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New Sol-Gel Transition Hydrogels Based on Pluronic-Mimicking Copolymers Grafted with Oligo(lactic acids)

*Yuhan Lee, Sung Young Park, Hyun Jung Chung, Tae Gwan Park**

Summary: A novel type of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) triblock copolymers grafted with oligo(D,L-lactic acid) (OLA) on the middle PPO segment was synthesized. The Pluronic-mimicking copolymers exhibited gel-to-sol transitions with increasing temperature over a wide range of copolymer concentrations, and formed more stable hydrogel structures than Pluronic F127 copolymer in aqueous solution. The gel-to-sol phase transition temperature was controllable depending on chain length of the grafted OLA on the PPO chain. The gel-to-sol transition occurring with raising temperature was attributed to thermal destabilization and expansion of the inner hydrophobic core, which disturbed the packing order of spherical micelles required for physical gelation. The OLA grafted Pluronic mimicking hydrogels not only showed highly retarded erosion at 37 °C but also sustained protein release profiles, compared to Pluronic F127 hydrogel.

Keywords: injectable hydrogel; pluronic; sustained protein release

Introduction

Injectable hydrogels, exhibiting an environment-responsive sol-gel transition behavior in an aqueous solution, have received much attention for versatile applications for macromolecular drug delivery and tissue engineering.[1–5] Various amphiphatic polymers, such as poly(N-isopropylacrylamide),[6] poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO),[7] poly(D,L-lactic-co-glycolic acid)-poly(ethylene glycol)-poly(D,L-lactic-co-glycolic acid) (PLGA-PEG-PLGA),[8–11] and modified polyphosphazenes[12] were demonstrated of thermally induced sol-gel transition around physiological temperature.

Among the polymeric injectable hydrogels, PEO-PPO-PEO triblock copolymers, also known as the Pluronics, are extensively used as injectable carriers for macromolecular drugs and cells.[13,14] Pluronic copolymers undergo an immediate thermoresponsive sol-gel transition around room temperature when the concentration becomes higher than 20% (w/w). However, when injected into the body, the in-situ formed Pluronic hydrogel structure was quickly disintegrated at the local site due to rapid dilution with the body fluid. The unstable nature of the physically cross-linked Pluronic hydrogels formed in the human body limits their practical use as depot materials for delivery.[15,16] Thus, there have been many efforts to modify the PEO-PPO-PEO copolymer structure to enhance their gel stability in aqueous solution.[17] They include multi-block formation and inclusion complex formation between the PEO segment and α-cyclodextrin.[18]

In this study, a new series of PEO-PPO-PEO copolymers having several hydroxyl groups on the PPO chain segment were synthesized from a PPO precursor to

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Republic of Korea
Fax: (+82) 42 869 5621
E-mail: tgpark@kaist.ac.kr

mimick the Pluronic tri-block copolymer structure. Oligo(lactic acid) (OLA) chains with various chain lengths were also grafted to the middle PPO chain to confer physical stability after thermo-gelation in the body fluid. Macromolecular drugs were incorporated within the gels and their release patterns were investigated using Pluronic F127 as a control.

Materials and Methods

Materials

Poly(propylene glycol) (Mn:2,000), methoxy-poly(ethylene glycol) (mPEG, Mn: 5,000), and stannous octoate were purchased from Aldrich (Milwakee, USA). D/L-lactide (Purac, The Netherlands) was recrystallized in ethyl acetate. Epichlorohydrin, ethanol, dioxane, and N,N′-dimethylethylenediamine were purchased from TCI (Tokyo, Japan). All solvents were dried by standard procedures and distilled before use.

Synthesis of PEO-PPO-PEO Triblock Copolymer Having Hydroxyl Groups on the PPO Segment

Synthesis of Epoxide-Terminated PPO and mPEG

Epichlorohydrin (7.44 g, 0.08 mol) was slowly added to 50 mL of anhydrous 1,4-dioxane containing PPO (40 g, 0.04 mol) and a pulverized NaOH pellet (5 g, 0.25 mol). The suspension solution was heated to 65 °C and stirred magnetically for 5 h. The cooled suspension was filtered and the organic phase was extracted with dichlromethane. The organic layer was dried over Na_2SO_4, and dried under vacuum. Epoxide-terminated mPEG was synthesized.

