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Injectable chitosan/dextran-polylactide/glycerophosphate hydrogels and their biodegradation

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ABSTRACT

Dextran-polylactide (Dex-PLA) copolymers were synthesized and the selected Dex-PLA with water-soluble characteristics was used together with chitosan and glycerophosphate (GP) to produce injectable chitosan/Dex-PLA/GP hydrogels. Some chitosan/Dex-PLA/GP solutions with designated compositions were able to form into hydrogel in a temperature range between around 32 and 35 °C, and their pH values were found to alter between 7.0 and 7.2. Elastic modulus of the optimal chitosan/Dex-PLA/GP gel could reach about 1.0 kPa or higher, and meanwhile, it was much higher than their viscous modulus, revealing that these chitosan/Dex-PLA/GP gels behave like mechanically strong ones. Compression measurements indicated that the certain chitosan/Dex-PLA/GP gels had around 8-fold modulus and strength higher than the chitosan/GP gel, confirming that greatly enhanced compressive properties for chitosan/Dex-PLA/GP gels have been achieved. After 8-week subcutaneous degradation in rats, some chitosan/Dex-PLA/GP gels showed significantly extended degradation endurance compared to the chitosan/GP gel, and the PLA content in the chitosan/Dex-PLA/GP gels was able to regulate the degradation rate of the gels in a controllable manner. These results suggest that the presently developed chitosan/Dex-PLA/GP gels have promising potential for injectable gelling applications where the gel with mechanically strong features and degradation tolerance is needed.

1. Introduction

Stimuli-responsive hydrogels, which usually undergo a phase transition in response to external stimuli: changes in temperature, pH, solvent, ionic strength, electric or magnetic fields and light, have become a topic of extensive research [1–3]. Of them, thermosensitive hydrogels having *in situ* gelling properties near physiological pH and temperature have received growing attention as temperature is the sole stimulus for their gelation without other requirements for chemical or environmental treatment [3,4]. In general, the hydrogels with thermosensitive features can be injected into tissue or organ cavity in a minimally invasive manner, and form into solid-like fillers with specific shapes exactly matching with the cavity, which makes them attractive for various biomedical applications [5]. To date, several hydrogels prepared with synthetically sourced polymers, such as *N*-isopropylacrylamide (NiPAAM), poly(ethylene oxide)-*b*-poly(propylene oxide)-*b*-poly(ethylene oxide) (PEO-PPO-PEO) and poly(ethylene glycol)-biodegradable polyester copolymers, have been largely investigated [6–8]. Despite injectability, their gel formation usually requires higher polymer concentration, and the resulting hydrogels have slow gel-conversion rate, which leads to limitations of their applications [9]. In particular, NiPAAM and PEO-PPO-PEO are known to be non-biodegradable, which could result in resistance to metabolism for their *in vivo* applications [9,10].

As an alternative, much attention for injectable hydrogels has been given to certain natural polymers [11,12]. Among them, polysaccharides have been largely investigated for *in situ* forming hydrogels since they have good biocompatibility and biodegradable features that are not possessed by many synthetic polymers [12,13]. Of optional polysaccharides, chitosan has been used as hydrogels for varied applications due to its well-demonstrated advantages [12,14]. By adding certain polyol salts such as sodium glycerophosphate (GP) into chitosan solution, a type of chitosan/GP gel has been developed, which appears to be a viscous liquid at room temperature or below, and is able to convert into a solid-like gel near 37 °C at physiological pH, meaning that they have well-defined injectability due to suitable pH and sol–gel transition temperature [15]. Nowadays, chitosan/GP hydrogels have been

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used as *in situ* gelling scaffolds for the repair or reconstruction of cartilage, bone, nerve and skin defects [11,16–18], and also, as vehicles for delivering drugs or bioactive molecules [19].

Despite mentioned advantages, the usage of chitosan/GP hydrogels is frequently limited because of their low mechanical strength and high *in vivo* degradation rate [14,20]. There have been a few efforts towards enhancing chitosan/GP gels so far. An effort was related to incorporation of collagen into the chitosan/GP gel considering that collagen is a natural polymer with doable tensile strength, and hence, it could be able to enhance the chitosan/GP gel while retaining the injectable nature of the resulting gel [21]. Another effort involved the addition of starch to the chitosan/GP gel, and the resulting chitosan/starch/GP gel had a lowered sol–gel transition temperature in comparison to chitosan/GP gel while showing certain enhanced strength [11,22]. In addition, some chopped silk fibers have also been incorporated into the chitosan/GP gel, and the resulting gels show enhanced strength while retaining thermosensitive characteristics [23]. However, addition of silk fibers into the chitosan/GP gel seems to be unfavorable for the injection of gels.

Although some other biomaterials can be used to mechanically enhance chitosan/GP gels, the resulting gels may lose their gelling properties or injectable characteristics, or otherwise, have an unsuitable sol–gel transition temperature at physiological pH if an inappropriate material is applied to the chitosan/GP gel. In the present study, an attempt was made to enhance the strength of chitosan/GP gel while slowing its degradation by using dextran-polylactide (Dex-PLA) as a complementary component. Polylactide (PLA) is known to be linear and biodegradable polyester, and has mechanically strong characteristics and slow degradation rate [24–26]. Nevertheless, it will be very difficult to directly incorporate PLA component into the chitosan/GP gel due to the water-insulable features of PLA. Accordingly, some Dex-PLAs with water-soluble properties were first synthesized, and they were then added into the chitosan/GP gel as an accessorial component. It is expected that Dex-PLAs will be able to enhance the strength of chitosan/GP gel while extending the degradation duration of the resulting gels. It was found that some chitosan/Dex-PLA/GP gels indeed showed well-defined phase-transition characteristics near physiological temperature and pH whilst having significantly enhanced mechanical strength and prolonged *in vivo* degradation in comparison to the chitosan/GP gel. Hence, some results for these gels were reported.

