A New Class of Sol-Gel Transition Hydrogels for Macromolecular Delivery

Yuhan Lee[1], *Sung Young Park*[1], *Hyun Jung Chung*[1], *Tae Gwan Park*[1]

[1]Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Daejeon, Korea

Introduction

Poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) triblock copolymers, also known as Pluronics, have received much attention for applications on macromolecular drug delivery and tissue engineering [1-2]. They exhibit thermo-responsive sol-gel transition behaviors at high concentration above 22 % (w/w) in aqueous solution. However, physically crosslinked Pluronic hydrogels showed rapid dissolution when injected into the body, limiting their practical uses as depot materials [2].

In this study, a new series of PEO-PPO-PEO and PPO-PEO-PPO copolymers having several hydroxyl groups on the PPO chain segment were synthesized, further modified with various poly(lactic acid) PLA oligomeric chains to confer physical stability after thermo-gelation in the body fluid. Gel stability was endowed by either increasing hydrophobic interaction between PLA chains or inducing stereocomplex formation between enatiomeric isomers of PLA chains. Macromolecular drugs were incorporated within the gels and their release patterns were investigated using Pluronic F127 as a control.

Experimental

Synthesis of Copolymers. A series of triblock copolymers were synthesized using methoxy-PEG (mPEG, Mw 5000) and low molecular weight PPO (Mw 2000 and 3,500) based on epoxide-amine chemistry as summarized in Figure 1. Low molecular weight PPO-diol and mPEG were first conjugated with epichlorohydrin in the presence of NaOH in 1,4-dioxane at 65 ℃ for 6 hours. The epoxide-terminated PPO and epoxide-terminated mPEG was filtered and precipitated in cold diethyl ether. The PPO precursor was then polymerized in a step growth manner using an equimolar amount of N,N'-dimethylethylenediamine in ethanol with refluxing for 40 hours to synthesize PPO prepolymer. The PPO prepolymer was then terminated with amine using excess amount of N,N'-dimethylethylenediamine. PPO prepolymer was purified by dialysis against distilled deionized water and lyophilized. Epoxide-terminated mPEG and amine-terminated PPO prepolymer were then conjugated in ethanol with refluxing for 40 hours. The final product was purified by dialysis and then lyophilized (EPE type copolymer). Each reaction step was monitored using gel permeation chromatography (GPC, Waters) and ^{1}H-NMR (Bruker Avance 400 spectrometer operating at 400 MHz) using chloroform and chloroform-d as solvents (Table 1). PPO-PEO-PPO type copolymer (PEP) was synthesized using a similar method except that PEO diol was used instead of mPEG and an excess amount of amine-terminated PPO prepolymer was used in the PEO-PPO conjugation process.

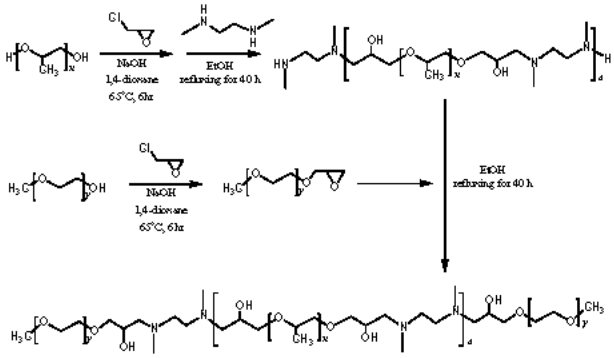

Figure 1. Synthesis and chemical structure of EPE copolymer

Synthesis of PLA-grafted copolymers. Three types of PLA ($P_{DL}LA$, P_DLA and P_LLA) were synthesized by ring-opening polymerization using EPE and PEP copolymer as an initiator and stannous octoate as a catalyst in toluene at 140 ℃ for 12 hours. The PLA-grafted copolymers were precipitated in cold diethyl ether and dried in vacuum. The degree of polymerization of PLA was determined by ^{1}H-NMR in $CDCl_3$ (Table 2).

