Effect of PLGA Block Molecular Weight on Gelling Temperature of PLGA-PEG-PLGA Thermoresponsive Copolymers

Noam Y. Steinman,1 Moran Haim-Zada,1 Isaac A. Goldstein,1 Ayelet H. Goldberg,1 Tom Haber,2 Jacob M. Berlin,2 Abraham J. Domb1

1Institute of Drug Research, School of Pharmacy-Faculty of Medicine, The Hebrew University of Jerusalem, Jerusalem 91120, Israel
2Department of Molecular Medicine, City of Hope Beckman Research Institute, 1500 East Duarte Road, Duarte, California 91010

Correspondence to: A. J. Domb (E-mail: avid@ekmd.huji.ac.il)

Received 23 August 2018; Accepted 3 October 2018; published online 25 November 2018
DOI: 10.1002/pola.29275

ABSTRACT: Thermoresponsive, biodegradable polymeric hydrogel networks are used widely in medicinal applications. Poly(lactic-co-glycolic acid)-b-poly(ethylene glycol)-b-poly(lactic-co-glycolic acid) (PLGA-PEG-PLGA) triblock copolymers exhibit a sol–gel transition upon heating. The effect of PLGA block and PEG chain molecular weights (MWs) on the gelling temperature of polymer aqueous solution (20% w/w) is described. All polymer solutions convert into a hard gel within 2 °C of the gelling temperature. The release properties of the gels were displayed using paracetamol as a representative drug. A linear relation is described between the gelling temperature and PLGA block MW. © 2018 Wiley Periodicals, Inc. J. Polym. Sci., Part A: Polym. Chem. 2019, 57, 35–39

KEYWORDS: biodegradable polymers; copolymerization; drug delivery systems; hydrogels; PEG-PLGA; thermoresponsive hydrogels

INTRODUCTION Effective controlled drug release provides the advantage of sustained therapeutic activity over a long time period. Poly(lactic-co-glycolic acid) (PLGA) has been studied extensively in this field due to its excellent biocompatibility and flexibility in terms of chemical composition.1–3 The potential to inject biodegradable polymers provides an attractive platform for the delivery of therapeutic materials directly to tissue without the need to remove unwanted byproducts.4

Polymeric hydrogels are three-dimensional systems that are able to absorb large amounts of water due to physical cross-linking of the polymer. “Smart” hydrogels have been developed to undergo a sol–gel transition as a response to a variety of external stimuli, namely, pH5 and temperature.6 In these systems, gelation is contingent upon the external stimulus; without it the polymer merely dissolves in the aqueous medium. This feature has allowed for the polymer to be injected to a site where the prescribed stimulus may be found, thereby causing the gel to form in situ.7

Biocompatible and biodegradable thermoresponsive hydrogel networks have gained popularity, particularly in the field of drug delivery, due to their ability to deliver peptides and drugs with low water solubility in situ.8–11 Specifically, these polymer networks have the special property that in dilute aqueous solutions they dissolve at room or cold temperatures, and at physiological temperatures undergo a sol–gel transition.

PLGA-b-poly(ethylene glycol)-b-PLGA triblock copolymers have been widely used to make safe, biocompatible, biodegradable, and crucially FDA-approved thermoresponsive hydrogels.12,13 The sol–gel transition temperature is affected by concentration, chain length of PEG, PLGA, the ratio between them, as well as the lactic acid/glycolic acid (LA:GA) ratio within the PLGA blocks.14–16 Polymer solutions with a sol–gel transition at physiological temperatures may be leveraged for their injectability and in situ gel formation.

While extensive research has been done on various applications of PLGA-PEG-PLGA triblock copolymers, including preparation of stimuli-responsive micelles,17 controlled release of proteins,10 or model small molecule drugs,19 and local delivery for bone regeneration,19 little attention has been given to role of block chain molecular weight (MW) in determining the gelling temperature of the thermoresponsive polymer gels. It is thus the objective of this study to systematically investigate the effects of both PEG and PLGA block chain lengths on the gelling temperature. Triblock copolymers of PLGA-PEG-PLGA with varying PEG lengths were synthesized and evaluated for gelling temperature and release of a model drug.
EXPERIMENTAL

Materials
PEG-1000 was purchased from Union Carbide Chemicals and Plastics Company Inc., Danbury, CT. PEG-1500 was purchased from BDH Chemicals Ltd., London, England. PEG-2000 and stannous octoate were purchased from Sigma Aldrich, Rehovot, Israel. Lactide and glycolide were purchased from Purac Biochem BV, Gorinchem, Netherlands. Dichloromethane (DCM) was purchased from Bio-Lab Ltd., Jerusalem, Israel.

