Carbohydrate Polymers 192 (2018) 10–18

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Thermosensitive and photocrosslinkable hydroxypropyl chitin-based hydrogels for biomedical applications

Meng Yuan, Bo Bi, Jiachang Huang, Renxi Zhuo, Xulin Jiang*

Key Laboratory of Biomedical Polymers of Ministry of Education & Department of Chemistry, Wuhan University, Wuhan, 430072, PR China

ARTICLE INFO

Chemical compounds studied in this article:
Chitin (PubChem CID: 6857375)
Glycidyl methacrylate (PubChem CID: 7837)
Irgacure 2959 (PubChem CID: 86266)
Propylene oxide (PubChem CID: 146261)
Urea (PubChem CID: 1176)
Sodium hydroxide (PubChem CID:14798)
Lysozyme (PubChem CID: 16129749)

Keywords:
Hydroxypropyl chitin
Methacrylation
Thermosensitive
Injectable hydrogel
Photocrosslinking

ABSTRACT

In situ forming injectable hydrogels based on thermosensitive polymers are being investigated for tissue engineering applications. However, the major limitations of this kind of hydrogels are low gel stability and weak mechanical properties under physiological conditions. Here, thermosensitive hydroxypropyl chitin (HPCH) was synthesized homogenously and subsequently functionalized with photocrosslinkable methacrylate groups *via* glycidyl methacrylate to generate glycidyl methacrylate-modified HPCH (GM-HPCH). The obtained new GM-HPCH polymers exhibited similar reversible thermosensitive sol–gel transition behaviors at a low concentration (2 wt% in PBS). The physical thermogelation GM-HPCH hydrogels were able to be photocrosslinked by UV irradiation under physiological conditions to form enhanced stable and mechanically strong hydrogels. The mechanical property, swelling and degradation behavior of the hydrogels could be tuned by controlling the degree of substitution of methacrylate groups and UV exposure time. Cytotoxicity test displayed that the photocrosslinked thermogels were non-cytotoxic. The photocrosslinkable GM-HPCH thermogels hold great potential for biomedical applications.

1. Introduction

Hydrogels are similar to natural extracellular matrix in that they have the ability to retain great quantities of water (Liu et al., 2016a; Lutolf & Hubbell, 2005; Omer, Hughes, Hama, Wang, & Tai, 2015). Because of their remarkable characteristics, such as high porosity, excellent biocompatibility, adjustable physicochemical properties, and biological properties, hydrogels have been extensively studied for biomedical applications (He et al., 2017; Jiang, Chen, Deng, Suuronen, & Zhong, 2014; Sun, Deng, Tian, & Lin, 2013; Wang et al., 2017; Xu et al., 2015; Zhang et al., 2015). Thermosensitive hydrogels that are usually spontaneously formed *in situ* under physiological conditions without any chemical reaction hold great potential for cell therapy and tissue regeneration, because of minimally invasive surgical procedures with the ease of handling and complete filling of the defect tissue (Bian et al., 2017; Li, Cho, Kwon, Janát-Amsbury, & Huh, 2013; Li et al., 2015; Yang, Yeom, Hwang, Hoffman, & Hahn, 2014). However, the physical nature of thermal gelation for thermosensitive hydrogels resulted in low gel stability and weak mechanical property under physiological conditions, which limits their potential applications (Cho et al., 2016; Dong et al., 2012; Potta, Chun, & Song, 2010a). Recently, many research colleagues have been working toward the development of injectable thermosensitive and *in situ* photocrosslinkable hydrogels with dual crosslinking structure to overcome the weak mechanical property (Cho et al., 2016; Coutinho et al., 2010; Tai et al., 2009; Vermonden et al., 2008). In general, thermogelling polymers can be chemically modified with photocrosslinkable (meth)acrylate groups. For example, Lee & Tae (2007) described a photo-polymerized hydrogel made of thermosensitive di-acrylated Pluronic F 127 (triblock copolymers). Vermonden et al. (2008) reported a photocrosslinkable thermosensitive A-B-A triblock copolymer p(HPMAm-lac)-PEG-p(HPMAm-lac) hydrogels, in which methacrylate groups were coupled to the side chains of the thermosensitive A blocks p(HPMAm-lac). However, they usually require a high polymer concentration (> 10 wt%) for thermogellation, and lack biodegradability. Cho et al. (2016) have proposed a chitosan based photocrosslinkable thermogelling polymer, methacrylated hexanoyl glycol chitosan. This polymer can thermogel at around 30 °C with a relatively low concentration (3–5 wt%), but the polymer synthesis needed complicated multi-step reactions using organic solvents or chemical agents, resulting in certain cytotoxicity and the difficulty in quality control. Therefore, it is of great interest to synthesize new biodegradable photocrosslinkable thermogels with less cytotoxicity and

