

Thermo-tunable Injectable Thermosensitive Hydrogel and its Application as Protein Carriers

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Abstract: It is known that polymer chemistries determine mechanical and physical properties of hydrogels and thus its drug delivery performance. In order to achieve desired drug release behavior, triblock copolyester PCT-PEG-PCT with proper hydrogel formulation has to be synthesized. This research has demonstrated a way to adjust hydrogel mechanical and gelation properties by simply physically mixing amphiphilic copolymers with different composition to achieve desired protein carriers. Tri-block copolymer poly (CL-*co*-TOSUO)-PEG-poly (CL-*co*-TOSUO), briefed as (PCT-PEG-PCT) with different composition have been successfully synthesized. These copolymers are thermosensitive and can form hydrogels in aqueous solution. Copolymers with higher percentage hydrophobic PCT blocks show higher mechanical stiffness and yet lower solubility. To reduce the crystallinity of the hydrophobic block and soften the hydrogel, the copolymer with long PCT blocks was physically mixed with ones with shorter PCT blocks. The polymer mixture demonstrated a moderated mechanical stiffness and desired solubility. The polymer mixture also achieved a gelation temperature at 37°C, which is desirable for drug delivery. Macromolecular drug, bovine serum albumin (BSA) was used as model drug for its release study. This protein drug was successfully loaded into the polymer mixture, and the drug release study shows the polymer mixture is able to extend a stable drug release for over 48 hours. This result confirms physical mixing of PCT-PET-PCT thermosensitive copolymers can tune gel properties and improve drug delivery performance without redesigning and synthesizing new polymers.

Keywords: Injectable Thermogel; Copolymer; Polymer Mixture; Sol-gel Transition; Drug Release.

1. Introduction

Hydrogel is a three-dimensional, hydrophilic molecular network that is able to retain up to thousands of times their dry weight in water [1]. The hydrogel network can be formed via physical, chemical and biochemical bonding. Thermosensitive hydrogel, a type of physical gel, undergoes reversible transition from liquid to a gel in situ in response to environmental temperature change at 37°C, and it has attracted much attention for its biomedical applications, such as drug delivery and tissue engineering [2]. At temperatures below 37°C, the thermogel remains as liquid, and thus therapeutical molecules, such as peptides, DNAs, RNAs and drugs can be mixed with the polymer solution, and then the solution mixture is injected into body, proven as a minimally invasive technology to avoid surgical trauma. At body temperature (37°C), the polymer solution solidifies due to physical reaction, trapping the biomolecules in the semisolid gel matrix without causing any chemical reactions [2-3].

The first reported and well-studied thermogel is Pluronics, which an amphiphilic block copolymer is consisted of a central hydrophobic propylene oxide (PO) block in between hydrophilic ethylene oxide (EO) block. However, the main issue with Pluronis is its poor biodegradability. Many studies have addressed this issue by replacing PO blocks with biodegradable polyesters, such as PLA, PCL, PGA, and PLGA [3, 4]. When the polymers are dissolved in the water, the hydrophobic blocks collapse to assemble into compact core while the hydrophilic PEG segments swell in water. At the moment, the assembled micelles are suspended in the aqueous solution and no gel is formed. When the temperature is further raised, the micelles start to closely pack together, and ultimately forming an interconnected, crosslinked 3D

hydrogel [5, 6]. The gelation temperature is very important for biomedical applications. A sample with low gelation temperature is difficult to store, transport and handle. While if the gelation temperature is above the body temperature, the polymer solution will not become a gel when injected into the body.

The gelation temperature as well as gel mechanical strength are determined by molecular design, such as block chemistry, length of the hydrophobic and hydrophilic segments, and block construction (AB, ABA or BAB linked types). A small change in these factors may cause the gelation window shifted or no gelation at all. For example, Yu, et. al [5] have synthesized a series of PLGA-PEG-PLGA block copolymers, and found sol-gel transition temperature of the polymer aqueous solutions was increased from 10 to 25 °C when the PEG/PLGA ratios was varied from 1: 2.8 to 1: 2.1. Thus, to achieve an appropriate polymer hydrogel, it may require tedious and time-consuming efforts. Yu et. al [7] and Abebe et. al [8] have reported a simple way to fine tune the gelation conditions of thermosensitive hydrogels by simply mixing polymers of different chemistries. The reports demonstrated forming thermogels by simply mixing polymers with non-gelling properties and the gelation temperature can be conveniently tailored by only adjusting polymer mix ratios. The micellar aggregations are formed by mixed polymer molecules, which lead to gelation properties drastically different from the ones formed by single polymer molecule.

