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Galactose functionalized injectable thermoresponsive microgels for sustained protein release

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ABSTRACT

Novel galactose functionalized thermoresponsive injectable microgels, poly(N-isopropylacrylamide-*co*-6-O-vinyladipoyl-D-galactose) P(NIPAAm-*co*-VAGA), have been fabricated using a combination of enzymatic transesterification and emulsion copolymerization. The microgels exhibit reversible temperature-responsive behavior, which can be tuned by varying the monomer feed ratio. The lower critical solution temperatures (LCSTs) of the materials are close to body temperature and can result in a rapid thermal gelation at 37 °C. Field emission scanning electron microscopy showed the resultant microgels to have porous structures, and dynamic light scattering experiments indicated a dramatic reduction in particle size as solutions of the polymers are heated through the LCST. The polymers can be loaded with protein (bovine serum albumin; BSA), and *in vitro* studies showed that the BSA release kinetics depend upon the temperature and copolymer composition. Microgels based on P(NIPAAm-*co*-VAGA) could hence serve as candidates for site-specific sustained release drug delivery systems.

1. Introduction

In recent years, thermoresponsive copolymers have attracted increasing interest, and applications as biosensors, for protein adsorption, drug delivery devices, and in tissue engineering proposed [1–4]. Thermoresponsive injectable microgels which undergo a sol–gel transition in the physiological temperature range have drawn particular attention for use as *in situ* gel formation systems. Such a system would comprise a free flowing and injectable liquid at ambient temperature, but once injected would rapidly gel under physiological conditions with minimal syneresis [5–7]. Gel formation post-injection brings a number of advantages: an injectable matrix can be implanted in the human body with minimal surgical wounds, and bioactive molecules or cells can be incorporated by mixing before injection. Following gelation, these matrices can become drug delivery reservoirs or cell-growth depots for tissue regeneration [8,9].

Gelation can occur *in situ* by ionic cross-linking or after a change in pH or temperature. Thermosensitive hydrogels undergo abrupt changes in solubility in response to increases in environmental temperature, and are especially attractive since spontaneous gelation can be achieved without the need for chemical treatment [10]. For instance, poly(N-isopropylacrylamide) (PNIPAAm) exhibits a reversible temperature-responsive phase transition throughout its lower critical solution temperature (LCST) at *ca.* 32 °C in aqueous media [11–13]. PNIPAAm based hydrogels can thus be easily applied through a syringe, and undergo a rapid sol–gel transition at the target site. When the PNIPAAm chains are entangled in a polymer network, the temperature-response is manifested as a volume collapse referred to as the "volume phase transition temperature" (VPTT); the VPTT is generally close to the LCST of the corresponding linear polymer [14]. Below the LCST, the PNIPAAm chains are soluble in water due to the formation of hydrogen bonds between water molecules and their amide side chains. When the temperature is increased, water is expelled from the microgel interiors, thus causing a drastic decrease in volume above the LCST of the polymer [15,16]. The LCSTs of microgels based on PNIPAAm can be modified to be appropriate for biomedical applications by copolymerization [17,18]. Recently, Hoare et al. reported the fabrication of a novel type of microgel based on N-isopropylacrylamide and acrylic acid. The copolymer was loaded with bupivacaine as a model drug; it was found that drug release could be sustained for up to 60 days [19]. Such drug delivery vectors can have high drug loading capacities, and through their confinement effect may protect unstable drugs from hostile environments. Drug release can be modulated in response to temperature changes.

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Efforts to incorporate multi-functional, biocompatible and hydrophilic comonomers within these responsive systems have been vigorously pursued. One family of materials which has been explored in this regard are carbohydrates, particularly glycolipids, glycopolymers and polysaccharides [20]: these compounds are ubiquitous on the surfaces of various cells, and thus could increase the biocompatibility of gel implants. Polymers with terminal galactose or N-acetylgalactosamine moieties have been designed to target the galactose-receptor on human hepatic parenchymal cells [21]. These polymers can assist in achieving the effectively targeted delivery of biomaterials (nanoparticles, hydrogels, nanofibers, *etc.*) into liver cancer cells because they can selectively bind to the asialoglycoprotein receptors that are overexpressed on the surface of hepatoma cells [22,23]. Zhang et al. recently fabricated pH-responsive microgels based on polymers of galactosylated chitosan-graft-poly(N-isopropylacrylamide) and used these as carriers of oridonin (ORI) for anti-cancer applications. The microgels were found to enhance the uptake of ORI into HepG2 cells *via* asialoglycoprotein receptor-mediated endocytosis [24].

A promising strategy for designing novel microgel drug delivery systems could therefore be to combine themoresponsive and hepatocellular carcinoma targeting attributes into a single polymer. To achieve this goal, galactose functionalized thermoresponsive polymeric microgels have been designed, fabricated and investigated in this paper. Following a thorough characterization, bovine serum albumin (BSA) was loaded into the gels, and its *in vitro* release kinetics probed at both 25 and 37 °C.

