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Preparation of injectable and thermoresponsive hydrogel based on penta-block copolymer with improved sol stability and mechanical properties

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ABSTRACT

New biocompatible, biodegradable and thermosensitive penta-block copolymer poly (*N*-isopropylacrylamide)–*b*-poly (ε-caprolactone)–*b*-poly ethylene glycol–*b*-poly (ε-caprolactone)–*b*-poly (*N*-isopropylacrylamide) (PNIPAAm–PCL–PEG–PCL–PNIPAAm) was synthesized by a combination of controlled ring-opening polymerization (ROP) and atom transfer radical polymerization (ATRP). This penta-block copolymer undergoes reversible sol–gel transitions between room temperature (22 °C) and human body temperature (37 °C). By incorporation of poly (*N*-isopropylacrylamide) (PNIPAAm) block at the end of PCL–PEG–PCL (PCEC) triblock copolymer, the resulting PNIPAAm–PCEC–PNIPAAm penta-block copolymer achieves improved mechanical properties and high sol stability while keeping its thermogelling property in the range of physiological temperatures 20–50 °C. The mechanical properties of these materials as a function of temperature were measured by rheometry. To confirm the sol stability, the crystalizability of penta-block copolymer was analyzed by the XRD. The sol-to-gel transition temperature of block copolymer aqueous solution is controllable by varying the composition and molecular weight of PEG and PNIPAAm. Their physicochemical properties in aqueous media were characterized by ^{1}H NMR spectroscopy and GPC techniques. This amphiphilic copolymer is self-assembled into spherical micelles in aqueous solution at room temperature. The critical micelle concentration (CMC) of these amphiphilic block copolymers was studied by fluorescence techniques. The size distribution and thermal responses of the polymeric micelles and aggregation to hydrogel in higher temperature were investigated by dynamic light scattering (DLS). The synthesized copolymer showed no apparent cytotoxicity on normal cells. *In vitro* release studies showed that the anticancer agent Na-HCl is effectively loaded and released by the temperature sensitive PNIPAAm–PCEC–PNIPAAm penta-block copolymer.

1. Introduction

Recently, controlled drug delivery systems using stimuli-sensitive and injectable polymeric materials have been attracted significant attention for biomedical applications [1,2]. Hydrogels prepared by chemical or physical crosslinking are a special class of polymer networks that can absorb a large amount of water while maintaining their shape. Due to porosity of hydrogels, the oxygen, nutrients, and other water-soluble metabolites can easily exchange inside the hydrogel networks. Injectable and biodegradable hydrogels that can change their structure in response to environmental stimuli (for example, temperature, pH, salt concentration, electric fields and so on) have been widely applied in drug/cell delivery and tissue engineering due to their various advantages such as hydrophilic network, prolonged delivery period, small body drug dosage and reduction of undesirable side effects by the protection of drugs from hostile environments [3–6]. These stimuli materials, specifically temperature stimuli, have ease of application because no surgical procedures are required for the insertion of gels into the body, therefore drugs, proteins and cells can be mixed homogeneously with the polymer solutions and administered by simple syringe injection at target sites [4,7–9]. Thus, thermosensitive hydrogels which use in drug/cell delivery and tissue engineering, should be sol at room temperature or lower, prior to subcutaneous injection, and should rapidly change to gel in the body temperature (37 °C), where ultimate degradation of the hydrogels is desired [4,10]. For biodegradability, aliphatic polyesters such as poly (ε-caprolactone) (PCl), poly(L-lactic acid) (PLLA), poly(glycolic acid)

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(PGA) or their copolyesters (PLGA) which their backbone can be cleaved by hydrolysis, are among the most attractive biodegradable polymers. So far, poly(ethylene glycol)/poly(lactic acid-*co*-glycolic acid) tri-block (PLGA–PEG–PLGA) and graft copolymers [11], poly(ethylene glycol) (MPEG)-poly(ε-caprolactone) (PCL) di-block copolymers [12], poly(ethylene glycol)/poly(ε-caprolactone) tri-block and penta-block copolymer [12–16], poly(ethylene glycol)/poly(propylene fumarate) [17], and polyphosphazene [18,19], were reported as typical thermoresponsive, biocompatible and biodegradable physical gels. The thermosensitive copolymers containing the PLGA segment have sticky paste morphology and thus they are usually difficult to transfer or weight. Furthermore, these copolymers required several hours of redissolution/reconstitution to be dissolved in water that is very difficult and time consuming [20,21]. Bae et al. [14] substituted the PLGA with the PCL in the hydrophobic polyester backbone and synthesized PCL–PEG–PCL (PCEC) tri-block copolymer as a biodegradable thermogelling polymer with a powder morphology that can transfer or weight easily. Also, this tri-block copolymer needs the easiest reconstitution/redissolution to be dissolved in water. However, because of high crystalizability of PCL, the tri-block copolymer aqueous solution (20 wt.%) becomes an opaque gel when left at room temperature (20 °C) over 30 min therefore, it must be used quickly after dissolution of the copolymer. This short time sol stability is an obstacle for the applications of these type materials as injectable hydrogels. The crystalizability of PCL is decreased as the molecular weight of PCL increases [22]. For this reason, PEG/PCL multi-block copolymer synthesized by coupling of PCL–PEG–PCL tri-block copolymers shows less crystalizability and partially improved sol stability due to the high molecular weight of polycaprolactone [23]. However, this system has high polydispersity (PDI = 2.3) and the precise control of the molecular weight of the multi-block copolymer is not easy. Park et al. [24] have synthesized end-group modified poly(ε-caprolactone-*co*-trimethylene carbonate)–*b*-poly(ethylene glycol)–*b*-poly(ε-caprolactone-*co*-trimethylene carbonate) tri-block copolymer to increase the amorphous character of the material, improving solubility in water and the sol stability, thus avoiding the precipitation problem in the PCL–PEG–PCL tri-block copolymer aqueous solution. Although some promising results have been achieved for this system, its weak mechanical properties are limiting its application as injectable gel. Poly(N-isopropylacrylamide) is also widely used as an excellent thermosensitive segment for *in vivo* drug delivery applications due to its lower critical solution temperature (LCST) (32 °C) that can change volume and shape abruptly and thermoreversibly at around the physiological temperature [25–28]. Copolymerization of PNIPAAm with hydrophilic polymer leads to thermogelling copolymer solution which is sol below the LCST and gel by increasing the temperature above the LCST [29,30].

