Acta Biomaterialia 12 (2015) 81–92

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Thermoreversible hydrogels based on triblock copolymers of poly(ethylene glycol) and carboxyl functionalized poly(ε-caprolactone): The effect of carboxyl group substitution on the transition temperature and biocompatibility in plasma

Nazila Safaei Nikouei [a], Mohammad Reza Vakili [a], Markian S. Bahniuk [b,c], Larry Unsworth [b,c,d], Ali Akbari [e], Jianping Wu [e], Afsaneh Lavasanifar [a,d,*]

[a] Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta T6G 2E1, Canada
[b] Department of Biomedical Engineering, University of Alberta, Edmonton, Alberta T6G 2V4, Canada
[c] National Research Council, National Institute for Nanotechnology, Edmonton, Alberta T6G 2M9, Canada
[d] Department of Chemical and Materials Engineering, University of Alberta, Edmonton, Alberta T6G 2V4, Canada
[e] Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada

ARTICLE INFO

Article history:
Received 16 July 2014
Received in revised form 15 September 2014
Accepted 1 October 2014
Available online 16 October 2014

Keywords:
In situ hydrogel
Thermoresponsive
Protein adsorption
Biodegradable gel
Biocompatibility

ABSTRACT

In this study we report on the development, characterization and plasma protein interaction of novel thermoresponsive in situ hydrogels based on triblock copolymers of poly(ethylene glycol) (PEG) and poly(α-carboxyl-co-benzyl carboxylate)-ε-caprolactone (PCBCL) having two different degrees of carboxyl group substitution on the PCBCL block. Block copolymers were synthesized through ring-opening polymerization of α-benzyl carboxylate-ε-caprolactone by dihydroxy PEG, leading to the production of poly(α-benzyl carboxylate-ε-caprolactone)–PEG–poly(α-benzyl carboxylate-ε-caprolactone) (PBCL–PEG–PBCL). This was followed by partial debenzylation of PBCL blocks under controlled conditions, leading to the preparation of PCBCL–PEG–PCBCL triblock copolymers with 30 and 54 mol.% carboxyl group substitution. Prepared PCBCL–PEG–PCBCL block copolymers have been shown to have a concentration-dependent sol to gel transition as a result of an increase in temperature above ~29 °C, as evidenced by the inverse flow method, differential scanning calorimetry and dynamic mechanical analysis. The sol–gel transition temperature/concentration and dynamic mechanical properties of the gel were found to be dependent on the level of carboxyl group substitution. Both hydrogels (30 and 54 mol.% carboxyl group substitution) showed similar amounts of protein adsorption but striking differences in the profiles of the adsorbed proteome. Additionally, the two systems showed similarities in their clot formation kinetics but substantial differences in clot endpoints. The results show great promise for the above-mentioned thermoreversible in situ hydrogels as biocompatible materials for biomedical applications.

1. Introduction

Thermogelling polymers that undergo a sol-to-gel transition in aqueous media at temperatures a few degrees below the normal physiological temperature of 37 °C are of great interest as vehicles for regional drug delivery or tissue engineering applications [1]. Thermogelling materials are injectable at room temperature, but immediately transform into standing in situ hydrogels when exposed to physiological temperature upon administration. This unique characteristic provides for the minimally invasive application of these materials. In addition, the in situ forming hydrogel will be able to take the shape of the body cavity in which it is inserted. Small molecule drugs or macromolecular therapeutic agents such as peptides and proteins can easily be incorporated into the gel in the sol state at room temperature [2,3]. At body temperature, however, the in situ forming hydrogels can provide sustained or stimulus-controlled delivery of the incorporated therapeutic.

A common problem associated with the use of existing thermogelling materials, as in situ hydrogels, for biomedical

* Corresponding author at: 2-142F Faculty of Pharmacy and Pharmaceutical Sciences, Katz Group/Rexall Centre for Pharmacy & Health Research, University of Alberta, Edmonton, Alberta T6G 2E1, Canada. Tel.: +1 780 492 2742; fax: +1 780 492 1217.
E-mail address: afsaneh@ualberta.ca (A. Lavasanifar).

http://dx.doi.org/10.1016/j.actbio.2014.10.001
1742-7061/

applications are concerns over their inadequate safety and/or degradation profile. One of the first examples of relatively safe and degradable thermogelling materials was reported in 1990s. This in situ gel-forming material was based on a poly(lactide-co-glycolide)–*block*–poly(ethylene glycol)–*block*–poly(lactide-co-glycolide) (PLGA–*b*–PEG–*b*–PLGA) triblock copolymer [4] that was water soluble at room temperature but formed a gel at concentrations of 5–30 wt.% and elevated temperatures [5,6]. This block copolymer, which was later given the trade name of ReGel®, was also shown to enhance the solubility of poorly soluble drugs like paclitaxel and cyclosporine A by 400 to >2000-fold. The gel was used for depot drug delivery, with release times ranging from 1 to 6 weeks. For instance, excellent control over the release of paclitaxel was achieved using ReGel® for approximately 50 days, while proteins like pGH, G-CSF, insulin and rHbsAg showed a continuous release for up to 10–14 days without any signs of burst release when incorporated in ReGel® [7]. Block copolymers consisting of PEG and a more hydrophobic polyester, poly(ε-caprolactone) (PCL) [8,9], have also been widely studied for drug delivery or tissue engineering applications [8,10–14].

