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Photocrosslinked methacrylated carboxymethyl chitin hydrogels with tunable degradation and mechanical behavior

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ABSTRACT

Photocrosslinked hydrogels are being investigated for many tissue engineering applications because of the ability to form these materials in-situ in a minimally invasive manner by injection of aqueous solution under physiological conditions. In this work, carboxymethyl chitin (CMCH) synthesized homogenously was further modified with methacrylic anhydride and photocrosslinked into hydrogel with tunable degradation and mechanical properties. This new methacrylated carboxymethyl chitin (Me-CMCH) hydrogel formed in-situ photocrosslinked under UV irradiation showed much higher storage modulus than that of the thermosensitive in-situ forming physical-crosslinking CMCH hydrogel. The Me-CMCH hydrogels remained stable under physiological conditions and could be degraded by lysozyme. Cytotoxicity test indicated that the photo-induced Me-CMCH hydrogels were non-cytotoxic. The mechanical property, morphology, swelling and biodegradation behavior of the Me-CMCH hydrogels could be tuned by controlling the degree of methacrylation of Me-CMCH. These biodegradable photocrosslinkable Me-CMCH hydrogels may hold great promises for various biomedical applications.

1. Introduction

Hydrogels have been intensively investigated as scaffolds in tissue engineering or as delivery vehicles for cells, bioactive molecules, or other cargo due to their structural similarity to natural extracellular matrixes (ECM) (Cho et al., 2016; Lee & Mooney, 2001; Xu et al., 2015; Zhang et al., 2015). Hydrogels are hydrophilic three-dimensional networks with high water content, excellent biocompatibility, tunable physicochemical properties, and high permeability for oxygen, nutrients, and other water-soluble metabolites (Cho et al., 2016; Hoffman, 2012). Photocrosslinking has been an interesting method to fabricate hydrogels for many translational medicine studies because of the ability to convert aqueous solution to a hydrogel in-situ in a minimally invasive manner as effective injectable systems under physiological conditions (Cho et al., 2016; Jeon, Bouhadir, Mansour, & Alsberg, 2009; Nguyen & West, 2002). Photocrosslinking of polymers for hydrogel preparation is conducted in the presence of cytocompatible photoinitiators rather than toxic crosslinkers (Burdick, Chung, Jia, Randolph, & Langer, 2005). This approach allows for temporal and spatial control (Shin, Olsen, & Khademhosseini, 2012) and rapid encapsulation of cells with minimal cell death due to sufficiently mild conditions (Amsden, Sukarto, Knight, & Shapka, 2007; Nguyen & West, 2002), resulting in hydrogels with strong mechanical properties (Tai et al., 2009). Recently, there has been increasing interest in developing photocrosslinkable materials by methacrylated polymers for protein drug delivery and tissue engineering. For example, methacrylation modification of hyaluronic acid (Burdick et al., 2005), alginate (Jeon et al., 2009), chitosan derivates (Amsden et al., 2007; Cho et al., 2016), gelatin (Gauvin et al., 2012; Nichol et al.,

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2010) and chondroitin sulfate (Khanlari, Detamore, & Gehrke, 2013; Li et al., 2004) for photocrosslinkable hydrogels have been reported.

Chitin, as a kind of abundant and important polysaccharide in nature, has been widely studied in biomaterial field due to its superior biocompatibility, biodegradability, and gel forming ability (Ding et al., 2015). However, strong intermolecular hydrogen bonding makes chitin hardly soluble in physiological solvents, which puts restriction on usage of chitin (Liu, Liu et al., 2016). To overcome this shortcoming, many endeavors have been made including modification of chitin with hydrophilic groups such as carboxyl (Liu, Yang, Zhang, Zhuo, & Jiang, 2016; Sini, Santhosh, & Mathew, 2005) and acylamino groups (Ding et al., 2013). Carboxymethyl chitin (CMCH) is one of the most attractive derivatives of chitin as biomaterial owing to its good solubility (Liu, Yang et al., 2016). But carboxymethyl chitin which can be completely dissolved in pure water exhibits no gelling ability (Liu, Yang et al., 2016) and weak mechanical properties.

In order to enable CMCH to gel and obtain hydrogel with improved strength and stiffness, methacrylate group, a kind of photoreactive group, is introduced to synthesize photocrosslinkable methacrylated carboxymethyl chitin (Me-CMCH). As far as we know, such a Me-CMCH hydrogel has not been reported. Here, Me-CMCHs with different degree of methacrylation (DM) were synthesized based on carboxymethyl chitin and methacrylic anhydride. The hydrogels were prepared via photocrosslinking in the presence of photoinitiator. The effects of the degree of methacrylation on the physicochemical properties (e.g., swelling, mechanics, and degradation) of the resulting hydrogels were studied. In addition, cell viability was investigated as a preliminary test for the potential use of photopolymerizable Me-CMCH networks as cell carriers for tissue regeneration.