Synthesis
PLGA-PEG-PLGA triblock copolymers were prepared by ring-opening polymerization (ROP) of PEG with β,β-lactide and glycolide in the presence of stannous octoate catalyst. A sample synthesis is as follows: 50 μL of a 100 mg mL⁻¹ solution of stannous octoate in DCM was added to a melt of PEG-1500 (595 mg, 0.397 mmol), β,β-lactide (696 mg, 4.83 mmol), and glycolide (94 mg, 0.81 mmol). Solvent was allowed to evaporate and the vial was purged with N₂. The mixture was stirred at 120 °C for 2 h, followed by overnight stirring at 150 °C. The crude polymer was taken up in DCM and precipitated into ether to afford polymer 8 in quantitative yield. Proton nuclear magnetic resonance (¹H NMR) (300 MHz, CDCl₃, δ): 5.22–5.14 (m, LA), 4.92–4.67 (m, GA), 3.64 (s, PEG), 1.56 (d, J = 6 Hz, LA); ¹³C NMR (75 MHz, CDCl₃, δ): 169 (C=O), 166 (C=O), 72 (LA, CH), 70 (PEG, CH₂), 69 (PEG, CH₂), 66 (LA, CH), 64 (GA, CH₂), 61 (GA, CH₂), 5.22 (m, LA), 4.92–4.67 (m, GA), 3.64 (s, PEG), 1.56 (d, J = 6 Hz, LA); ¹³C NMR (75 MHz, CDCl₃, δ): 169 (C=O), 166 (C=O), 72 (LA, CH), 70 (PEG, CH₂), 69 (PEG, CH₂), 66 (LA, CH), 64 (GA, CH₂), 61 (GA, CH₂), 5.22 (m, LA), 4.92–4.67 (m, GA), 3.64 (s, PEG), 1.56 (d, J = 6 Hz, LA); infrared (IR) (NaCl): ν = 1750 cm⁻¹, 1452 cm⁻¹, 1350 cm⁻¹, 1184 cm⁻¹, 1086 cm⁻¹.

Nuclear Magnetic Resonance
¹H and ¹³C NMR spectra were obtained on a Varian 300 MHz spectrometer with CDCl₃ as the solvent and tetramethylsilane as shift reference.

IR Spectroscopy
IR spectroscopy (2000 FTIR; PerkinElmer, Ra’anana, Israel) was performed on polymer samples cast onto NaCl plates.

Determination of Gelling Temperature
Total of 20% w/v aqueous polymeric solutions were incubated at a given temperature for 10 min, and the vial was inverted to test for gelling. If the gel did not flow, the temperature was recorded as the gelling temperature of the solution (T_gel). Results are accurate to ±2.0 °C.

Release Study
Paracetamol was dissolved in 1 mL of 20% aqueous PLGA-PEG-PLGA solution at a ratio of 5:100 paracetamol:polymer (w/w). The solution was heated to 37 °C until gel was formed. Total of 4 mL 0.1 M phosphate-buffered saline (PBS) solution was added on top of the gel at 37 °C. Paracetamol released was measured by ultraviolet (UV) absorbance at 243 nm.

RESULTS AND DISCUSSION

Rational Design of PLGA-PEG-PLGA Copolymer Series
Qiao et al. have demonstrated that the gelling temperature of aqueous solutions of PLGA-PEG-PLGA triblock copolymers was lowered by increasing either the lactide:glycolide (LA:GA) ratio in the PLGA block or polymer aqueous concentration.15 Here, we fixed a 6/1 LA:GA molar ratio and a 20% w/v aqueous polymer concentration in order to isolate the effect of PLGA/PEG weight ratio on gelling behavior of the copolymer (Fig. 1). A series of such polymers (Fig. 2) was thereby synthesized with varying PEG and PLGA MWs and ratios, while keeping constant a 6:1 LA:GA molar ratio in the feed. Gelling temperature of 20% w/v aqueous solutions of each polymer were then tested (Table 1). Polymers with different MW PLGA blocks were obtained by altering the ratio of combined LA and GA monomers relative to PEG in the feed.