Our research group has developed a new family of biodegradable di- and triblock copolymers based on PEG and carboxyl-functionalized PCL [15,16]. The current manuscript describes the results of our investigations on the potential of triblock copolymers based on PEG and carboxyl-functionalized PCL for the formation of thermoresponsive and biocompatible in situ hydrogels. In this context, we defined the sol–gel transition temperature of the aqueous solutions of triblock copolymers as a function of polymer concentration by different methods and investigated the mechanism of thermoresponsive gel formation. We then assessed the potential biocompatibility of the gels using human platelet-poor plasma for the express purpose of studying protein adsorption and plasma clotting kinetics. This adsorbed protein layer has been shown to direct numerous responses, including coagulation, immune, complement and platelet activation, in addition to directing the interactions between the hydrogels and host cells [17]. A kinetic study of the clotting response of recalcified human plasma in response to the polymer hydrogel material will build upon the information gleaned from the adsorption experiments and further discern the biocompatibility of this system.

2. Materials and methods

2.1. Materials

Dihydroxy poly(ethylene glycol) (PEG, MW = 1450 Da) and palladium, 10% on activated charcoal, were purchased from Sigma, St. Louis, MO. α-Benzyl carboxylate-ε-caprolactone (BCL) was synthesized by Alberta Research Chemicals Inc (ARCI), Edmonton, Canada. Cyclosporine A was purchased from LC laboratories, USA. All other chemicals and reagents were analytical grade.

2.2. Synthesis of triblock copolymers

Synthesis of triblock copolymer was carried out following a previously reported two-step procedure [16] with some modifications. First, triblock copolymers composed of PEG in the middle and poly(α-benzyl carboxylate)-ε-caprolactone (PBCL) on the sides were synthesized through ring-opening polymerization of BCL in the absence of any catalyst. The polymerization was completed with 3 h of preheating at 140 °C followed by 15 h of heating at 160 °C. The product was purified by dissolving the polymer in dichloromethane and precipitation in ether followed by repeated washing with ether. The PBCL–*b*–PEG–*b*–PBCL block copolymer was then reduced using continuous hydrogen gas in the presence of palladium on activated charcoal at a concentration of 5 wt.% of the polymer. Dry tetrahydrofuran was used as the solvent. The degree of reduction was controlled by keeping the hydrogen gas flow rate to 19–20 ml min^{-1} and changing the reaction time between 30 to 90 min. For purification, the product was dissolved in dichloromethane and precipitated in hexane three times. The degree of polymerization and the number of carboxyl groups for each copolymer was measured by ^{1}H NMR spectroscopy, as described before [16]. As a result, poly[(α-carboxyl-co-α-benzylcaboxylate)-ε-caprolactone]–*b*–PEG–*b*–poly[(α-carboxyl-co-α-benzylcaboxylate)-ε-caprolactone] (PCBCL–*b*–PEG–*b*–PCBCL) triblock copolymers with 30 and 54% carboxyl substitution on the lateral blocks were produced.

2.3. Characterization of sol–gel transition temperature of triblock copolymer solutions in water

2.3.1. Visual observation of sol–gel transition by tube inversion method

The sol-to-gel transition of the synthesized triblock copolymer solutions was first determined by the tube inversion method [13,18]. For this purpose, PCBCL–*b*–PEG–*b*–PCBCL block copolymers were dissolved in distilled water at concentrations of 7, 10 and 15 wt.%. Vials containing 1 ml of polymer solution were immersed in a water bath at a temperature of 25 °C. The temperature was allowed to increase by 1 °C every 5 min up to 60 °C. The phase transition (flow/no flow) was assessed by inverting the tube vertically to allow a visual assessment of the aqueous polymer sample. The copolymer solution was considered to be a gel when the solution did not flow for 1 min upon inversion of the vial. The incubation temperature at which this phenomenon was observed, was recorded as the sol-to-gel transition temperature.

2.3.2. Modulated differential scanning calorimetry

Thermal analysis was performed with a Q series™ 2000 modulated differential scanning calorimeter. Triblock copolymer solutions in deionized water at concentrations of 7, 10 and 15 wt.% were prepared and kept in a refrigerator overnight. Each sample (10 mg) was hermetically sealed in an aluminum pan. The samples were first heated to 70 °C, then kept at this temperature for 5 min to eliminate their thermal history. Heating thermograms were recorded using a modulated mode with an amplitude of 0.2 °C in 60 s and a ramping of 0.5 °C per min. Scans were conducted from 8 to 10 °C. This was followed by holding the sample at 10 °C for 15 min, then increasing the temperature to 70 °C with the same rate under nitrogen gas. A pan filled with distilled water was used as a reference.

2.3.3. Dynamic mechanical analysis (DMA)

The storage and loss moduli were determined for polymer solutions as a function of temperature using a Physica MCR xx0 rheometer equipped with a Peltier heating/cooling plate. Polymer solutions in distilled water (7, 10 and 15 wt.%) were placed between two parallel plates. Temperature sweeps (20–60 °C) were conducted at a fixed strain of 1% using an angular frequency of 10 rad s^{-1}. Sweeps were conducted at 1 °C min^{-1}. The changes in the storage and loss moduli as a function of temperature were plotted. The transition temperature of a solution was estimated by dividing the loss modulus value by the storage modulus value. The sol-to-gel transition temperature was calculated as the temperature at which the ratio of loss modulus to storage modulus fell below one (graph not shown).