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Preparation and characterization of oxidized konjac glucomannan/carboxymethyl chitosan/graphene oxide hydrogel

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ABSTRACT

Polysaccharide hydrogels have been widely used as biomaterials in biomedical field. In this article, composite hydrogels were prepared through the Schiff-base reaction between the aldehyde of oxidized konjac glucomannan (OKGM) and the amino of carboxymethyl chitosan (CMCS). Meanwhile, different amount of graphene oxide (GO) was added as nano-additive. The hydrogels have been characterized by various methods including Fourier transform infrared spectroscopy (FT-IR) and Surface morphology (SEM). Through the observation of SEM, the hydrogels' scaffolds present a homogeneous interconnected pore structure after lyophilizing. In addition, the influence of different GO content on properties including gelation time, swelling ability, water evaporation rate and mechanical properties was investigated. The results indicate that the hydrogels have short gelation time, appropriate swelling ability and water evaporation rate. Especially, the compressive strength and modulus increase 144% and 296% respectively as the GO content increase from 0 to 5 mg/ml. Moreover, MTT assay was applied to evaluate the biocompatibility of hydrogels. The result indicate that hydrogels with GO show better biocompatibility. Therefore, due to the appropriate water absorption capacity, the similar compressive modulus with soft tissue and excellent biocompatibility, the composite hydrogels have potential application in wound dressings.

1. Introduction

Skin is an important natural barrier organ for protecting internal organs from the external environment and preventing body dehydration, and it would lose its protected mechanism upon damage [1]. In the same way, an intact barrier following the occurrence of a wound is of critical importance [2]. In recent years, as an important class of biomaterials, hydrogels have a wide range of applications in pharmaceutical and biomedical areas due to its unique properties including biocompatibility, biodegradability, non-toxicity [3]. The capacity of hydrogels to maintain a moist environment is important to facilitate the wound-healing process [4]. Their high water content as well as swelling ability to absorb large amount of body fluid facilitates also creating a moist environment that encourages rapid granulation tissue formation and reepithelialization [5]. Furthermore, hydrogels are structurally similar to the native extracellular matrices (ECM) [6,7], they can provide three dimensional structures for cell adhesion, proliferation, transportation of cytokines, nutrients and metabolic waste [8,9]. Therefore, hydrogels are very suitable to be used as wound dressings.

Natural polymers have similar components with native extracellular matrices (ECM) and are widely used for biomedical applications. Chitosan and its derivatives are among the most frequently used biomaterials [10]. Chitosan (CS) is composed of N-acetyl-Dglucosamine and D-glucosamine units linked by β-(1–4) bonds. It has always been used as a wound healing promoter [11–14]. However, it is suffering due to its poor water-solubility which largely restricts its application [15,16]. As a modified chitosan, carboxymethylated chitosan (CMCS) is an important water-soluble chitosan derivative, which exhibit low toxicity, biodegradability, biocompatibility, stability in blood and a good ability to form films and hydrogels [17–19]. So CMCS has been extensively used in many biomedical materials such as moisture-retention agents, bactericides, wound dressings, artificial tissue, blood anticoagulants and drug-delivery matrices [20–22]. Furthermore, CMCS is capable of stimulating the extracellular lysozyme activity of fibroblasts, promoting the proliferation of normal skin fibroblasts and inhibiting the proliferation of keloid fibroblasts [16].

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There are many reports on cross-linking of CMCS to form hydrogels, where small molecular cross-linking agents are generally involved but they have cytotoxicity potential [23–25]. Konjac glucomannan (KGM) is a natural polysaccharide and it is composed of D-mannose and D-glucose units linked by β-(1, 4) bonds. Being a β-(1, 4) linked polysaccharide, KGM can be oxidized by reacting with sodium periodate to produce OKGM [26,27]. Through the oxidation reaction, carbon–carbon bonds of the *cis*-diol group in the KGM molecular chain are cleaved and generate reactive aldehyde functions, which can chemically cross-link with CMCS via Schiff-base reaction between the free amino groups of CMCS and the aldehyde groups of the OKGM.

Conventional hydrogels consist of natural or synthetic polymers, usually exhibit relatively poor mechanical properties, which limits their practical applications as dressings [28]. To deal with this issue, researchers have paid special attention to graphene oxide, a precursor of chemically converted graphene, which consists of a two-dimensional sheet and has a large number of oxygen-containing groups such as hydroxyl, epoxide and carboxyl groups on the basal planes and edges [29,30]. These oxygen-containing groups impart GO sheets with the function of strong interaction with polymers to form GO intercalated or exfoliated composites [31]. Meanwhile, this property makes GO can be also easily combination with polymers for enhancing the mechanical properties of hydrogels [32]. In addition, studies have shown that graphene and graphene oxide own the ability to support cellular proliferation, adhesion, and differentiation with little or non-cytotoxic effects [33–35]. Moreover, it has been reported that GO shows pro-angiogenic properties [36], which can promote wound healing. Therefore, we consider adding graphene oxide into CMCS/OKGM hydrogels to improve the mechanical properties and biocompatibility.