Characterization
Polymer MW and experimental LA:GA ratio were defined by ¹H NMR (Fig. 3). To determine MW, the known integration value of the PEG peak (Fig. 3, Peak A) was compared to the integrations of the lactide and glycolide peaks (Fig. 3, Peaks C and D). The postpurification LA:GA ratio was also determined from the relative integration of the C (lactide CH) and D (glycolide CH₂) peaks of the ¹H NMR spectrum. Ester formation was confirmed by ¹³C NMR and IR. The carbon that experienced a chemical shift of 169 ppm corresponds to PLGA polyester and of 166 ppm to the ester connectivity between PEG and PLGA blocks (Fig. 4, Peaks B and P). The IR ester band at 1750 cm⁻¹ also indicates ester bond formation (Fig. 5). The spectroscopy and gelling results for all 26 synthesized polymers can be found in Table 1.
The mechanism of PLGA-PEG-PLGA triblock copolymer gel formation has been described. In short, at temperatures of about 0–25 °C, the interchain hydrogen bonding between PEG segments dominates the solution energy profile, and the polymer dissolves in water. As the temperature increases, these hydrogen bonds weaken, and interchain hydrophobic interactions between the PLGA segments of the copolymer strengthen, leading to a three-dimensional physically crosslinked system that does not exclude water, resulting in the hydrogel. As temperature is further increased, the hydrophobic interactions are further strengthened and the polymer crashes out of solution (Fig. 6). The PLGA/PEG ratio is therefore crucial in determining the sol–gel transition temperature ($T_{gel}$), as a low amount of hydrophobic interchain PLGA interactions relative to those of PEG would require a higher amount of energy to overcome the hydrophilic PEG-water and PEG–PEG interactions, and a high PLGA/PEG ratio would require less energy to overcome this barrier. Consequently, one would expect that a higher PLGA/PEG ratio would lead to a lower $T_{gel}$.

By controlling the LA:GA ratio, PEG MW, and polymer concentration, we were able to isolate the effect of PLGA block length on gelling temperature. As expected, increase of hydrophobic PLGA block lead to a lower gelling temperature, as the system required less energy for the PLGA–PLGA hydrophobic interactions to overcome the hydrogen bonding between hydrophilic PEG segments. Indeed, a linear relationship was found between descending PLGA block length and gelling temperature (Fig. 7).

### Controlled Release of a Representative Drug

The controlled release of paracetamol as representative water soluble drug from within the gel to external physiological media was tested to prove the ability of a therapeutic agent to be released from the gel matrix. To this effect, paracetamol was dissolved in an aqueous solution containing 20% w/v PLGA-PEG-PLGA 13, then heated to 37 °C to form gel. PBS was added on top of the gel, and it was exchanged for fresh PBS each morning until almost 90% of paracetamol had been released.
observed in the exchanged media. We chose paracetamol as a representative drug as its release profile was easy to follow by UV absorbance of the exchanged media. Over 50% of the paracetamol was released within 16 h, and 90% released within 64 h (Fig. 8). It should be noted that the gel maintained its robustness in the release media for over 1 week with almost no erosion or change in viscosity. These results are consistent with previously reported water-soluble drug release from PLGA-PEG-PLGA thermoresponsive hydrogels.\textsuperscript{15,21}

**FIGURE 3** Representative $^1$H NMR spectrum of PLGA-PEG-PLGA triblock copolymer (16) with peak assignments. LA:GA ratios were calculated by comparing the integration of their respective peaks (Peak C represents the CH of lactide and Peak D the CH$_2$ of glycolide), and overall polymer MW was determined by using the known integration of the PEG Peak (A) and adding to it the total LA and GA content. [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 4** Representative $^{13}$C NMR spectrum of PLGA-PEG-PLGA triblock copolymer (16) with peak assignments. Important to note are Peak B which represents PLGA block ester bonds and Peak F which represents the ester bridge between PEG and PLGA blocks. [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 5** Representative IR spectrum of PLGA-PEG-PLGA triblock copolymer (16). A strong band at 1750 cm$^{-1}$ may be observed for the formed polyester. [Color figure can be viewed at wileyonlinelibrary.com]

**FIGURE 6** Representative phase diagram of PLGA-PEG-PLGA (19) aqueous solutions. As temperature increases the solution turns to gel, and upon further heating a precipitate is formed. [Color figure can be viewed at wileyonlinelibrary.com]
Due to the robustness of the hydrogel, and its optimized sol-gel sharp transition, it can be injected into a physiological environment at room temperature as a liquid, and gel in tissue. When representative therapeutic material was dissolved in the room-temperature solution, controlled release from the gel was achieved. This finding may allow for the targeted delivery and controlled release of any therapeutic material at an injectable site.

CONCLUSIONS

We have quantified here the correlation between PLGA block MW and gelling temperature of PLGA-PEG-PLGA triblock copolymers at several MW PEGs. In this way, a polymer can be rationally designed in order to provide any gelling temperature desired for a given application. In addition, therapeutic agents may be released from the gel and into surrounding physiological medium at a controlled rate, affording controlled release at any injectable site.

ACKNOWLEDGMENTS

This work was supported by a grant from the Israel Cancer Research Fund (ICRF). This study was supported by an International Collaboration Grant from the Jacki and Bruce Barron Cancer Research Scholars’ Program, a partnership of the ICRF and City of Hope, as supported by The Harvey L. Miller Family Foundation.

REFERENCES AND NOTES