Table 1. List of pluronic-mimicking copolymers

Polymer Name	Mw (PPO)[a]	Mw (PPO-prepolymer)[a]	Mw (copolymer)[a]
EPE	2,000	4,200	14,400
PEP	725	1,900	12,600

a: determined by GPC

Table 2. List of PLA-grafted pluronic-mimicking copolymers

Polymer Name	Initiator	DP[a,b]	Mw (PLA)[a]	Mw (total)[a]
Plu-PLA 10	EPE	10	4320	18,720
Plu-PLA 15	EPE	15	6480	20,880
Plu-PLA 20	EPE	20	8640	23,040
Plu-PLA 25	EPE	25	10800	25,200
EPE-PDLA	EPE	20	8640	23,040
EPE-PLLA	EPE	20	8640	23,040
PEP-PDLA	PEP	13	9230	21,830
PEP-PLLA	PEP	13	9230	21,830

a: determined by ^{1}H-NMR
b: Degree of Polymerization

Results and discussion

Sol-gel transition behavior of Plu-PLA copolymer hydrogels. Copolymer solutions in pH 7.4 phosphate buffered saline showed gel-sol transition behaviors depending on temperature in a wide range of copolymer concentration as shown in Figure 2. The gel-to-sol transition temperatures for copolymer hydrogels increased with increasing concentration of the copolymers. At low temperature, they were in a gel state, but at a higher temperature above the transition curves, they became sol. In addition, the gel-to-sol transition temperatures increased with increasing chain length of oligomeric PLA segment grafted on the PPO segment, suggesting that Pluronic micelles were packed more compactly due to increased hydrophobic interaction between grafted PLA chains.

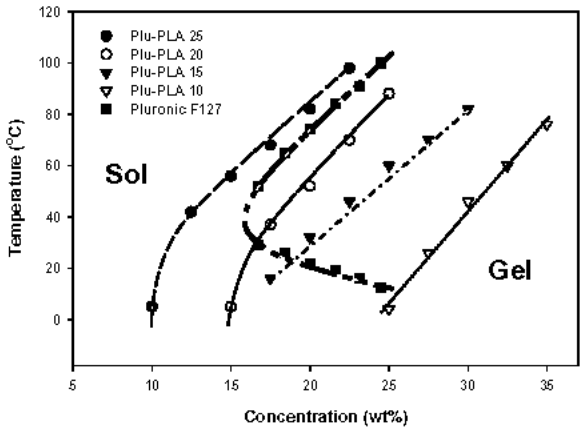

Figure 2. Sol-gel transition phase diagram of Plu-PLA copolymer solutions in pH 7.4 PBS solution.

It is also notable that, the gel-to-sol transition temperatures of Plu-PLA 20, Plu-PLA 15, and Plu-PLA 10 were lower than that of Pluronic F127 at the same concentration, while the gel-to-sol transition temperature of Plu-PLA 25 was higher than that of Pluronic F127. The prepolymer (EPE multiblock copolymer having multiple hydroxyl groups on the PPO segment without grafted PLA chains) did not exhibit gelation behaviors.

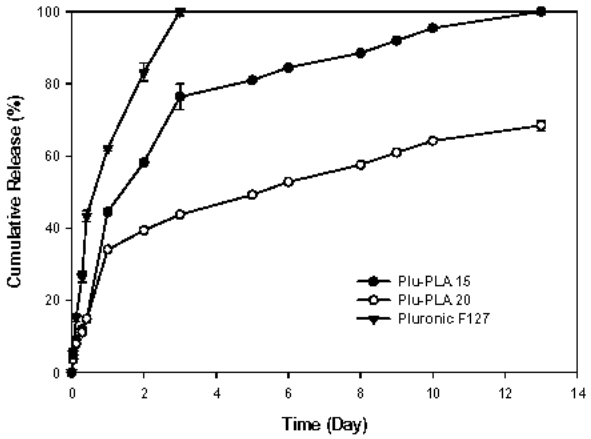

Figure 3. Protein (BSA) release profile of 25 wt% Plu-PLA copolymer hydrogels and 25 wt% Pluronic F127 hydrogel

Protein release profile of Plu-PLA copolymer hydrogels. The Plu-PLA hydrogels exhibited controlled and sustained protein release characteristics as shown in Figure 3. While 25 wt% Pluronic F127 hydrogel was dissolved rapidly and released 100% of BSA protein within 3 days, Plu-PLA copolymer series released BSA in a more sustained and controllable manner for over 2 weeks depending on the grafted PLA chain length (over 3 weeks in the case of 25 wt% Plu-PLA 20 hydrogel). This indicates that hydrophobically modified Pluronic-mimicking copolymers with grafted PLA chains stabilized the gel at body temperature by inter-locking the closely packed Pluronic micelles. The retarded dissolution of Plu-PLA copolymer solution at high concentration was likely to control the BSA release rates.