^{1}H-NMR ($CDCl_3$): δ = 3.88–3.08 (br, 2H of PPO), 3.08 (m, oxirane), 2.78 (m, OCH_2), 2.42 (m, oxirane), 1.16–1.03 (br, 3H of PPO).

Synthesis of PPO Multi-Block Having Hydroxyl Groups on the Chain

The PPO multi-block precursor copolymer having OH group in the backbone was prepared by reacting epoxide-terminated PPO (21.1 g, 0.01 mol) with N,N′-dimethyl-ethylenediamine (0.088 g, 0.01 mol) in ethanol (200 mL) at 100 °C for 40 h. After 1 day, an excess amount of N,N′- dimethylethylenediamine was added to the solution to produce terminally end-capped amine groups. The reaction mixture was stirred overnight at 100 °C, dried under vacuum, dissolved in dichloromethane (80 mL), and followed by washing with saturated NaCl solution (10 mL). The organic layer was dried over $MgSO_4$. The polymer was washed with distillated water to remove the unreacted N,N′-dimethyl-ethylenediamine, and dried for 1 day.

^{1}H-NMR ($CDCl_3$): δ = 3.88–3.06 (br, 2H of PPO), 2.3–2.25 [br, $(CH_3)CH_2CH_2N$-(CH_3)], 1.14–1.04 (br, 3H of PPO).

Synthesis of PEO-PPO-PEO Triblock Copolymer

A PEO-PPO-PEO triblock copolymer having OH group in the PPO backbone was prepared by reacting with multi-block PPO (15 g, 0.0036 mol) with terminal amine groups and mPEG with terminal epoxide group (55 g, 0.01 mol) in ethanol (100 mL) at 100 °C for 40 h. To produce a triblock copolymer structure, an excess amount of epoxide-terminated mPEG was used. The resultant polymer was precipitated in cold diethyl ether and then dried. The polymer was purified by dialysis (molecular weight cut-off: 6,000–8,000, Spectrum Laboratories, Inc.) in a cold room for 2 days.

^{1}H-NMR (D_2O): δ = 5.21–5.14 (br, 1H of lactide), 3.88–3.06 (br, 2H of PPO and PEO), 2.3–2.25 [br, $(CH_3)CH_2CH_2N$-(CH_3)], 1.2–1.05 (br, 3H of PPO).

Grafting of OLA Chains on the PPO Segment of Triblock Copolymer

OLA chains were grafted on the middle PPO segment by ring-opening polymerization of D,L-lactide monomer using pendant hydroxyl groups as an initiator and stannous octoate as a catalyst. Briefly, 4 g of PEO-PPO-PEO triblock copolymer prepolymer, a desired amount of D,L-lactide monomer, and 0.05% (w/w) stannous octoate catalyst were added in a clean

round-bottomed two-neck flask and the flask was purged with N_2 gas to provide inert atmosphere. Thirty milliliter of anhydrous toluene was then added to the flask and the reaction was maintained at 140 °C for 12 h. The resultant copolymer was purified by precipitation in cold diethyl ether (−20 °C) in a dropwise manner. The product was then dried in vacuum, and then stored in a freezer until use. The degree of polymerization was determined by ^{1}H-NMR (Bruker Avance 400 spectrometer operating at 400 MHz) by using d-chloroform as the solvent. The degree of polymerization values of the products are listed in Table 1.

^{1}H-NMR ($CDCl_3$): δ = 5.21–5.14 (br, 1H of lactide), 3.88–3.06 (br, 2H of PPO and PEO), 1.51–1.46 (br, 3H of lactide), 1.36–1.33 (t, 2H of CH_2CH_2), 1.16–1.05 (br, 3H of PPO).