* Corresponding author at: Key Laboratory of Biomedical Polymers of Ministry of Education & Department of Chemistry, Wuhan University, Luojia Hill, Wuhan, 430072, PR China.
E-mail addresses: yuanm@whu.edu.cn (M. Yuan), bibo_91@whu.edu.cn (B. Bi), jiachang_huang@whu.edu.cn (J. Huang), bmp@whu.edu.cn (R. Zhuo), xljiang@whu.edu.cn (X. Jiang).

https://doi.org/10.1016/j.carbpol.2018.03.031
Received 18 October 2017; Received in revised form 21 January 2018; Accepted 13 March 2018
Available online 15 March 2018
0144-8617/

Table 1
Characteristics of HPCH and GM-HPCHs used in this work.

Code	Molar feed ratio[a]	MS[b] (%)	DA[c] (%)	DM[d] (%)	M_η[e]($\times 10^{-4}$)	Tgel[f](°C)
HPCH	–	76	85	–	41.4	22.0
GM4-HPCH	5	76	85	4.1	44.1	20.6
GM8-HPCH	11	76	85	8.0	47.0	19.1
GM8-HPCH-2	16	76	85	8.0	47.2	19.0

[a] Feed molar ratio of glycidyl methacrylate to saccharide unit.
[b] Molar substitution of hydroxypropyl.
[c] Degree of acetylation.
[d] Degree of substitution of methacrylate groups determined by the peak integration of ^{1}H NMR.
[e] Viscosity-average molecular weight according to the Mark–Houwink equation $[\eta] = 7.92 \times 10^{-4} M_\eta^{1.0}$ (mL/g).
[f] Sol–gel transition temperature measured by rheological test in PBS with polymer concentration of 2 wt%.

better mechanical properties.

Chitin has been considered as new functional biomaterial of high potential in biomaterial field, due to its biocompatibility, biodegradability, non-toxicity, antimicrobial properties and bioactivity (Kang, Bi, Zhuo, & Jiang, 2017; Liu et al., 2016a). In our previous study, we have synthesized a new hydroxypropyl chitin (HPCH) with thermosensitivity homogeneously *via* the etherification of hydroxyl groups of chitin with propylene oxide in "green" solvent aqueous NaOH/urea solution (Jiang, Liu, Yang, & Zhuo, 2016; Jiang et al., 2017). This polymer is soluble in water at low temperature and gels rapidly under physiological conditions at a relatively low concentration (0.5–4 wt%). Hydrogels based on this HPCH polymer are enzymatically degradable, non-cytotoxic and good *in vivo* biocompatibility (Jiang et al., 2016, 2017). It can be injected and gel quickly inside the animal body and promote 3D cell growth.

The present study aimed to improve the stability and the mechanical properties of the formed thermogels, in which the methacrylate groups were introduced to the side chains of the thermosensitive HPCH polymer. The obtained new photocrosslinkable thermogels were prepared by photocrosslinking of glycidyl methacrylate modified hydroxypropyl chitin (GM-HPCH) in the presence of photoinitiator under physiological conditions. The effects of the degree of substitution of methacrylate groups and UV exposure time on the physicochemical properties (*e.g.*, swelling, mechanics, and degradation) of the resulting GM-HPCH hydrogels were investigated. Additionally, the cytotoxicity of the photocrosslinked GM-HPCH hydrogels was also studied as a preliminary test for the potential use as cell carriers for tissue regeneration.