In this report, thermogels prepared by mixing PCT-PEG - PCT copolymers have shown enlarged gelation window and improved solution stability tailored for drug delivery. The hydrophobic PCT segment is consisted of ϵ -CL (ϵ -capro lactone) and pendant cyclic ketals substituted TOSUO (1, 4, 8-Trioxa [4,6] Spiro-9-Undecanone) where TOSUO modifies

the crystallinity of ϵ -CL and consequently the gelation behavior and mechanical strength of the thermogels [9]. The addition of TOSUO to the hydrophobic PCL segments will reduce the crystallinity of the hydrophobic core and thus reduce the gelation temperature as well as gel mechanical property. This paper studied mixtures of different PCT-PEG-PCT triblock copolymers and their corresponding thermogels. The mixture gels are able to broaden gelation window and thus the sol-gel stability for drug delivery. The gel mechanical strength as well as its drug delivery performance is also tuned by polymer mixing. The resultant mixed thermogel demonstrates a moderate gel strength and sustainable drug release of protein drug bovine serum albumin (BSA).

2. Materials and Methods

2.1. Materials and Reagents

1,4-dioxaspiro[4,5]-8-decanone ($C_8H_{12}O_3$), M-chloroperoxy benzoic acid (m-CPBA) and BSA (bovine serum albumin) was purchased from Sigma-Aldrich and used as received. ϵ -CL was obtained from Acros and was dried over CaH_2 for 2 days at room temperature and distilled under reduced pressure prior to use. PEG with molecular weight $M_n = 1500$ was acquired from Shanghai Aladdin Bio-Chem Technology Co. Stannous Octoate ($Sn(Oct)_2$, 99.9%) was supplied by Aldrich and distilled under reduced pressure just before use. All other reagents and solvents were purchased from Guangzhou Chemical Company and were purified prior to use.

2.2. PCT-PEG-PCT Preparation and Characterization

Synthesis of 1,4,8-trioxaspiro [4.6]-9-undecanone (TOSUO) was synthesized via Baeyer-Villiger oxidation based on literatures [9-10]. Briefly, $C_8H_{12}O_3$ was dissolved in dichloromethane (DCM) and was allowed to react with m-CPBA in reflux for 16 hours at 40 °C after mixing for 1 hour at 0 °C. After the reaction, pure TOSUO was obtained after recrystallization. Triblock PCT-PEG-PCT copolymer were synthesized via ring-opening polymerization of ϵ -CL and TOSUO using PEG ($M_n = 1500$) as macro-initiator follows the Scheme 1 shown below. Anhydrous PEG macro-initiator was dissolved in toluene with presence of ϵ -CL and TOSUO followed with addition of catalyst $Sn(Oct)_2$. The molelar ratio of ϵ -CL and TOSUO monomer mixture was monitored and added for reaction. During the synthesis, anhydrous PEG macro-initiator was first dissolved in toluene with mixtures of ϵ -CL and TOSUO followed with addition of catalyst stannous octoate. The reaction was carried out at 120 °C for 16 hrs under vaccum. The final product was first precipitated in diethyl ether and then was washed in methylene chloride for second precipitation. The final PCT-PEG-PCT polymer was filtered and dried under vacuum.

Molecular weight and weight distribution of the synthesized copolymers were determined by gel permeation chromatography (GPC) on Breeze 2 HPLC System, Waters.

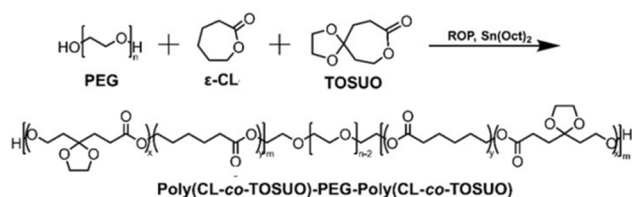


Figure 1. Synthesis schematic of PCT-PEG-PCT

2.3. Gel Formulation and its Thermosensitive Gel Behavior Characterization

The PCT-PEG-PCT micelle solution was prepared by solvent-exchange method. PCT-PEG-PCT copolymers with desired weight ratios were first dissolved in acetone. Then the solution was slowly added in DI water in dropwise. Then acetone was completely removed via evaporation under vigorous stirring, leaving injectable PCT-PEG-PCT micelle solution and the final polymer aqueous solution was stored at 4 °C.

