement of drug-loading amount [22]. Therefore, GO-based drug delivery systems have drawn increasing attention in recent years.

In the present work, by using calcium ion (Ca^{2+}) as the crosslinker, hybrid aerogels of CS, CMC and GO are synthesized through electrostatic self-assembly approach followed by freeze-drying treatment. The as-prepared hybrid aerogels,

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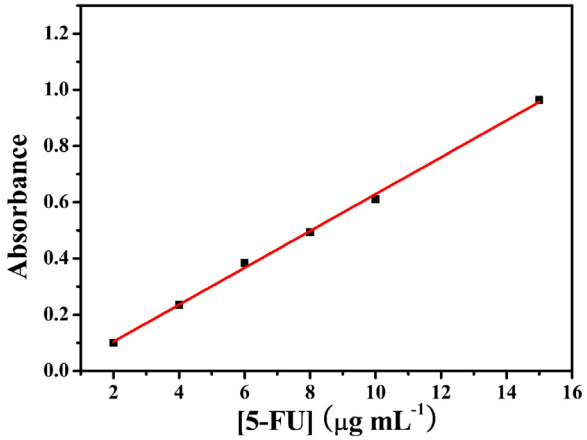

Fig. 1. Calibration plot of the absorbance of 5-FU to its concentration.

CS/CMC/Ca^{2+}/GO, are used as the carrier for drug loading and delivery by using 5-fluorouracil (5-FU) as the model drug. The results show that the delivery of 5-FU from the CS/CMC/Ca^{2+}/GO is pH-sensitive, and the investigation on the release kinetics demonstrates that the delivery of 5-FU from the hybrid aerogels is controlled by Fickian diffusion.

2. Experimental

2.1. Reagents and apparatus

Chitosan (CS), sodium carboxymethyl cellulose (NaCMC) and flake graphite were purchased from Sinopharm Chemical Reagent Co., Ltd. (SCRC, China). 5-Fluorouracil (5-FU) was obtained from Yika Biotechnology Co., Ltd (Shanghai, China). Other chemicals not mentioned were of analytical grade and used as received without further purification. All solutions were prepared using ultrapure water of 18.2 MΩ (Milli-Q, Millipore).

Fourier transform infrared (FT-IR) spectra were recorded on a Shimadzu FTIR-8400S spectrophotometer (Japan). The morphologies of CS/CMC/Ca^{2+}/GO were characterized on a Supra55 field emission scanning electron microscope (FESEM, Zeiss, Germany). A FD-1A-50 vacuum lyophilizer (Yuming Instrument Co., Ltd. Shanghai, China) was used for the freeze-drying treatment. The concentrations of 5-FU were precisely measured by using a Shimadzu UV160 UV–vis spectrophotometer (Japan) according to the predetermined calibration plot (Fig. 1).

2.2. Preparation of the hybrid aerogels

Firstly, GO was prepared from flake graphite according to the method reported by Marcano et al. [24], and then 100 mg GO and 300 mg NaCMC were dissolved in 150 mL of 0.1 M acetic buffer (pH 5.0). After continuous stirring for 60 min, 5 mL of 8 mg mL^{-1} $CaCl_2$ was added into this solution of GO and NaCMC with constant stirring for another 30 min. Secondly, 100 mg CS was dissolved in 50 mL of 0.1 M acetic buffer (pH 5.0) under stirring for 60 min, and the obtained CS solution (2 mg mL^{-1}) was added to the mixture of GO, NaCMC and Ca^{2+}. Finally, NaOH aqueous solution (2 wt%) was added to the mixture for pH adjustment (from 5.0 to neutral), and the hybrid hydrogels were precipitated. After being thoroughly washed with ultrapure water for three times, the hybrid hydrogels were freeze-dried in the vacuum lyophilizer under −45 °C for 24 h, and the hybrid aerogels (CS/CMC/Ca^{2+}/GO) were obtained. For a comparison, the hybrid aerogels of CS/CMC were also prepared via the similar procedures with the exception of the addition of GO and Ca^{2+} crosslinker.

