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Facile fabrication of P(OVNG-*co*-NVCL) thermoresponsive double-hydrophilic glycopolymer nanofibers for sustained drug release

Mu-Ru Xu[a,1], Meng Shi[a,1], David H. Bremner[b], Kan Sun[a], Hua-Li Nie[a], Jing Quan[a,c,*], Li-Min Zhu[a,*]

[a] College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai, 201620, PR China
[b] School of Science, Engineering and Technology, Kydd Building, Abertay University, Dundee DD1 1HG, Scotland, UK
[c] Key Laboratory of Science &Technology of Eco-Textile, Donghua University, Shanghai 201620, PR China

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ABSTRACT

The thermoresponsive double-hydrophilic glycopolymer (DHG), Poly (6-*O*-vinyl-nonanedioyl-D-galactose-*co*-*N*-vinylcaprolactam) (P(OVNG-*co*-NVCL)) was synthesized via a chemo-enzymatic process and a free radical copolymerization and the resulting nanofibers were fabricated using an electrospinning process. The desired lower critical solution temperature (LCST) between 32 and 40 °C of the DHG polymers was achieved by adjusting the molar fraction of galactose monomer in the copolymers during the synthesis. The thermoresponsive DHG polymers were found to have good cytocompatibility with Hela cells as determined by the MTT assay, and special recognition of the protein peanut agglutinin (PNA). The drug release properties of these newly designed thermoresponsive DHG P(OVNG-*co*-NVCL) nanofibers are temperature regulated, can target specific proteins and have the potential application in the field of sustained drug release.

1. Introduction

Stimuli-responsive polymer nanocarriers are widely used in controlled-release systems [1,2]. These materials may change their affinity for water so that interior channels open or close in response to external stimuli such as temperature or pH change [3]. Thermoresponsive double hydrophilic copolymers are one of the most important types of biomaterials and have the capability of reversible micellization and dissociation responding to the change of temperature [4]. These copolymers, containing at least one functional unit such as temperature-responsive, light-sensitive and pH-sensitive monomers, have recently attracted significant interest for potential applications in drug delivery systems [5,6]. Thus, novel double hydrophilic polymers have been developed which can undergo a reversible change of morphology in response to the environmental stimuli to provide smart therapy with active drugs [7].

In order to modify functionality, to produce smart stimuli-responsive copolymers with bioactive molecules, much attention has been focused on the fabrication of glycopolymers due to their biocompatibility, biodegradability and the hydrophilic nature of the carbohydrate moieties [8–9]. The glycopolymers have been used to mimic biological systems such as binding lectins through multipoint interactions [10]. In our previous research, thermoresponsive double hydrophilic glycopolymers have been investigated and these glycopolymers have shown promising capability for protein binding [11].

Recent advances in micro and nanotechnology show that polymer microparticles or nanoparticles (NPs), micelles, nanogels, liposomes, dendrimers, and composite nanofibers can be used for drug delivery applications [12]. Compared with the nanoparticulate systems, electrospun nanofibrous scaffolds have high specific surface areas, high porosity, and three-dimensional network structures which can, for instance, enhance the adhesion, the proliferation and growth of cells [13]. In addition, using tissue engineering, the fibers can also be used as carriers in controlled drug release, delivering prolonged or better targeted drug delivery [14]. To the best of our knowledge, few reports relating to the thermoresponsive double hydrophilic glycopolymers nanofibers for drug controlled release are available.

* Corresponding authors.
E-mail addresses: jquan@dhu.edu.cn (J. Quan), lzhu@dhu.edu.cn (L.-M. Zhu).
[1] These authors are co-first authors.

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Table 1
The specific situation of L9 (3)[4] orthogonal of electrospinning.

Sample ID	Voltage (kV)	Collection Distance (cm)	Feed rates (mL/h)	Solution concentration (wt%)
1	12	12	0.3	35
2	12	15	0.7	37
3	12	18	0.5	39
4	13.5	15	0.5	35
5	13.5	18	0.3	37
6	13.5	12	0.7	39
7	15	18	0.7	35
8	15	12	0.5	37
9	15	15	0.3	39

In this work, thermoresponsive double-hydrophilic glycopolymeric (TDHG) nanofibers were prepared using free radical copolymerization and electrospinning methodology [15]. Drug-loaded TDHG nanofibers were also prepared in order to investigate the influence of temperature, pH and the nature of the polymer on drug release [16]. The cytotoxicity of TDHG nanofibers were also investigated for their potential as drug carriers in controlled drug delivery for targeting hepatic cells.