Sol-Gel Transition Phase Diagram

The sol-gel phase transition temperatures of Plu-OLA hydrogels in pH 7.4 phosphate buffered saline (PBS) solution were determined using a test tube inverting method with a 2-ml test tube with temperature increment of 1 °C. Each sample with a given concentration was added with PBS solution for 12 h at 4 °C, heated to 60 °C (or higher temperature if needed) for 10 min, and then kept at 4 °C for 2 h before measurement. A gel state was determined by inverting the vial when no fluidity was visually observed for 1 min. The sol-gel phase diagram determined by this method is known to have a precision of ±0.5 °C.[4]

Erosion Properties of Plu-OLA Hydrogels

To determine erosion rates, the Plu-OLA sample was added with 1 ml of pH 7.4 PBS solution at a concentration of 23 wt% using a 2-ml test tube at 4 °C. The copolymer solution was then heated to 60 °C (85 °C in the case of Plu-OLA 25) for 10 min, kept at 4 °C for 2 h, and then equilibrated at 37 °C. One milliliter of 37 °C equilibrated PBS solution was added to the hardend hydrogel layer. The upper solution was replaced with the same volume of fresh PBS solution at predetermined time intervals. To determine erosion rate, the remaining hydrogel in the bottom layer was retrieved and freeze-dried to measure dry weight. Triplicate samples were used for the determination.

Bovine Serum Albumin (BSA) Protein Release

Plu-OLA copolymers were dissolved in 1 ml of pH 7.4 PBS solution containing 1 mg/ml BSA at 60 °C for 10 min, kept at 4 °C for 2 h, and then equilibrated to 37 °C. One milliliter of 37 °C PBS solution was added to the hydrogel layer and equilibrated at 37 °C. The upper solution was carefully collected and replaced with the same volume of fresh buffer solution at predetermined time intervals. The collected samples were analyzed by the MicroBCA assay method to determine the amount of protein released. Triplicate samples were used.

Results and Discussion

Pluronic mimicking tri-block copolymer having hydroxyl groups in the PPO

Table 1.
Lists of Plu-OLA copolymers studied.

Polymer Name	Mw of PEO-PPO-PEO			DP [b,c]	Mw (PLA)[b]	Mw (total)[b]
	PPO	PPO-prepolymer[a]	Final Copolymer[a]			
Plu-OLA 10	2,000	4,200	14,400	10	4320	18,720
Plu-OLA 15				15	6480	20,880
Plu-OLA 20				20	8640	23,040
Plu-OLA 25				25	10800	25,200

[a] Determined by GPC.
[b] Determined by ^{1}H-NMR.
[c] Degree of polymerization.

