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Preparation and characterization of chitosan – collagen peptide / oxidized konjac glucomannan hydrogel

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ABSTRACT

In this paper, the microbial transglutaminase (MTGase) was used as a catalyst to graft the collagen peptide (COP) molecules on the amino group of chitosan to obtain water-soluble chitosan-collagen peptide (CS-COP) derivatives. The preparation of composite hydrogel was via the Schiff-base reaction between the amino of CS-COP and the aldehyde of oxidized konjac glucomannan (OKGM). The hydrogels were characterized by various techniques including Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM). The results of SEM showed that the hydrogel sample had a clear and stable three-dimensional network structure. Meanwhile, these effects of the addition of OKGM on gelation time, swelling behaviors, water evaporation rate and blood coagulation capacity were investigated. The shortest gelation time for hydrogels was 99.3 s. The hydrogels showed a good swelling ability and appropriate water retention capacity. The maximum swelling ratio of the hydrogel was 265%. Dynamic blood clotting test showed that the hydrogels materials had good blood coagulation capacity. Moreover, The biocompatibility of hydrogels was evaluated with NIH-3T3 cells by MTT method. The results indicated that the hydrogels exhibited better biocompatibility. Therefore, this hydrogel has a promising potential to be applied as wound dressing.

1. Introduction

Human skin is an important organ of the body against the external environment. One of its most vital functions is protection against external mechanical aggressions, which is ensured by using reversible deformation of its structure [1,2]. The loss that large portions of the skin would lead to a lesion can bring out major disability or even death [3]. Hydrogels with characteristic properties such as desired functionality, reversibility, sterilizability and biocompatibility have many incredible purposes in tissue engineering, biology and pharmaceutical sciences [4–7]. Hydrogels can maintain a moist environment at the wound interface which is important for the wound-healing process [8]. Hydrogels can also absorb body fluids and simultaneously prevents their excessive loss, being permeable to oxygen, nutrients, and other water-soluble metabolites [9,10]. Furthermore, there are many similarities between hydrogels and the native extracellular matrix (ECM) [11,12], and they are a three-dimensional network structure which is beneficial to cell adhesion, proliferation, transportation of cytokines, nutrients and metabolic waste [13].Thus, hydrogels are well suited for use as wound dressings.

As a unique triple-helical structural protein, collagen is the primary component of extracellular matrices existing in all multicellular animals [14].Therefore collagen has been widely used in biomedical applications fields including hemostatic [15], drug delivery systems [16], wound dressings [17], and tissue engineering scaffolds [18]. COP is hydrolyzed from collagen, which has good antioxidant properties [19] and could be particularly beneficial for wound healing application [20].

Chitosan is a natural polymer derived from crustacean shells, insect exoskeletons, fungi, and algae, can effectively promote blood coagulation [21]. Chitosan possesses desirable properties, such as biodegradable, biocompatible, nontoxic and bioactive [22]. So, it has a beneficial effect as a wound healing promoter [23–25]. However, it is suffering due to its poor solubility in water which restricts biomedical application to some extent [26,27]. Accordingly, to exploit its application, increasing the solubility of chitosan will

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be a prerequisite. Compared to chemical modification, Enzyme-catalyzed reactions are constantly investigated as an attractive alternative to toxic, environmentally unfriendly and non-specific chemical approaches [28]. MTGase, an extracellular enzyme of the class of transferases [29], is used in many applications to attach proteins and peptides to small molecules, polymers, surfaces, DNA, as well as to other proteins at present [30]. And it catalyses an acyl transfer reaction between γ–carboxyamide groups of peptide-bound glutamine residues and ε-amino groups of lysine or primary amino groups of some polyamines [31]. In the previous work, we used MTGase as the catalyst to graft the COP on the amino group of chitosan to obtain the CS-COP derivative, and its solubility was greatly improved [32].

Konjac glucomannan (KGM) is a natural polysaccharide that is made up of β-1,4-linked D-mannose and D-glucose [33]. KGM can be prepared into various derivatives easily because of its good biocompatibility and biodegradable activity [34]. As a β-(1,4) linked polysaccharide, KGM can react with sodium periodate to obtain OKGM [35]. After the oxidation reaction, the carbon–carbon bonds of the *cis*-diol group in the molecular chain of KGM are cleaved and forms an aldehyde structure, which can react with CS-COP via Schiff-base reaction.

In this paper, we have prepared composite hydrogels from OKGM and CS-COP without employing any extraneous cross-linking agents, which were verified by FT-IR and SEM. The physical properties of the resulting hydrogels, such as gelation time, swelling ability, water evaporation rate were studied by varying the amount of OKGM. The hemostatic properties of the hydrogels were also evaluated in vitro. In addition, Cytotoxicity of gels was evaluated by methyl thiazolyl tetrazolium (MTT) assay.