The solution-gel (Sol-gel) phase transition diagrams of the PCT-PEG-PCT micelle solution were also determined by reverse vial method with different gel concentration. Briefly, vials with 1ml containing given concentrations of the PCT-PEG-PCT micelle solutions as described above (10%, 12.5%, 15%, 17.5%, 20%, and 25%) were immersed in a water bath starting at 15 °C at a heating rate of 1 °C/min and equilibrated at each given temperature for 15 min. At each temperature point, the sol-gel transition was examined by the inversion of the vial, and the sample was regarded as a gel in the case of no flow within 30 s.

The gelation dynamics of the PCT-PEG-PCT gel were determined by a Fluids Rheometer (Malvem, Kinexus Pro). The polymer aqueous solution of 20 wt% was kept below 4 °C before was placed between a 2° core-plate with a diameter of 60 mm and a gap of 0.07 mm for temperature sweep. Briefly, under amplitude sweep determine liner viscoelastic region, the solution was evaluated at heating rate of 1 °C/min under a controlled stress of 0.01 Pa and a frequency of 1.0 Hz. The storage modulus (G') and loss modulus (G'') were plotted with temperature in the range of 15-55 °C by single frequency (1 Hz) and constant shear (1%) measurement. The temperature at which storage modulus (G') was larger than loss modulus (G'') was determined as gel point where the gel was formed.

Field-emission SEM (ZEISS ULTRATM 55, Germany) was employed to investigate the gel microstructure. Hydrogel of interest was first vitrified in liquid nitrogen and then was freeze-dried for 48 hours. The dried sample was gold-coated and then was observed under SEM.

2.4. Release Behavior Study of BSA Loaded PCT-PEG-PCT Hydrogel

The in vitro protein release of BSA loaded PCT-PEG-PCT thermogel was evaluated with a BSA concentration varies as 12.5, 6.25, and 3.125 mg/ml with two different gel concentration (15% and 20%). BSA loaded PCT-PEG-PCT hydrogel was prepared by dissolving BSA in the above prepared PCT-PEG-PCT micelle solution with chosen concentration. To record the BSA release curves, each group take 5 mL polymer micelle solution to 15 mL centrifuge tube, under 37°C make glue and let stand for 30 min to make its stability, and then add 10 mL warm up to 37 °C to tube of PBS solution, at 37 °C (100 RPM) in the table, from a particular point in time (2 to 50 day), every time point takes 4 mL release liquid, and replace it with 4mL fresh PBS solution. Subsequently, BSA concentration in the released solution was detected by ultraviolet spectrophotometer, and the mass of BSA in the released solution was calculated according to the pre-determined standard curve, and thus the total released drug during the period of time was calculated.

3. Result and Discussion

3.1. PCT-PEG-PCT Characteristics

Copolymers PCT-PEG-PCT with varied TOSOU contents was successfully synthesized. The molecular weight, PDI and copolymer chemistries are listed in Table 1. The polymerization was well controlled so that the TOSOU to CL molar percentage was tuned from 11.4% to 16.6%. Since both polymers were synthesized using the same PEG macro-initiators, the PEG blocks of all copolymers remain the same. The PDIs of the copolymers maintains in the range of 1.2 to 1.3, indicating a controlled reaction.

Table 1. Structure, composition and TOSOU content of PCT-PEG-PCT copolymers

	PCT-PEG-PCT Structure	M_n	M_w	PDI	TOSOU/PCT [mol%]
P2	(ϵ -CL _{13,2} -co-TOSOU _{1,5})-(EG) ₃₄ -(ϵ -CL _{13,2} -co-TOSOU _{1,5})	5016	4617	1.3	11.4%
P3	(ϵ -CL _{13,2} -co-TOSOU _{2,2})-(EG) ₃₄ -(ϵ -CL _{13,2} -co-TOSOU _{2,2})	5250	5205	1.2	16.6%

The mixtures aqueous solutions of P2 and P3 were prepared and their gelation conditions were investigated. As shown in Table 2, the polymer mixtures can only undergo sol-gel transition when the copolymer weight percentage is in between 15% to 20%. As the weight percentage of P3 increases, the gelation temperature range starts to rise. Among all mixing conditions, the mixture P23, where mix weight ratio of P2: P3 = 2: 3, is well dissolved in water at low temperature and is maintained as solution at room temperature, and then turn into a dense gel when the temperature is raised to 37 °C.