2. Material and methods

2.1. Materials

Chitin powder was supplied by Golden-Shell Biochemical (Zhejiang, China). The viscosity-average molecular weight (M_η) was determined to be 3.75×10^5 Da according to the Mark–Houwink equation reported (Li et al., 2010). Glycidyl methacrylate was from Aladdin Reagent (Shanghai, China). TSP (2, 2, 3, 3-d4-3-(trimethylsilyl) propionic acid sodium salt) was purchased from Alfa Aesar (MA, USA), 2-Hydroxy-4-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure 2959) was bought from BASF (China). Lysozyme (biological grade, ≥20,000 U/g) was purchased from Sinopharm Chemical Reagent (Shanghai, China). All other chemicals were used without further purification.

2.2. Synthesis of hydroxypropyl chitin

Hydroxypropyl chitin (HPCH) was homogeneously prepared in 11 wt% NaOH/4 wt% urea solution (Fig. S1) as described in the previous work (Jiang et al., 2016, 2017). Briefly, 2 wt% chitin in NaOH/urea solution and propylene oxide were reacted under vigorous stirring at 15 °C for 24 h. The reaction solution was neutralized with 3.0 M HCl, and then dialyzed against deionized water using dialysis membrane (MWCO 8–14 kDa) for five days and freeze dried. Carboxymethyl chitin (CMCH) was synthesized in a similar way (Liu, Yang, Zhang, Zhuo, & Jiang, 2016b) with DS (degree of substitution of carboxymethyl) 0.34 and DA (degree of acetylation) 0.85.

2.3. Synthesis of GM-HPCH

Glycidyl methacrylate modified hydroxypropyl chitin was synthesized based on the literature (Cho et al., 2016). In brief, 0.5 g of HPCH was dissolved in 100 mL of deionized water and the pH was adjusted to 9 using 3.0 M NaOH at 25 °C. A certain amount of glycidyl methacrylate (1.4–3.9 mL) was added to the HPCH solution and stirred for 48 h at pH 7–9 maintained by addition of 3.0 M NaOH. The reaction solution was then neutralized by 1.0 M HCl, dialyzed against deionized water and lyophilized to obtain the purified GM-HPCH samples (the number in the sample code represents the percentage of the degree of methacrylation in Table 1). Glycidyl methacrylate modified carboxymethyl chitin (GM8-CMCH, DS 0.34, DA 0.85, and DM (degree of substitution of methacrylate groups) 0.08) was prepared in a similar way (Cho et al., 2016).

2.4. Characterization of GM-HPCH

^{1}H NMR spectra were measured on a Unity Inova-600 spectrometer (600 MHz, Varian, USA). TSP was used as an internal standard (at 0.00 ppm). HPCH and GM-HPCHs were hydrolyzed in 20% DCl at 50 °C for 36 h at a concentration of 1.0 wt% before NMR test (Liu et al., 2016b).

The intrinsic viscosity ([η]) of HPCH and GM-HPCHs in 0.1 mol/L NaCl aqueous solution was measured at 30 °C by using an Ubbe-lohde viscometer, and the solution was filtered by a G3 sand filter before measurement. M_η was calculated according to the Mark–Houwink equation: $[\eta] = 7.92 \times 10^{-4} M_\eta^{1.0}$ (mL/g) (Chen, Du, Wu, & Xiao, 2002).

Ninhydrin assay was used to determine the quantitative amount of primary amino groups of HPCH and GM-HPCHs (Leane, Nankervis, Smith, & Illum, 2004; Woranuch & Yoksan, 2013), see Supplementary data.

2.5. Thermogelling property

The thermal gelation of HPCH and GM-HPCH solutions (2 wt%, PBS) were performed on a rotating rheometer (HAAKE Rheo Stress 6000, Thermo Scientific, Germany) equipped with a temperature controlled water bath and a 40 mm diameter parallel plate geometry. The aqueous solution was loaded between parallel plates of a gap of 500 μm. A temperature sweep was conducted between 4 °C and 45 °C at a heating or cooling rate of 1 °C/min. The experiments were performed with a frequency of 1 Hz and 1% strain within the linear viscoelastic region. All the samples were sealed by a thin layer of silicon oil to