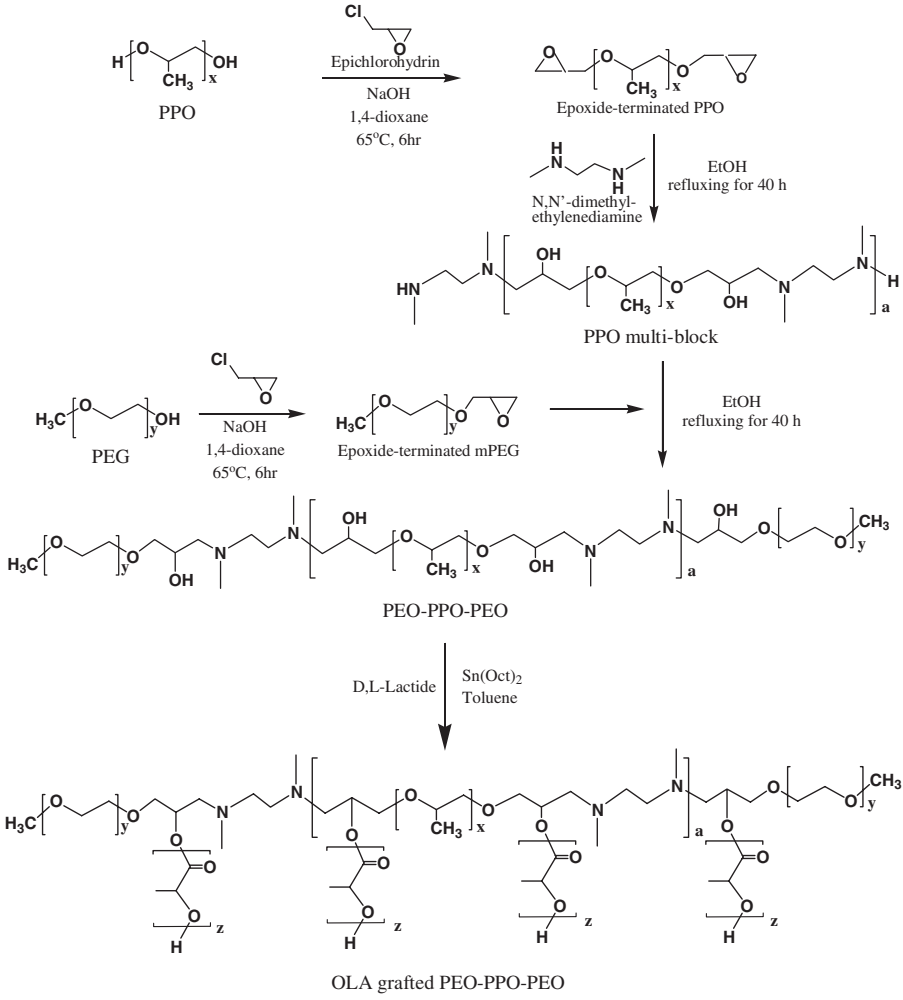

Figure 1.
Synthesis and chemical structures of Plu-OLA copolymers.

backbone was synthesized as shown in Figure 1. Under basic condition, bi- functional epoxide-terminated PPO was first synthesized using PPO and epichlorohydrin. The PPO multi-block copolymer bearing secondary amine groups at the terminal ends was prepared by reacting epoxide-terminated PPO with N,N′-dimethylethylenediamine as a chain extender. The molecular weight of the PPO pre-polymer was evaluated by gel permeation chromatography (GPC) after treatment with N,N′-dimethylethylenediamine to end-cap the terminal oxirane groups. PPO multi-block copolymer (6 OH groups in the backbone) with a molecular weight of 4,200 was obtained, indicating that two PPO precursors (Mw = 2,000) were combined in the PPO chain extension reaction. In the ^{1}H-NMR spectra, a peak at 2.72 ppm assigned to a terminal oxirane group of PPO disappeared, while a new peak appeared around 2.31 ppm assigned to a methyl group of $-N(CH_3)CH_2CH_2N(CH_3)$ group. This indicates that the PPO pre-polymer was end-functionalized with secondary amine groups. The Mw of the PPO block of 4,200 is highly desirable for

mimicking Pluronic F-127 having PPO Mw of 4,060 (70 units of propylene oxide monomer).

Pluronic mimicking copolymer was prepared by reacting the bi-functional amine terminated PPO-multi-block pre-polymer with mPEG terminally derivatized with an oxiranylmethyl group. From GPC analysis, it was found that the resultant PEO-PPO-PEO tri-block copolymer with Mw of 14,000 was successfully synthesized and purified. Oligo(D,L-lactic acid) with different chain lengths was then grafted to the PPO backbone of PEO-PPO-PEO by ring opening polymerization.

Figure 2 shows sol-gel transition phase diagrams for four kinds of Plu-OLA copolymers with different OLA chain lengths as function of concentration and temperature. The Plu-OLA copolymers exhibited gel-to-sol transition behaviors with increasing temperature, but the gel-to-sol transition temperatures were highly dependent on the chain length of grafted OLA. The phase diagram of Pluronic F127 is also shown as a reference. It can be seen that with increasing temperature, the Plu-OLA copolymers demonstrated only gel-to-sol transitions, whereas Pluronic F127 showed sol-to-gel-to-sol transitions above a critical gelation concentration. It is known that sol-gel transition of Pluronic F127 at lower temperature (lower curve part) occurs due to the formation of closely packed spherical Pluronic micelles at a lower critical temperature, and gel-to-sol transition at higher temperature (upper curve part) takes place by dehydration of the PEO shell layer in the packed micelles at a higher critical temperature.[9]