Table 2. Gelation condition of PCT-PEG-PCT mixtures in water at different mixing ratios and weight percentage

Mix ratio P2:P3 (w/w)	10%	15%	20%	25%
1:9	--	28-38 °C	--	--
1:4	--	--	28-46 °C	--
2:3	--	34-46 °C	32-48 °C	--
3:2	--	36-46 °C	--	--

Although P2 and P3 have similar molecular structure, their gelation behaviors are distinctively different. The phase diagram in Figure 2 suggests a downward shift of gelation temperature as the TOSOU contents in PCT segment increases from 11.4% to 16.6%. This shift is due to TOSOU molecules. TOSOU pendant is confirmed to disrupt the regular crystalline structure of PCT hydrophobic segment, and the higher TOSOU content, the more irregularity in crystalline structure. Our study [9] shows the crystal irregularity promotes intermicellar percolation and cross-linking, and thus is beneficial to hydrogel formation. This tendency to gelation leads to a lower gelation temperature.

According to the phase diagram in Figure 2, neither P2 nor P3 is desirable for biomedical applications since P2 will remain as sol at 37 °C while P3 will turn into gel even at room temperature, making it hard to handle. Mixing P2 and P3, however, is able to tune the gelation property of the copolymer solution. As P3 content is raised in the mixture, the crystallinity of the micelles of mixing P2 and P3 is disrupted

as a result, and consequently its gelation temperature is reduced. Copolymers P23 demonstrated a gelation temperature at 37 °C in a wide range of polymer solutions, and thus are considered a good system for drug delivery application. P23 mixture was then used as the system for the following discussion. This study proves the validity of gelation modification by simply adjusting mixture ratios of copolymers of different chemistries.

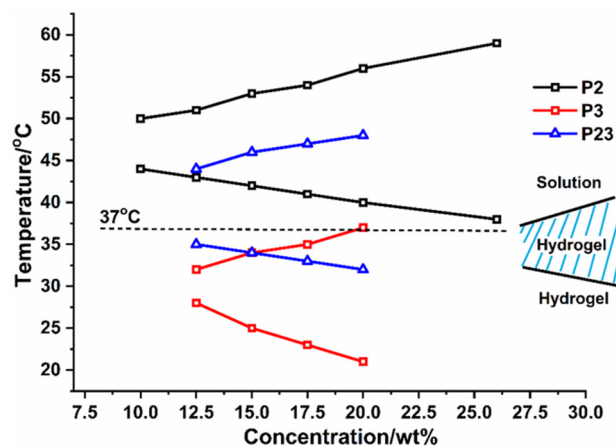


Figure 2. Phase diagrams of P2, P3 and P23 mixture aqueous solutions.

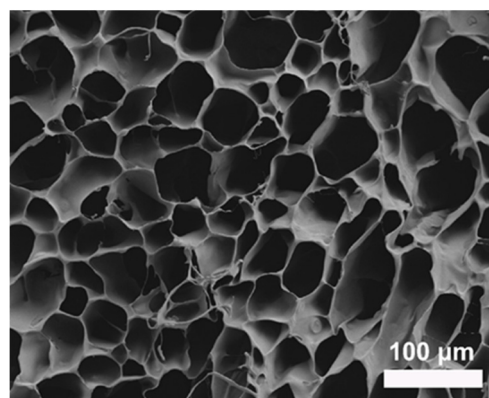


Figure 3. SEM micrograph of freeze-dried P23 hydrogel.

The microstructure of P23 gel was characterized by FESEM, presented in Figure 3. The SEM image of the P23 hydrogel reveals a porous network that is three-dimensionally connected. This porous microstructure is also observed other hydrogels made of mixing copolymers [8].

3.2. Gel Property and Drug Release

The gelation dynamics of the copolymer P2, P3 and P23 20% aqueous solutions were monitored with temperatures. Figure 4 shows the storage modulus (G') with temperature. The change of storage modulus confirms P23 formed dense hydrogel at around body temperature. While P3 gel was formed at lower temperature and its gel started to disintegrate at 37 °C. On the other hand, P2 did not even form a gel until 46 °C.

The gel property directly determines drug release performance. When drug is trapped in a stable and densely formed hydrogel, a sustainable drug release will be achieved since the gel matrix remains intact. When the gel structure is less stable, a quick drug release is observed due to matrix erosion. At 37 °C, P23 forms the most stable and dense hydrogel, and thus its drug release performance is of the most

interest.

