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Preparation and characterization of macromolecule cross-linked collagen hydrogels for chondrocyte delivery

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ABSTRACT

Collagen hydrogels are widely used in cartilage tissue engineering for their mimicked chondrogenic environment. Due to the rapid degradation nature and weak mechanical property, collagen hydrogels are often cross-linked in application. In this work, collagen hydrogels were soaked into oxidized alginate solution which used as macromolecular cross-linker to prepare the cross-linked hydrogels. Soaking method could retain the self-assemble property of collagen and also bring in a cross-linking network. The compressive modulus and degradation properties of collagen hydrogels were ameliorated after cross-linked, and chondrocytes encapsulated in the cross-linked hydrogels proliferated well and maintained the cell phenotype. This study implied that collagen hydrogels cross-linked by oxidized alginate may have a great potential for application in cartilage tissue engineering.

1. Introduction

Articular cartilage damage is a common problem in clinical, and once damaged, the self-repair ability of articular cartilage is limited due to its avascular, alymphatic nature [1]. Tissue engineering strategies have been used to repair articular cartilage defects and have made initial successes [2]. For a tissue engineering strategy, the cells are seeded and cultured in a suitable scaffold. A fair amount of materials including both natural and synthetic polymers have been made to serve as scaffolds for the study of chondrogenesis [3,4]. Among these materials, hydrogels are promising scaffold materials for cartilage tissue engineering because of the property of high water retention and the ability to maintain the spherical morphology of encapsulated cells [5–7]. In special, collagen hydrogels are suitable scaffolds for chondrogenic differentiation [8,9].

Type I collagen (Col) is the primary structural protein of the multicellular animals' extracellular matrix (ECM) [10,11]. It is easy for the neutral collagen solution to form hydrogels by self-assembly when the temperature is up to 37 °C. Due to the good biocompatibility and unique biological properties, collagen hydrogels have been widely used in many clinical applications, such as burn dressing and drug delivery [12,13]. They also play a pivotal role in tissue reconstruction and regeneration [14,15]. However, some properties of collagen could not meet the medical application requirements, such as mechanical strength, rapid degradation rate, etc. As an engineered scaffold, when collagen hydrogel is used to repair some long-cycle renewable tissue, the rapid degradation nature will limit its use in clinical practice. Therefore, to prolong the degradation rate of collagen is of great importance.

It is evident that the presence of even a few stable cross-links in the skin protein could greatly improve the mechanical strength and the resistance of leather to deterioration [16]. Usually, cross-linking is adopted to improve the strength and prolong the degradation duration of collagen-based biomaterials in vivo [17]. Cross-linkers used in tissue engineering can be roughly divided into two catalogs, chemically synthesized cross-linkers and biological cross-linker. At present, the commonly used chemically synthesized cross-linkers include glutaraldehyde, ethylene glycol diglycidyl ether, formaldehyde, etc. There are some shortcomings in chemically synthesized cross-linkers when they are used to deal with biological tissues. Firstly, the cytotoxicity of chemically synthesized cross-linker is relatively high [18,19]; secondly, the materials are permanently cross-linked by chemically synthesized cross-linkers and unable to be remodeled in vivo [20]; thirdly, when scaffolds cross-linked by these agents are implanted in vivo, they may have an effect on the growth of normal tissues. Biological cross-linker abstracted from natural, such as genipin, has a lower cytotoxicity and better biocompatibility compared with chemically synthesized cross-linking agent [21]. Though genipin is widely used in tissue engineering, it still has its disadvantage. When the scaffolds are cross-linked with genipin, a dark blue pigment spontaneously shows up during the reaction [21]. It may affect the appearance and color of the scaffolds. Thus, it might limit the use of genipin.

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Another type of biological cross-linker is obtained by moderately modifying the non-toxic biomaterials with good biocompatibility. Some polysaccharide molecules have the vicinal diol structure which could be oxidized by sodium periodate and transformed into aldehyde group. Aldehyde group can react with the amino-groups in active protein, polypeptide, specific biological active substance, etc. [22]. It can be used to modify the material or prepare the composite hydrogel.

As a natural polysaccharide extracted from seaweed, alginate has been intensively investigated in a variety of biomedical applications such as cell and drug delivery [23,24]. Alginate is a linear copolymer with homopolymeric blocks of (1-4)-linked β-D-mannuronic acid (M units) and its C-5 epimer α-L-guluronic acid (G units) residues linked together randomly. Studies have reported that alginate has the possibility to maintain the chondrocyte phenotype and promote the chondrogenic differentiation of stem cells [25,26]. Based on the feature that the vicinal diol groups in alginate can be partially oxidized to dialdehyde groups, alginate dialdehyde (ADA) is used as the cross-linker to crosslink collagen hydrogel [27]. The purpose is to prolong the degradation time and enhance the mechanical strength of the collagen-based hydrogel.