It is noticeable that the grafted OLA part seemed to play an important role in forming a hydrogel structure and exhibiting a characteristic gel-sol transition behavior. The gel-to-sol transition temperatures of Plu-OLA 20, Plu-OLA 15 and Plu-OLA 10 were lower than that of Pluronic F127 at the same concentration, while the gel-to-sol transition temperature of Plu-OLA 25 was higher than that of Pluronic F127. It should be noted that the PEO-PPO-PEO tri-block pre-polymer having hydroxyl groups on the PPO segment did not exhibit any sol-gel transition behavior. This is probably

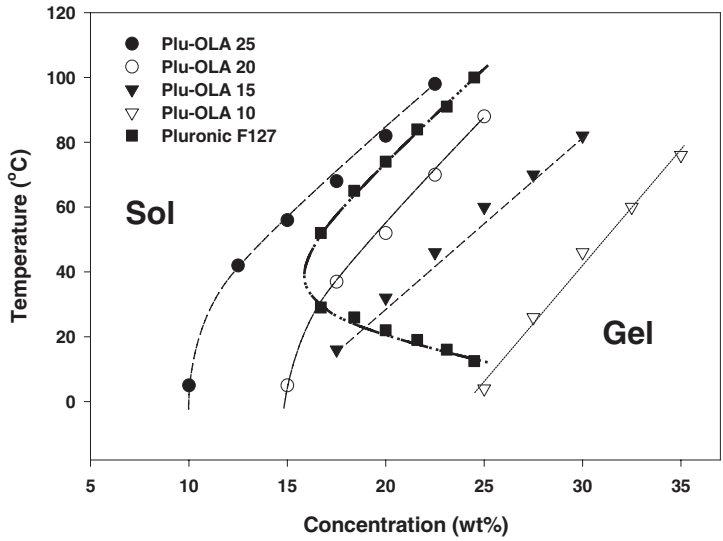

Figure 2.
Sol-gel transition phase diagram of Plu-OLA copolymer solutions in pH 7.4 PBS solution. Each symbol indicates Plu-OLA 25 (●), Plu-OLA 20 (○), Plu-OLA 15 (▼), Plu-OLA 10 (▽) and Pluronic F127 (■), respectively.

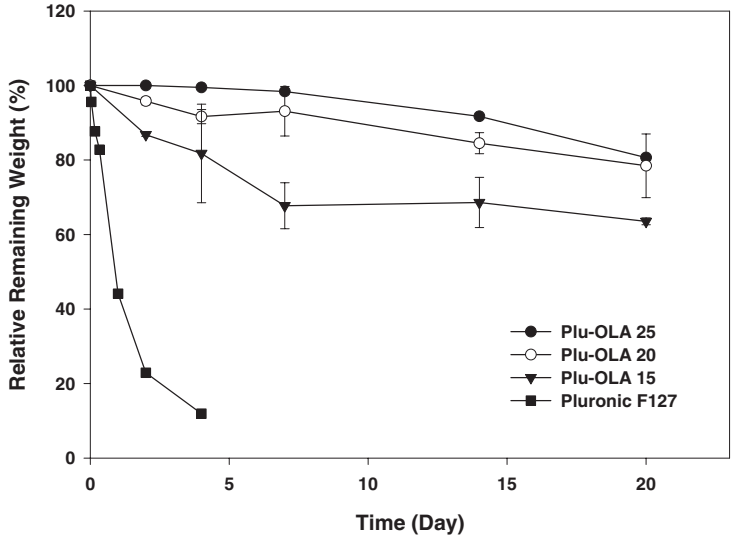

Figure 3.
Enhanced hydrogel stability of 23 wt% Plu-OLA compared to 23 wt% Pluronic F127 hydrogel. Each symbol indicates Plu-OLA 15 (▼), Plu-OLA 20 (○), Plu-OLA 25 (●) and Pluronic F127 (■), respectively. All the samples were triplicate.