2. Experimental

2.1. Materials

Chitosan was purchased from Aofulong Bio-Technology, China. To increase the degree of deacetylation (DDA) of chitosan, the received sample was treated in a 50% NaOH aqueous solution for 2 h at 95 °C and the alkali treatment was repeated once. Viscosity-average molecular weight and DDA of processed chitosan were measured as 7.15(±0.16) × 10^5 and 92.4(±1.8)%, respectively, following reported methods [24]. Dextran (Dex, M_w:23.8 k), L-lactic acid (LA), *N*,*N*′-carbonyldiimidazole (CDI), 4-(*N*,*N*-dimethylamino) pyridine (DMAP), β-glycerophosphate (GP) disodium salt, and other chemicals were of analytical grade and purchased from SinoPharm, China.

Dex-PLA copolymers were synthesized according to some methods described elsewhere [27–29]. Lactic acid oligomers with terminated mono-hydroxyl groups were first synthesized by ring-opening polymerization of LA with ethanol as an initiator and stannous octoate as a catalyst, and the average degree of polymerization (DP) was tailored by the monomer/initiator ratio [27]. The resulting lactic acid oligomers were processed with preparative HPLC (column: ACE C18, 10μ, 21.2 × 250 mm) to remove short oligomers by using acetonitrile as the solvent, and a gradient was run from 100% A (water/actonitrile 95:5) to 100% B (acetonitrile/water 95:5) in 50 min at a flow rate of 5 mL/min [28,29]. The oligomers with DP changing between 20 and 22 were collected for the follow-up coupling reaction. The hydroxyl group of the oligomers was activated using CDI and Dex-PLAs were synthesized via a coupling reaction between dextran and activated oligomers [27]. In a typical procedure, 240 mg of Dex and 220 mg of DMAP were dissolved in dry DMSO (8 mL). To this mixture, hydroxyl-activated oligomers (DP: 20–22, 180 mg) dissolved in dry DMSO (5 mL) was added, and the reaction was allowed to perform with stirring at room temperature for 4 days in a nitrogen atmosphere. After that, the reaction was stopped by addition of HCl to neutralize DMAP and imidazole. The obtained precipitate was collected by centrifugation and was washed with acetone. The traces of uncoupled PLAs were removed by Soxhlet extraction using acetone and the product was dried under vacuum to yield Dex-PLA powder sample.

Dex-PLAs with various PLA weight percentages were synthesized by mainly changing the feed ratio of Dex to oligomer, and in some cases, regulating the reaction time (4–6 days) and/or temperature (25–60 °C) [27,30]. The amount of lactic acid oligomers grafted to dextran was calculated using the integral area ratio of CH_3 of lactate oligomer at 1.41 ppm to the anomeric proton of dextran at 4.65 ppm in ^{1}H NMR spectra [28].

2.2. Preparation of chitosan/Dex-PLA/GP solution

A serious of Dex-PLA solutions was prepared by dissolving from 50 to 200 mg of selected Dex-PLA in 9 mL of 0.1 M HCl at a step-size of 50 mg. To each Dex-PLA solution, 200 mg of chitosan was introduced with stirring, and accordingly, four types of chitosan/Dex-PLA solutions with varied weight ratios of chitosan to Dex-PLA at 4:1, 4:2, 4:3 and 4:4 were produced. These solutions were cooled down to ca. 4 °C, and to each of them, 1 mL of 50 (w/v)% GP solution in distilled water was added dropwise. The resultant chitosan/Dex-PLA/GP solutions were additionally stirred to obtain homogenous ones and each solution had a final volume of 10 mL.

2.3. Characterization

^{1}H NMR measurements were recorded on a Bruker AV 500 spectrometer using dimethyl sulfoxide-d_6 (DMSO-d_6) as solvent.

Gelation performance was assessed using the inverted tube test. Typically, 2 mL of chitosan/Dex-PLA/GP solution were stirred for around 5 min in an ice/water bath and the solution was then introduced into a glass vial. Gelation time was measured as a function of incubation time that was recorded starting from the incubation of the glass vial in a water bath at 37 °C. The flowability of the solution was examined every 20 s by inverting the vials. The time at which the gel stopped flowing was designated as the gelation time.

2.4. Rheological measurement

Rheological measurements were carried out using a Kinexus Pro KNX2100 Rheometer (Malvern). The values of the strain amplitude were optimized to ensure that measurements were performed in a linear viscoelastic region in which the elastic modulus (G′) and viscous modulus (G″) were independent of the strain amplitude. Aliquots of the chitosan/Dex-PLA/GP solution (2 mL) were introduced onto parallel plate and the temperature dependency of G′ and G″ was measured from 25 to 45 °C at a temperature-elevated rate of 1 °C/min. To determine incipient gelation temperature (T_i),