In this study, macromolecule cross-linked collagen hydrogels were fabricated by soaking the preformed collagen hydrogels into the partially ADA solution. ADA used as the macromolecule biological cross-linker can avoid the possible cytotoxicity caused by chemical cross-linker. Compared with the direct mixing of ADA and collagen solutions, this fabrication process could retain the self-assemble property of collagen and also brought in a covalent cross-linking between ADA and collagen. Meanwhile, it is easy to operate. The effect of crosslink on hydrogels as potential cartilage tissue engineering scaffold was characterized by the compressive modulus, swelling ability, enzymatic degradation rate and the cellular compatibility.

2. Materials and methods

2.1. Materials

Alginate was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Type I collagen (Col) was extracted from calf skin and suspended in 0.5 mol/L acetic acid solution. Sodium periodate, ethylene glycol, sodium hydroxide, hydroxylamine hydrochloride were obtained from Chengdu Kelong Chemical Co.

2.2. Preparation and oxidizing degree of ADA

ADA was prepared according to a reported procedure with slight modification [28]. A 1% solution of sodium alginate (1.0 g, 5.05 mmol uronate) dissolved in distilled water was mixed with an aqueous solution of sodium periodate (0.14 g,10 mL), and the mixture was stirred for 4 h in the dark at room temperature. The equimolar ethylene glycol to sodium alginate was added into the mixture to quench the reaction, keeping for half an hour [29]. The solution was purified by dialysis for at least 3 days against distilled water to remove the excess NaIO4. The purified product was obtained by freeze-drying and the ADA used in this work was marked as ADA1, ADA2, ADA3.

The degree of oxidation (DO) was determined by the quantitative reaction of hydroxylamine hydrochloride solution and aldehyde group, producing Schiff base and releasing hydrochloric acid (HCL) [30]. Through a titration method to calculate the amount of HCL, the concentration of aldehyde group can be calculated.

2.3. Preparation and cross-linking of collagen hydrogel

The acidic collagen solution (7 mg/mL) was firstly adjusted to neutral (pH 7.4) by adding 2 M NaOH at 4 °C, then the neutral solution was poured into molds and incubated at 37 °C to permit the formation of collagen hydrogel. The collagen hydrogel was cross-linked by oxidized alginate. At first, according to the fixed mass ratio of ADA and Col, amount of ADA1, ADA2, ADA3 was dissolved in PBS (pH 7.4), respectively, to form an aqueous solution. Then the hydrogels were immersed into the ADA1, ADA2, and ADA3 solution for 24 h at 37 °C and marked as ADA1-Col, ADA2-Col, and ADA3-Col, respectively. Uncross-linked collagen hydrogels were used as the contrast.

2.4. Morphology observation

Morphology of hydrogels was observed by scanning electron microscopy (SEM, S-800, HITACHI, Tokyo, Japan). At first, the hydrogels were frozen at −20 °C and lyophilized at −50 °C, and then the lyophilized gels were coated with ultrathin layer of gold/Pt in an ion sputter and observed by SEM.

2.5. Infrared (IR) spectroscopic measurement

An FTIR spectrometer was used to identify the functional group of oxidized alginate and cross-linked hydrogel. Samples used for analysis were milled with potassium bromide (KBr) which is transparent to infrared. All samples were recorded with FTIR spectrometer against a blank KBr pellet background.

2.6. Equilibrium swelling

Swelling study was conducted on Col, ADA1-Col, ADA2-Col, and ADA3-Col. The hydrogel samples were frozen at −20 °C and lyophilized at −50 °C, and then the freeze-dried samples were immersed in PBS and taken out at different predetermined time, and wiped with filter paper to remove excess liquid. The weight of freeze-dried samples before and after immersion was weighted and recorded as M_d and M_w, respectively. The swelling ratio (SR) was calculated according to the equation:

$$SR = \frac{(M_w - M_d)}{M_d}.$$

2.7. Degradation properties of ADA cross-linked collagen hydrogels

Proteolytic enzymes such as the collagenases solution (100 μg/mL) and four kinds of hydrogels (Col, ADA1-Col, ADA2-Col, ADA3-Col) were prepared. The hydrogels were washed by PBS, then wiped with filter paper and weighted, W_1. The hydrogels were transferred into a 24-well plate, and 1 mL enzyme solution was added into each well. The plate was put into a water-bath box and maintained at 37 °C. At a specific time point, the samples were removed, blotted gently with filter paper to remove surface water, and the swollen hydrogel was weighed (W_2). The degradation rate of hydrogels was calculated using the following formula: $(W_1 - W_2)/W_1 \times 100\%$.

2.8. Compressive modulus

Four kinds of hydrogels (Col, ADA1-Col, ADA2-Col, ADA3-Col) were prepared (11 mm diameter, 5.3 mm height, $n = 3$) for compression tests using a dynamic mechanical analyzer (CMT4104, Shenzhen SANS Testing Machine Co. Ltd). Samples were swelled in distilled water for 24 h and then compressed at a constant stress