because the modified middle PPO block was not hydrophobic enough to form an inner core structure. Only Plu-OLA copolymers exhibited gel-to-sol transitions, indicating that they self-associated to form spherical micelles and closely packed together above a critical concentration at any temperature below the gel-to-sol transition temperature. It was likely that hydrophobic OLA chains participated in self-association of the PPO middle blocks to form an inner hydrophobic core, thereby producing a core/shell micellar structure. It also appeared that the gel-to-sol transition

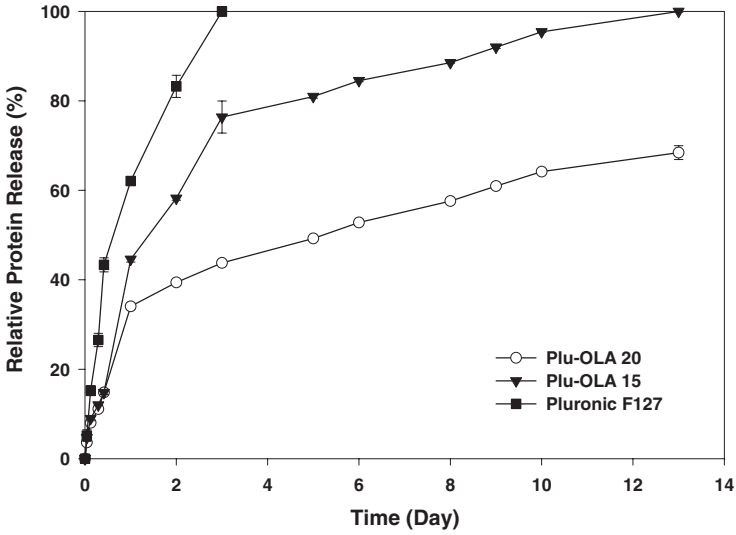

Figure 4.
Protein (BSA) release profile of 25 wt% Plu-OLA copolymer hydrogels and 25 wt% Pluronic F127 hydrogel. Each symbol indicates Plu-OLA 15 (▼), Plu-OLA 20 (○), and Pluronic F127 (■), respectively.

curves for Plu-OLA copolymers shifted to the lower concentration region with increasing chain length of OLA. This implies that the longer OLA chain stabilized the individual micellar structure more efficiently and concomitantly produced a more densely compacted micellar packing structure for gelation.

Plu-OLA copolymer hydrogels exhibited greatly enhanced gel stability as demonstrated in Figure 3. Pluronic F127 hydrogel was dissolved out rapidly within 4 days, while over 80% of the Plu-OLA hydrogels remained after the same period and depending on OLA chain length, 60% to 80% remained even after incubating for 20 days. As expected, Plu-OLA hydrogels having a longer OLA chain length eroded more slowly. This suggests that the enhanced stability of Plu-OLA hydrogels was most likely due to their more dense micellar packing structure.

The Plu-OLA hydrogels exhibited controlled and sustained BSA release characteristics as shown in Figure 4. While the Pluronic F127 hydrogel prepared at a concentration of 25 wt% released out 100% of the encapsulated BSA within 3 days, Plu-OLA hydrogels exhibited more sustained BSA release profiles for over 2 weeks dependent on the OLA chain length (over 3 weeks in the case of 25 wt% Plu-OLA 20 hydrogel) with some initial bursts. Since water-soluble proteins are well-dissolved in the aqueous inter-micellar region within the micellar packed hydrogel structure, protein release was likely to be governed mainly by erosion rates of Plu-OLA hydrogels. Consequently, the BSA release results suggest that inter-micellar interaction and packing density within the physically crosslinked Plu-OLA hydrogels were important factors in controlling protein release.

Conclusions

We have shown that OLA grafted PEO-PPO-PEO copolymers synthesized in this study formed physically crosslinked hydrogels in aqueous solution. Aqueous solutions of the Plu-OLA copolymers exhibited gel-sol transition behaviors over a wide range of copolymer concentrations. They seemed to have a more densely compacted micellar packing structure for gelation compared to that of PluronicF127. Erosion and release studies showed that the hydrogels exhibited enhanced stability and sustained release profiles of model protein. The adjustable sol-gel transition behaviors by controlling OLA chain length could be applied in injectable hydrogel systems for controlled release of various protein drugs.

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