To the best of our knowledge, up to now, there are no reports of any sol–gel system based on copolymers that contain both PCL–PEG–PCL tri-block copolymer and *N*-isopropylacrylamide. In this study, we assumed that the end-group modification of PCL–PEG–PCL tri-block copolymer with well-defined poly(*N*-isopropylacrylamide) using the atom transfer radical polymerization (ATRP) yields: 1) simple dissolution of copolymer in water, 2) improving the sol stability of hydrogel in room temperature (20 °C) by decreasing crystalinity of PCL chain, 3) improving mechanical properties, 4) precise adjusting of the composition and temperature-induced phase transition behavior of the synthesized hydrogel because the living radical polymerization allows the synthesis of the well-defined block copolymers with low polydispersitiy [25,26,31]. This method can remove some limitations such as, high polydispersity and unwanted random or branched polymerization that cause the masking of experimental results and make its intractable to study the effect of the hydrophobic/hydrophilic balance of PEG and PNIPAAm. The LCST and gelation concentration of polymer are regulated by changing the length of the PNIPAAm and PEG block and the temperature of the aqueous medium. The resulting copolymers were characterized by FT-IR, ^{1}H NMR, gel permeation chromatography (GPC), transmission electron microscopy (TEM), Dynamic light scattering (DLS) and X-ray diffraction (XRD). Some properties of hydrogel copolymer such as the LCST and critical micelle concentration (CMC) were also investigated.

2. Materials and experimental methods

2.1. Materials

Poly (ethylene glycol) (PEG, M_n = 1000 and 2000 g/mol) purchased from Fluka and was dried by azeotropic distillation using anhydrous toluene. ε-Caprolactone (CL) was purchased from Sigma and purified with CaH_2 by vacuum distillation. Stannous octoate ($Sn(Oct)_2$, 95%), 2-bromoisobutyryl bromide (98%), CuCl (99.9%), *N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA, 99%), and naltrexone hydrochloride (Na–HCl) were purchased from Aldrich and used as received. *N*-Isopropylacrylamide (NIPAAm, 97%; Aldrich) was purified by recrystallization from a toluene/hexane mixture. THF and 1,4-dioxane (>99%) were obtained from Aldrich Chemical Co. and dried by refluxing over sodium and distilled just prior to use. Dichloromethane (DCM) was purchased from Merck Chemical Co. and dried over calcium chloride ($CaCl_2$) and distilled. Triethylamine (TEA) was dried over calcium hydride (CaH_2) and distilled. All other commercially available solvents were purchased from Merck Chemical Co.

2.2. Synthesis of the thermosensitive PNIPAAm–PCEC–PNIPAAm penta-block copolymer

The thermosensitive PNIPAAm–PCEC–PNIPAAm penta-block copolymer was synthesized from the following three-step synthetic method: (1) Synthesis of hydroxyl-terminated PCL–PEG–PCL triblock copolymer (PCEC) by bulk ring-opening polymerization. (2) Esterification reaction of the terminal hydroxyl groups of the PCL with 2-bromoisobutyryl bromide to obtain macroinitiator (Br–PCEC–Br) for ATRP. (3) ATRP of N-isopropylacrylamide using difunctional PEG–$(PCL–Br)_2$, as macroinitiator. The reaction scheme is shown in Scheme 1.

2.2.1. Tri-block copolymer synthesis

The PCL–PEG–PCL (PCEC) triblock copolymer was synthesized using a ring-opening polymerization of ε-caprolactone in the presence of PEG as a macroinitiator and stannous octoate as a catalyst. Briefly, to synthesize the PCEC triblock copolymer, the PEG (M_w = 1000) (10 g, 10 mmol) was added in flame-dried flask and stirred at 80 °C until PEG was melted completely. Then 160 mmol (18.26 g, 17.73 mL) of ε-caprolactone (CL) and 0.16 mmol (0.064 g) of $Sn(Oct)_2$ were added to the reaction mixtures and stirred at 120 °C for 24 h. The product was recovered by precipitating into ice-cooled diethyl ether. The polymer was redissolved in methylene chloride and then reprecipitated by slowly adding diethyl ether. The resultant precipitate was filtered out and dried overnight under vacuum (product yield: 92%).

2.2.2. Synthesis of bromo-terminated PCL–PEG–PCL [PEG–(PCL–Br)$_2$]

Bromo-terminated (PEG–$(PCL–Br)_2$) was synthesized by the esterification of the terminal hydroxyl groups of the PCL with 2-bromoisobutyryl bromide in the presence of triethylamine (TEA) in DCM. Typically, 2 g (PCL–PEG_{23}–PCL) (0.77 mmol, M_n = 2597 g/mol) was dissolved in 50 mL anhydrous DCM,