2.3. Encapsulation of 5-FU to the hybrid aerogels

The CS/CMC/Ca^{2+}/GO were used for 5-FU encapsulation. Typically, 200 mg of the hybrid aerogels were dispersed in 100 mL of ultrapure water, and then 50 mg of 5-FU was added to this dispersion. Next, the mixture was shielded from light and continuously stirred for 24 h. Finally, the products were filtered, thoroughly washed and freeze-dried under −45 °C for another 24 h to form 5-FU loaded hybrid aerogels (CS/CMC/Ca^{2+}/GO–5-FU). The whole process showing the preparation of 5-FU loaded hybrid aerogels is illustrated in Fig. 2. According to the same procedure, 5-FU loaded CS/CMC aerogels were also prepared. After the CS/CMC/Ca^{2+}/GO–5-FU was filtered, the concentration of residual 5-FU in the filtrate was determined by the UV–vis spectra at the maximum adsorption of 5-FU (265 nm), and the encapsulation capacity of 5-FU could be calculated based on the following equation: Encapsulation capacity (%) = 100 M_{enc}/M_{add}, where M_{enc} is the mass of 5-FU encapsulated, and M_{add} is the mass of 5-FU added [25,26].

2.4. pH-Controlled delivery of 5-FU from the hybrid aerogels

pH-Controlled delivery of 5-FU from the hybrid aerogels was investigated. Three samples of CS/CMC/Ca^{2+}/GO–5-FU (15 mg) were accurately weighted and filled into three dialysis bags (molecular weight cut-off, 3500), and then these dialysis bags were placed into 50 mL of 0.1 M phosphate buffer saline (PBS) of pH 1.2, pH 5.5 and pH 7.4, respectively, at the temperature of 37 °C. During the release process of 5-FU, 3 mL of the PBS was taken out every 30 min for the quantification of the released 5-FU and an equal volume of fresh PBS (3 mL) was added to the testing solution to replenish it. The cumulative release (%) of 5-FU from the hybrid aerogels was expressed as the total percentage of 5-FU released at time *t* versus the mass of 5-FU encapsulated in the hybrid aerogels [16,27]. For the control experiments, the delivery of 5-FU from the drug loaded CS/CMC aerogels was also investigated in 0.1 M PBS of pH 7.4.

2.5. Measurements of swelling ratio

Swelling kinetics is of great importance in the understanding of controlled drug delivery behaviors [28], and thus the swelling ratios (*SR*) of CS/CMC/Ca^{2+}/GO were measured. First, dry aerogels (150 mg) were immersed in 100 mL of 0.1 M PBS (37 °C) of various pH values (1.2, 5.5 and 7.4), respectively. After a predetermined interval, the swollen samples were separated and the excess fluid was drained by filter paper. The *SR* value can be calculated based on the following equation: $SR = (m_t - m_0)/m_0$, where m_t and m_0 represent the weight of the swollen and dry sample, respectively, and *SR* is obtained as grams of water per gram of CS/CMC/Ca^{2+}/GO.

3. Results and discussion

3.1. Formation of the hybrid aerogels

The acidity coefficient (pKa) values of GO and CMC are 4.3 [29] and 4.0 [30], respectively, and therefore both GO and CMC are negatively charged at pH 5.0. As a result, the incorporation of CMC to the GO sheets becomes difficult owing to the electrostatic repulsion between GO and CMC. As previously reported, Ca^{2+} is an effective crosslinker for polysaccharides [31,32], so it is expected that Ca^{2+} also plays a crucial role in the combination of CMC and GO in this work. Different from GO and CMC, CS (pKa = 6.2 [33]) is positively charged at pH 5.0 owing to the protonation of the amino groups ($-NH_2$) on CS chains, and thus CS could be easily integrated to the negatively charged CMC through the electrostatic attraction. As Cerchiara et al. [34] reported, the complexs of CS and CMC are nearly spherical in shape. The FESEM images of the CS/CMC/Ca^{2+}/GO are