2. Materials and methods

2.1. Materials

N-vinyl caprolactam (NVCL) and Phosphate Buffered Saline (PBS) (0.01 M, pH 7.4) were purchased from Sigma–Aldrich (Shanghai) Trading Co., Ltd., Alkaline protease from Bacillus subtilis (EC 3.1.1.3, powder, a crude preparation of alkaline serine protease, powder, 200 u/mg) was acquired from the Wuxi Xuemei Technology Co., Fluorescein isothiocyanate (FITC), bovine serum albumin (BSA), peanut agglutinin (PNA) were purchased from Shanghai BioSun Sci & Tech Co., Ltd. Hela cell, fetal calf serum, trypsin (2.5 g/L) and Dulbecco's modified eagle's medium (DMEM) were acquired from Hang Zhou jinuo bio-pharmaceutical Technology Co., Ltd. Ethyl acetate, azelaic acid, mercuric acetate, copper acetate, petroleum ether, 2,2′-Azo-bis-iso-butyronitrile (AIBN, 97%), methyl alcohol, tetrahydrofuran, pyridine, sodium bicarbonate, sodium carbonate, sodium chloride, and dimethyl sulfoxide (DMSO) were purchased from the China National Medicines Corporation Ltd., (Beijing, China). β-Lactose and calcium chloride were of analytical grade and were purchased from the Sinopharm Chemical Reagent Co., Ltd. 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. All other reagents were used as received. Water was distilled before use.

2.2. Preparation of P(OVNG-co-NVCL)

The polymerizable galactose derivatives, 6-*O*-vinyl-nonanedioyl-D-galactose (OVNG), were synthesized, using alkaline protease as a catalyst, according to a previously published protocol in anhydrous pyridine at 50 °C, with stirring at 250 rpm for 3–4 days [17–18]. A series of P(OVNG-*co*-NVCL) were subsequently synthesized in dimethylformamide (DMF) with 2, 2′-azodiisobutyronitrile (AIBN) as an initiator using molar input ratios of OVNG–NVCL of 1:1, 1:4, 1:6, 1:8 (mol/mol). In a typical protocol, OVNG (0.2 g) and NVCL (0.0915 g/0.1489 g) were dissolved in DMF (1.0 mL), AIBN (0.00583 g/0.010978 g, 2% of total mass, w/w) was subsequently added after the two monomers dissolved under an atmosphere of N_2. The polymerization was carried out at room temperature for 8 h. The resulting products were subjected to three dissolutions (in methanol) — coaguline (by diethyl ether) cycles and then dried under vacuum. Giving a yield of 78%–80%.

2.3. Characterization

^{1}H NMR measurements were performed on an Advance (Bruker, Rheinstetten, Germany) Unity Plus 400 MHz nuclear magnetic resonance spectrometer using D_2O as the solvent. FT-IR spectra were obtained between 400 and 4500 cm^{-1} on a Nicolet NEXUS-670 (Nicolet Instrument Corporation, WI, USA) at room temperature. The spectra. The number-average molecular weight (*Mn*), weight-average molecular weight (*Mw*), and polydispersity (*Mw/Mn*) of the polymers were determined by gel permeation chromatography (GPC) at 35 °C using a Waters 1525 chromatograph equipped with a Waters 2414 refractive index detector. Tetrahydrofuran (THF) was used as the mobile phase at a flow rate of 1 mL/min. The GPC

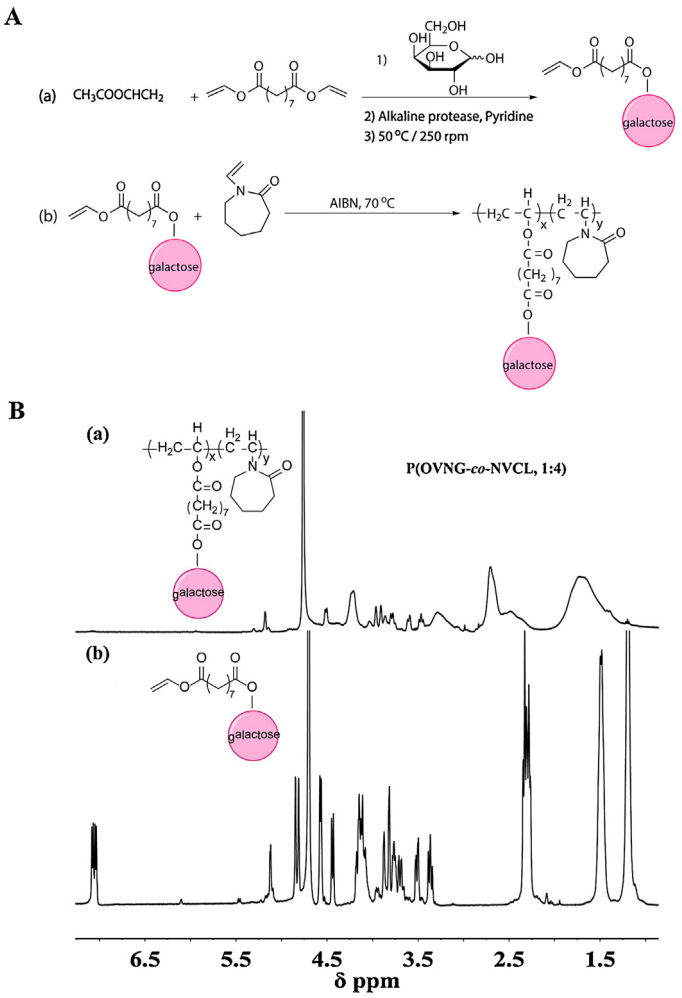

Fig. 1. (A) The synthesis of P(OVNG-*co*-NVCL) by controllable regioselective enzymatic transesterification and free radical polymerization; (B)^{1}H NMR spectra of (a) P(OVNG-*co*-NVCL) and (b) monomer OVNG.