2. Material and methods

2.1. Materials

Chitin powder was purchased from Golden-Shell Biochemical (Zhejiang, China), which is in the form of α crystalline structure (Liu, Yang et al., 2016). Methacrylic anhydride was bought from Energy Chemical (Shanghai, China). 2-Hydroxy-4-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959) was purchased from BASF (China). Lysozyme (biological grade, ≥20,000 U/g) was supplied by Sinopharm Chemical Reagent (Shanghai, China). All other chemicals were reagent grade and used without further purification.

2.2. Synthesis of methacrylated carboxymethyl chitin

Methacrylated carboxymethyl chitin was prepared by sequential chemical modifications, carboxymethylation and methacrylation, of chitin. Firstly, carboxymethyl chitin (CMCH) was homogeneously synthesized in NaOH/urea solution (2 wt%) according to our previous paper (Liu, Yang et al., 2016). In brief, chitin was reacted with sodium monochloroacetate at 15 °C for 24 h in 11 wt% NaOH/4 wt% urea aqueous solution. Then the reaction solution was neutralized with HCl before dialysis against deionized water and lyophilization to obtain purified CMCH sample.

Secondly, methacrylated carboxymethyl chitin (Me-CMCH) was synthesized through the reaction between CMCH and methacrylic anhydride, according to the method to prepare methacrylated hyaluronic acid (Burdick et al., 2005). Briefly, 50 mL of CMCH solution (1 wt% in deionized water) was adjusted to pH 8 under magnetic stirring in ice bath, prior to the addition of a certain amount of methacrylic anhydride. The reaction was carried out for 12 h at pH 7–8 maintained by addition of 5 M NaOH. Afterwards, the reaction mixture was precipitated in acetone and washed with ethanol to remove methacrylic sodium and methacrylic anhydride. The acquired product was again dissolved in deionized water and then dialyzed using dialysis membrane (MWCO 8–14 kDa) against ultrapure water for two days at 4 °C followed by lyophilization to obtain purified Me-CMCH samples.

2.3. ^{1}H NMR and FTIR characterization

^{1}H NMR analysis was performed on a Mercury VX-300 spectrometer (300 MHz, Varian, USA). TSP (2,2,3,3-d4-3-(trimethylsilyl) propionic acid sodium salt) was used as an internal standard (at 0.00 ppm). CMCH was hydrolyzed in 20% DCl at 50 °C for 36 h (Liu, Yang et al., 2016) and Me-CMCHs were dissolved in 20% DCl at room temperature before NMR test. The FTIR spectra were recorded in a wavenumber range from 500 to 4000 cm^{-1} on a FTIR spectrometer (Perkin-Elmer Spectrum One FTIR spectrometer, USA).

2.4. In-situ formation of methacrylated carboxymethyl chitin hydrogel through photocrosslinking

Me-CMCH solution (1 wt%) was prepared by dissolving Me-CMCH into deionized water containing Irgacure 2959 (0.2 wt%) as photoinitiator. Centrifugation for Me-CMCH solution was performed to remove entrapped bubbles and then the solution was transferred into cylindrical molds. Me-CMCH hydrogels were attained via exposing the Me-CMCH solution to UV light (λ = 365 nm, ~0.85 mW/cm^2) at room temperature around 25 °C. The photocrosslinking process was traced with ^{1}H NMR analysis by observing the change of the characteristic peaks belonging to carbon-to-carbon double bond in methacrylate groups as a function of UV light irradiation time. As the representative sample, 1 wt% of Me-CMCH-3 solution was exposed to UV light for 5, 10, 25, 40 and 60 min to get a series of hydrogels (8 mm diameter × 5 mm height) with different degree of crosslinking reaction. These hydrogels were then freeze-dried and hydrolyzed in 20% (w/w) DCl for ^{1}H NMR measurement.

2.5. Rheological characterization

Dynamic rheological measurements of Me-CMCH hydrogels (1 wt%) were carried out at room temperature on a controlled stress rheometer (HAAKE Rheo Stress 6000, Thermo Scientific, Germany) equipped with a temperature-manageable system and with a 40 mm diameter parallel plate geometry. First, viscoelastic regime was determined by oscillatory strain sweep test at a frequency of 1 Hz. Then, the storage modulus (G') and loss modulus (G") of hydrogels were characterized as a function of frequency at 1% strain set based on viscoelastic regime.

2.6. Compressive mechanical property

The compressive mechanical property of Me-CMCH hydrogels was assessed on a universal testing machine (SANS, MTS system corporation, China) at room temperature. Cylindrical samples (8 mm diameter × 5 mm height) were compressed until failure. During the test the compressing rate was set as 1 mm/min and stress-strain curves were recorded. The compressive modulus was acquired from the slope of the initial linear region of the stress-strain curve.

2.7. Hydrogel morphology

The internal morphology of the photocrosslinked Me-CMCH hydrogels was observed through scanning electron microscopy (SEM; Zeiss SIGMA FESEM). The freshly prepared cylindrical