In this article, we have prepared CMCS/OKGM/GO composite hydrogels, which were verified by FT-IR and SEM. And the effects of different GO content on the properties such as gelation time, swelling ability, water evaporation rate and mechanical properties was studied. In addition, the biocompatibility was evaluated by methyl thiazolyl tetrazolium (MTT) assay. On account of their biomimic composition and green fabrication procedure, the composite hydrogels are expected to have potential applications as wound dressings.

2. Experimental

2.1. Materials

Konjac glucomannan (The content of glucomannan is above 85%) was purchased from Konson konjac Corp. (Wuhan, Hubei, China). Chitosan (degree of deacetylation = 92%) was purchased from Zhejiang Yuhuan Ocean Biochemistry Co. Let. (China). Graphite power was purchased from Aladdin. Monochloro acetic acid, sodium hydroxide, sodium periodate, ethylene glycol, potassium permanganate, Diphosphorus pentaoxide, Sodium nitrate, 30%wt H_2O_2 and other reagent used in this article were of analytical grade and without further purification. They were purchased from Sinopharm Group Chemical Reagent Corp.

2.2. Preparation of carboxymethyl chitosan (CMCS)

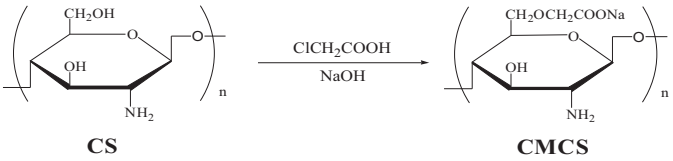
CMCS was prepared according to our previously research with slight modification [15]. Briefly, chitosan (6 g) was added into 50%wt NaOH solution and the mixture was kept at −20 °C for 24 h. Then the thawed chitosan was dispersed in isopropanol and monochloro acetic acid (9 g) was added. The mixture was stirred vigorously at room temperature. Then the mixture was heated to 60 °C for 5 h. The reaction product was dialyzed against distilled water for 3d through the 8000–10,000 molecular weight cut-off dialysis tubing, and vacuum-dried at 50 °C to obtain the purified CMCS and the dried samples were stored in vacuum desiccators for further use. The reaction is shown in Scheme 1.

2.3. Synthesis of oxidized konjac glucomannan (OKGM)

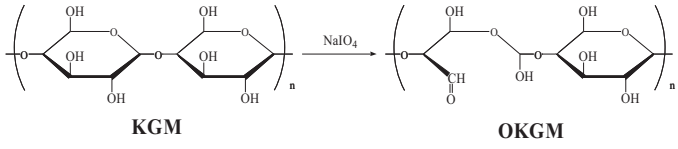
OKGM was prepared according to the previous research with slight modification [26]. Konjac glucomannan was oxidized using sodium periodate. In 600 ml of 1% (w/v) aqueous dispersions of KGM, 1.58 g of sodium periodate was added and the mixture was stirred vigorously at 30 °C in the dark for 12 h. Then 10 ml ethylene glycol was added to reaction mixture to reduce unreacted periodate and stirred for another 2 h. The reaction product was dialyzed against distilled water for 3 d until the dialysate was free from iodate (checked with silver nitrate). Then the reaction product was centrifuged for 20 min at 3000 rpm. The supernatant was vacuum-dried at 50 °C to obtain the purified OKGM and the dried samples were stored in vacuum desiccators for further use. The procedures of synthesizing OKGM were as follows in Scheme 2.

2.4. Synthesis of graphene oxide (GO) sheets

GO was prepared from graphite power by a modified Hummers' method [29,37]. Graphite powder (2 g), $K_2S_2O_8$ (1 g), P_2O_5 (1 g) were put into a flask, concentrated H_2SO_4 (20 ml) were added into the flask to preoxidize graphite power. The mixture was stirred vigorously at 80 °C for 5 h. Then the mixture was slowly cooled to room temperature over a period of 6 h. The mixture was filtered and washed until the filtrate was neutral. Then the filter cake was dried overnight at 50 °C. The preoxidize graphite power (2 g), $NaNO_3$ (2 g) and H_2SO_4 (100 ml) were put into a flask with stirring in an ice bath. Meanwhile $KMnO_4$ (6 g) was added slowly in portions to keep the reaction temperature below 10 °C. After stirring for 30 min, the mixture diverted to an oil bath with further stirring for 5 h at 35 °C, then the mixture was diluted with distilled water (500 ml) and followed by 15 ml of H_2O_2 (30%wt) was gradually added to terminate the reaction. This product was washed by diluted hydrochloride acid (1:10 in volume). After centrifugation at 8000 rpm for 10 min, the precipitation was washed until the supernatant was neutral. Then the product was dispersed in distilled water and sonicated for 3 h at 100 W and followed by the dispersion was dialyzed against distilled water for 3d. The dispersion was freeze-dried to obtain the purified GO.



Scheme 1. The synthesis of Carboxymethyl Chitosan.



Scheme 2. The synthesis of Oxidized Konjac Glucomannan.