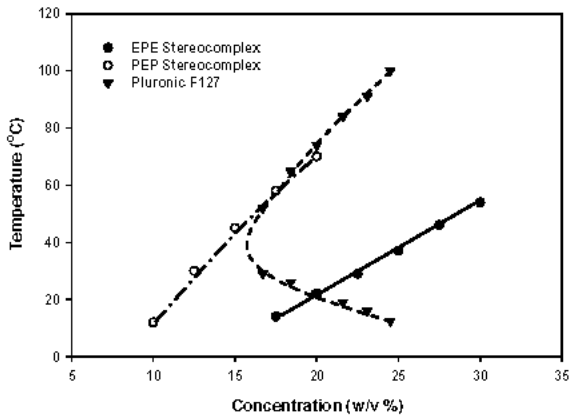

Figure 4. Sol-gel transition phase diagram of EPE-stereo and PEP-stereo copolymer hydrogels in pH 7.4 PBS solution.

Sol-gel transition behavior of EPE-stereocomplexed and PEP-stereocomplexed copolymer hydrogels. A 50/50 blend mixture of EPE-PDLA and EPE-PLLA or PEP-PDLA and PEP-PLLA was prepared to produce more stable Pluronic hydrogels via the formation of stereocomplex crosslinking between Pluronic micelles. EPE-stereocomplexed and PEP-stereocomplexed copolymer solutions at high concentrations in pH 7.4 phosphate buffered saline solution exhibited thermo-sensitive sol-gel transition behaviors as shown in Figure 4. It is of interest to note that EPE- and PEP-stereocomplexed hydrogels, having the same unit of PLLA or PDLA chain length, had different gel-to-sol transition temperatures. PEP-stereocomplexed hydrogel had a higher gel-to-sol transition temperature than that of the EPE-stereocomplexed hydrogels at the same concentration. Inter-micellar interaction through the formation of stereocomplex between packed Pluronic micelles seems to be the major factor to demonstrate the higher gel-to-sol transition temperature for the PEP-stereocomplexed hydrogel, thereby stabilizing PEP-stereocomplexed hydrogel to a greater extent.

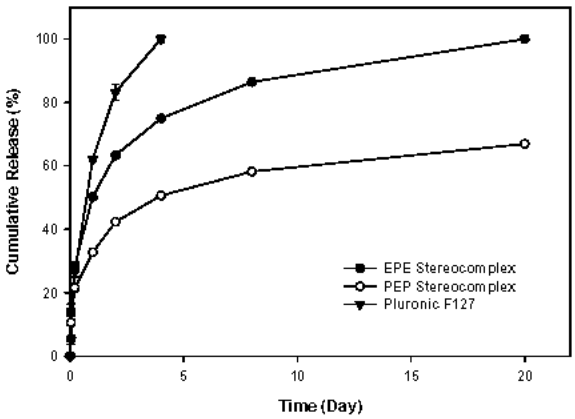

Figure 5. Protein (BSA) release profile of 24 wt% EPE-stereo and EPE-stereo hydrogels and 24 wt% Pluronic F127 hydrogel

Protein release profile of EPE-stereocomplexed and PEP-stereocomplexed hydrogels. Since PEP- and EPE-stereocomplexed hydrogels had enhanced physical stability compared to Pluronic F127 hydrogels, they showed far better controlled and sustained protein release characteristics as shown in Figure 5. EPE-stereocomplexed hydrogel and PEP-stereocomplexed hydrogel released BSA for up to 20 days. The protein release profile supports the fact that the physical stability of PEP- EPE-stereocomplexed hydrogels had been enhanced rendering it resistant to the rapid dissolution when incubated in the large volume of release medium.

Conclusions

We have shown that PLA grafted PEO-PPO-PEO and PPO-PEO-PPO copolymers synthesized in this study formed stable hydrogels in aqueous solution though increased hydrophobic interaction. The blend mixture of PLLA and PDLA grafted PEO-PPO-PEO and PPO-PEO-PPO copolymers further stabilized the Pluronic gels by stereocomplex formation. The adjustable sol-gel transition behaviors by changing PLA chain length provided the hydrogels with a wide range of potential applications as injectable hydrogel systems useful for controlled release of various protein drugs.

References

[1] Jeong, B.; Bae, Y. H.; Lee, D. S.; Kim, S. W. *Nature.* **1997**, *388*, 860.
[2] Cohn, D.; Sosnik, A.; Levy, A. *Biomaterials.* **2003**, *24*, 3707.