2. Experimental

2.1. Materials

Konjac glucomannan (The content of glucomannan is above 85%) was purchased from Konson konjac Corp. (Wuhan, Hubei, China). Chitosan (M_w 520,000) with a 92% degree of deacetylation was purchased from Zhejiang Yuhuan Ocean Biochemistry Co. Let. (China). Collagen peptide (M_w 800) was purchased from Sichuan Mingrang Biological Technology Co. Ltd., Sichuan, China, without further purification. Microbial transglutaminase was purchased from Huashun Biological Technology Co. Ltd., Wuhan, China. Sodium hydroxide, sodium periodate, ethylene glycol, acetic acid, sodium dihydrogen phosphate, disodium hydrogen phosphate, calcium chloride 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 oxidized konjac glucomannan (OKGM)

The preparation of OKGM is described in [36] with slight modification. Into 5 g KGM dissolved in 500 mL distilled water. Fifty milliliter 0.15 mol/L NaIO4 solution was added dropwise and the mixture was stirred vigorously at 30 °C in the dark for 12 h. The degree of oxidation was found by determining the concentration of unreacted periodate by iodometry after 12 h [37]. Briefly, an aliquot (5 mL) of the reaction mixture was neutralized with 10 mL of 10 wt% sodium bicarbonate solution, and iodine was liberated by the addition of 20% potassium iodide solution (2 mL).After keeping in dark for 15 min, the liberated iodine was titrated with standardized sodium thiosulphate solution using starch as the indicator. And the oxidation degree of OKGM was determined to be 28%.Then 10 mL ethylene glycol was added to reaction mixture to remove unreacted periodate and stirred for another 2 h. After reaction, solutions were dialyzed against distilled water for 3 d with several changes of water till the dialyzate was periodate-free (checked with silver nitrate). The dialyzate was then centrifuged for 20 min at 3000 rpmThe supernatant was dried at 50 °Cto constant weight for the subsequent measurements. The procedures of synthesizing OKGM were as follows in Supplementary Data S1.

2.3. Preparation of chitosan – collagen peptide (CS-COP)

The details of preparation of CS-COP can be found in our previous report [32] .Briefly, appropriate amount of MTGase was dissolved in 50 mL of PBS (0.2 mol/L, pH 6.0) buffer solution. After the solution was centrifuged, the supernatant was vacuum filtered. The filtrate was dialyzed through the 8000–10,000 molecular weight cut-off dialysis tubing for 72 h and lyophilized to obtain purified MTGase lyophilized powder. Two grams of chitosan dissolved in 1% acetic acid solution was mixed with COP (2 g) dissolved in PBS (0.2 mol/L, pH 6.0) and pH was adjusted to 6.0. Then 0.2 g of purified MTGase lyophilized powder was added and mechanically stirred at 40° C for 1.5 h. Then the solution was treated in boiling water for 10 min and later cooled to room temperature. The CS-COP solution was obtained after filtering. Subsequently, the solution was neutralized with 20% (w/w) aqueous NaOH. Finally, the solution was dialyzed with distilled water through the 8000–10,000 molecular weight cut-off dialysis tubing for 72 h, and freeze-dried to obtain the purified CS-COP. The degree of substitution of CS-COP was confirmed by ultraviolet-visible spectroscopy.CS-COP (0.05 g/l) was dissolved in deionized water, and its absorption was measured at 200 nm. A standard curve of collagen was established in the range of 0.001–0.05 g/l. The degree of substitution of CS-COP is 0.554.The reaction is shown in Supplementary Data S2.

2.4. Preparation of CS-COP/OKGM hydrogel

A certain amount of CS-COP and OKGM was added to distilled water, magnetic stirred continuously at room temperature until dissolved to a final concentration of 6%wt, respectively. Volumes of 2, 4, 6, 8 and 10 mL of OKGM solution were added to the CS-COP solution. The mixture stood for some time to get the CS-COP/OKGM hydrogel. According to the amount of OKGM, the hydrogels were marked as OKCP-2, OKCP-4, OKCP-6, OKCP-8, and OKCP-10, respectively. The procedures of synthesizing hydrogels were as follows in Scheme 1.

2.5. Gelation time test

In this study, gelation time was assessed according to the previously reported method [38]. A mixture of CS-COP and OKGM solution was added in a 15 mL flat bottom vial (diameter 26 mm) and stirred using a Teflon magnetic stir bar (diameter 5 mm, length 10 mm) at 155 rpm. Gelling time was noted as the time required for the stir bar to stop using a stop watch.The experiments were performed in triplicate

2.6. Swelling measurements

The hydrogel samples (column, diameter 20 mm and height 10 mm) were lyophilized with a freeze-dryer. Then the samples were immersed in PBS buffer solution (PH = 7.4) at room temperature. After a period of time, the hydrogel can achieve swelling equilibrium, and the samples were removed from the tube and gently absorbed with filter paper to remove the excess of liquid