2. Experimental

2.1. Materials

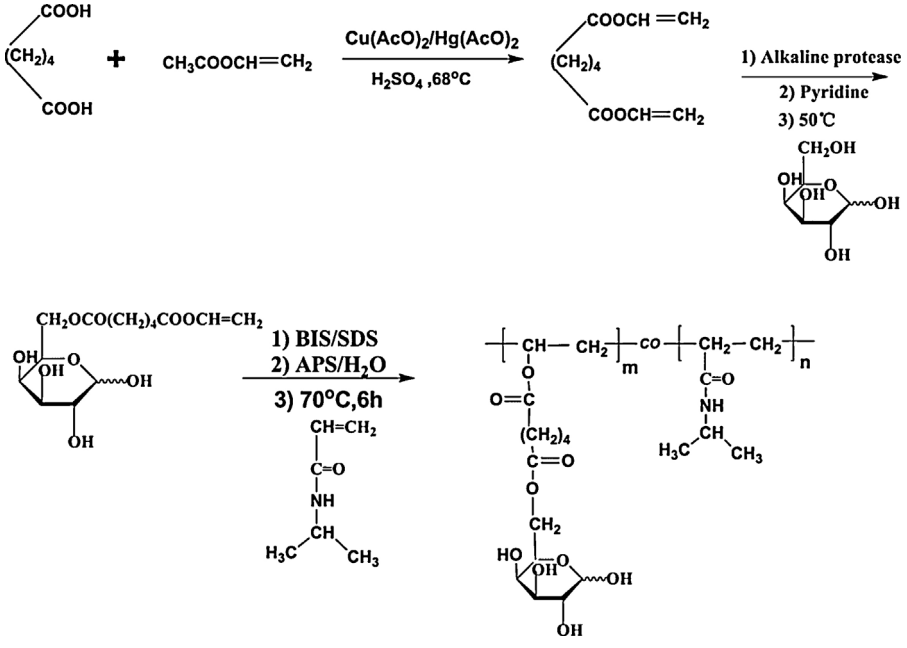
Alkaline protease from Bacillus subtilis (EC 3.4.21.14, a crude preparation of alkaline serine protease, power, 100 U/mg) was purchased from the Wuxi Xue Mei Technological Co. Ltd. N-isopropylacrylamide (NIPAAm), N,N′-methylenebis(acrylamide) (BIS), ammonium persulfate (APS), sodium dodecyl sulfate (SDS), and galactose were purchased from the Sinopharm Chemical Reagent Co., Ltd. NIPAAm was recrystallized from a n-hexane/toluene mixture. APS was recrystallized from deionized water and dried under vacuum. All solvents used in this work were of analytical grade and were dried by storing over activated 4 Å molecular sieves for 24 h prior to use.

2.2. 6-O-vinyladipoyl-α-D-galactose (VAGA) synthesis

6-O-vinyladipoyl-D-galactose (VAGA) was prepared by controllable regioselective enzymatic transesterification with alkaline protease as a catalyst, in anhydrous pyridine [25–27]. Galactose (3.96 g), adipic acid vinyl ester (13.14 g) and alkaline protease (1.0 g) were mixed with 100 mL anhydrous pyridine and reacted at 50 °C under 250 rpm stirring for 4 days. After reaction, the mixture was filtered and concentrated. The crude products were purified by silica gel column chromatography (mobile phase: ethyl acetate). ^{1}H NMR (DMSO-*d*6, *δ*, ppm): 7.21 (1H, dd, *J* = 6.3, *J* = 14.1 Hz, —CH═), 4.88 (dd, 1H, *J* = 14.1 Hz, *J* = 6.3 Hz, ═CH_2), 4.57 (dd, 1H, *J* = 6.3 Hz, *J* = 1.5 Hz, ═CH_2), 5.28 (d, *J* = 3.59 Hz, 0.4H, H-1 of α-D-galactose), 4.59 (d, *J* = 7.92 Hz, 0.6H, H-1 of β-D-galactose), 4.50 (m, 2H, CH_2—O), 4.30 (m, 2H, H-6,6′ of β-D-galactose, H-6,6′ of α-D-galactose), 4.04–3.66 (other α H or β H of D-galactose), 3.53 (t, *J* = 8.03 Hz, 0.6H, H-2 of β-D-galactose), 2.39 (m, 4H, 2—CH_2—), 1.68 (m, 4H, 2—CH_2—). ^{13}C NMR (DMSO-*d*6): 172.8, 170.4 (C═O), 141.2 (—O—CH═), 98.0 (═CH_2), 60.72, 60.94 (C-6α and C-6β of D-galactose), 70.19 (C-5α of D-galactose), 75.46 (C-5β of D-galactose), 68.09 (C-2α of D-galactose), 68.48 (C-4β of D-galactose), 68.9 (C-3α of D-galactose), 71.63 (C-2β of D-galactose), 73.53 (C-3β of D-galactose), 93.01 (C-1α of D-galactose), 97.18 (C-1β of D-galactose).

2.3. Microgel synthesis

A series of galactose functionalized crosslinked microgels of poly(N-isopropylacrylamide-*co*-6-O-vinyladipoyl-D-galactose) [P(NIPAAm-*co*-VAGA)] were sythesized *via* free radical precipitation emulsion polymerization, with some modification to the synthesis reported in the literature [28,29]. Polymerizations were performed in a three-necked flask with condenser attached. 1.13 g of the NIPAAm monomer, the desired amount of VAGA, 30.8 mg of the crosslinker N,N′-methylenebis(acrylamide) (BIS), and 58 mg of sodium dodecyl sulfate (SDS) were dissolved in 95 mL of deionized water. The solution was maintained at a temperature of 70 °C



Scheme 1. The synthesis of P(NIPAAm-co-VAGA) by controllable regioselective enzymatic transesterifications and emulsion polymerization.