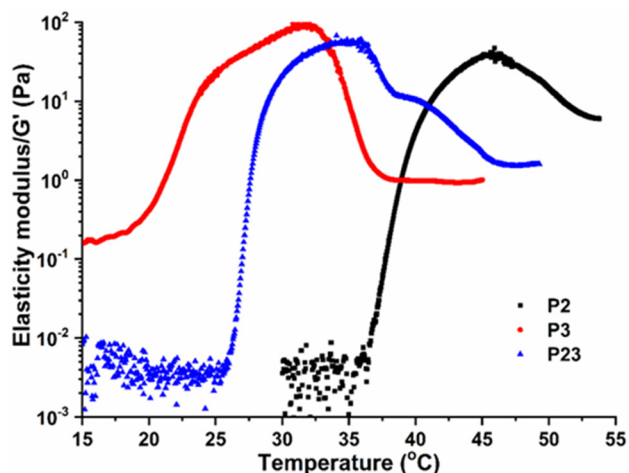


Figure 4. Storage modulus G' of P2, P3 and P23 solution (20 wt%) with temperature via rheologic measurement

The in vitro release behaviors of BSA from the copolymer P23 20% aqueous solutions were examined and the drug release profile is summarized in Figure 5. The drug release profile in P23 hydrogel is closely related to the drug concentration. When the drug loading was low at 3.125 and 6.25 mg/ml, a burst release was observed. The drug release profile is much improved when BSA concentration was around 12.5 mg/ml. The system decreased the burst release to a relatively low level (about 58% in first 12 hours) and increased the sustainable drug release up to 36 hours during which 58% to 75% drug released.

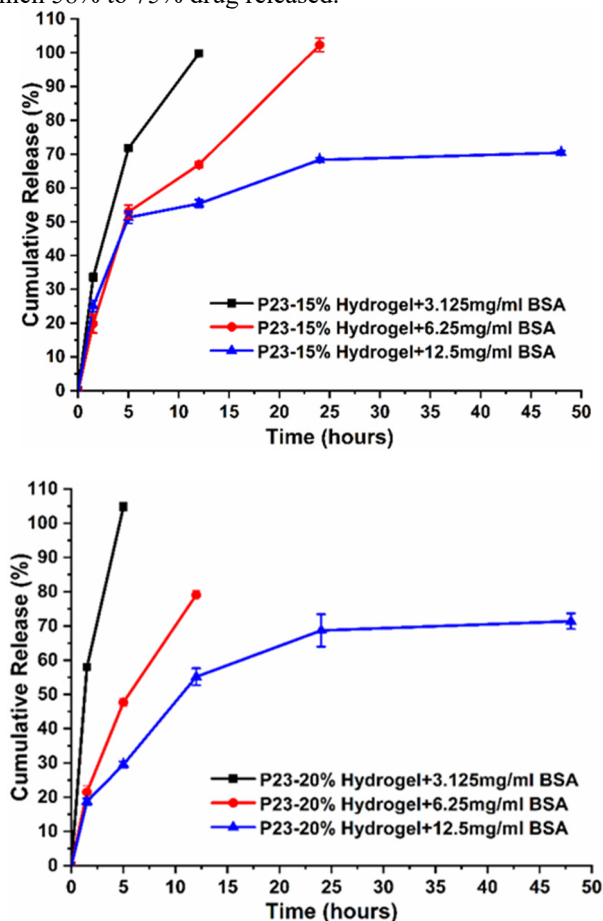


Figure 5. Cumulative release of BSA from P23 15% and 20% hydrogel with different BSA concentration at 37 °C

4. Conclusion

The thermosensitive hydrogel PCT-PEG-PCT was used as the protein carrier to construct an injectable thermosensitive hydrogel sustained release system. PCT-PEG-PCT hydrogels have good temperature sensitivity and its sol-gel transition temperature could be modified by only varying mix ratios of copolymers. The polymer mixture formed a highly porous but widely connected 3D structure. The copolymer mixture with preferred gelation condition and stably BSA release profile was identified. A sustainable protein release was achieved when the gel matrix was at its most stable and dense condition. The PCT-PEG-PCT could be used as the effective loading of protein active molecules, and its drug release behavior can be conveniently adjusted by mixing the hydrogel with different composition to meet different application demands.

Acknowledgments

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