공학석사학위논문

Host-Guest Interaction Mediated Pluronic F127 Based Hydrogel for Delivery of Therapeutic Agents

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심 성 보

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이 논문을 공학석사학위논문으로 제출함

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Abstract

Host-Guest Interaction Mediated Pluronic F127 Based Hydrogel for Delivery of Therapeutic Agents

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Poloxamer composed of poly(ethylene oxide)- poly(propylene oxide)-poly(ethylene oxide) triblock copolymer which is called Pluronic F127 is representative material for showing reversible sol-gel transition by temperature change. This behavior is achieved by micelle packing mechanism

above critical gelation concentration. Micelle structure is obtained around 15 ℃ and more micelles are formed as temperature increases because each block has different low critical solution temperature. This thermoreversible hydrogel has attractive characteristics for therapeutic agent delivery carriers due to its high water contents and similar mechanical property like the extracellular matrix. However, it has limitation for using in clinical application due to its low stability.

To overcome the critical drawback of Pluronic F127 hydrogel, the host-guest interaction was utilized to enhance packing ability of micelles. Due to strong host-guest interaction, it was possible to achieve highly improved mechanical stability. However, the viscosity of the blended solution was too high for injection due to existing strong host-guest interaction at injection condition (at 4 ℃). Thus, the system was still hard to deliver therapeutic proteins and cells.

To maintain long-term stability of hydrogel and improve injection ability, multi-guest molecules were conjugated at the end of Pluronic F127 for strengthening the micelle packing while reducing the amount of each polymer needed. Because of increased host-guest complex at a reduced concentration, critical gelation concentration of blended solution decreased comparing with

conventional Pluronic F127 hydrogel and mono guest conjugated F127 hydrogel system. As a result, the viscosity of multi-guest conjugated F127 / CDP blended solution at the injectable condition largely decreased comparing with the conventional method and the high stability was maintained in the physiological condition. In addition, this host-guest interaction based gel system enabled affinity based protein release. Host molecule modified protein showed sustained protein release profile in this system. Consequently, multi-guest conjugated Pluronic F127 hydrogel which has overcome its limitations while maintaining existing merits is expected to be used for various biomedical application

Keywords: Pluronic F127, hydrogel, injectable system, host-guest interaction, multi-guest, micelle packing mechanism, protein delivery system, cell delivery system

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1. INTRODUCTION

One of widely known temperature-responsive polymer Pluronic F127 which is composed of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) have got interests by its rapid in situ gelling system in physiological condition and soft texture like an extracellular matrix for injectable cell therapy and protein delivery [1], [2]. Highly concentrated solution (>16 wt%) of Pluronic exhibits temperature-responsive sol-gel transition behaviors at a low critical solution temperature (LCST) [3], [4]. However, conventional Pluronic hydrogel with self-assembled structure exhibits low mechanical property and poor stability, which limit the clinical application [5], [6].

Previously, we tried to utilize host-guest interaction in micelle packing mechanism to improve the stability of Pluronic F127 hydrogel. Host-guest interaction is strong noncovalent binding between two or more molecules. Common host molecule is β-cyclodextrin (CD) which composed of 7 dextrose units connected *via* α-1,4-glucosidic linkages. β-CD has 21 hydroxyl groups at the outer shell of the molecule and hydrophobic interior cavity which offers binding sites to hydrophobic guest molecules such as adamantane (Ad) or cholesterol *via* strong Van der Walls interaction [7], [8]. β-cyclodextrin polymer

(CDP) was used to provide binding sites for enhanced micelle packing of Ad conjugated F127 (F127-Ad). This system showed decrement of critical gelation concentration and significantly increased stability compared with unmodified F127 hydrogel. However, the viscosity of the host-guest interaction introduced system is too high for injection even the lowest gelation concentration. This high viscosity can give damage to cells due to strong mechanical agitation.

To decrease viscosity as well as maintaining the enhanced stability of host-guest interaction based hydrogel, multi-guest molecules were introduced to end group of Pluronic F127 to reduce critical gelation concentration by maintaining an absolute number of host-guest complexes. The gelation behavior was observed by completing phase diagram and the gelation mechanism and size of micelle by modification was verified by dynamic light scattering (DLS) method. Viscosities of F127-mono-Ad, F127-multi-Ad / CDP blended solution at the lowest gelation condition were compared using rheometrics mechanical spectrometry (RMS) analysis. The stability of F127-multi-Ad / CDP blend hydrogel in the physiological environment was finally evaluated by *in vitro* experiment.

In addition, β-CDs have been used extensively both in clinical

applications and in drug formulations, especially in stabilizing and solubilizing hydrophobic compounds [9]-[11]. There were many trials to use cyclodextrin to increase the half-life and prolonged therapeutic effects [12], [13]. In a similar manner, host (CD) and guest (Ad) modified gelatin was loaded into the host-guest interaction based hydrogel to confirm sustained protein release behavior. In addition, specific aromatic rings (Tyr, Phe) of insulin, diabetes regulating hormone, made the strong interaction between β-CD [14]. Studies of β-CD-based insulin delivery were widely done [15], [16]. Thus, release behavior of insulin was also estimated in the hydrogel system to check this host-guest based hydrogel as potent protein delivery carrier.

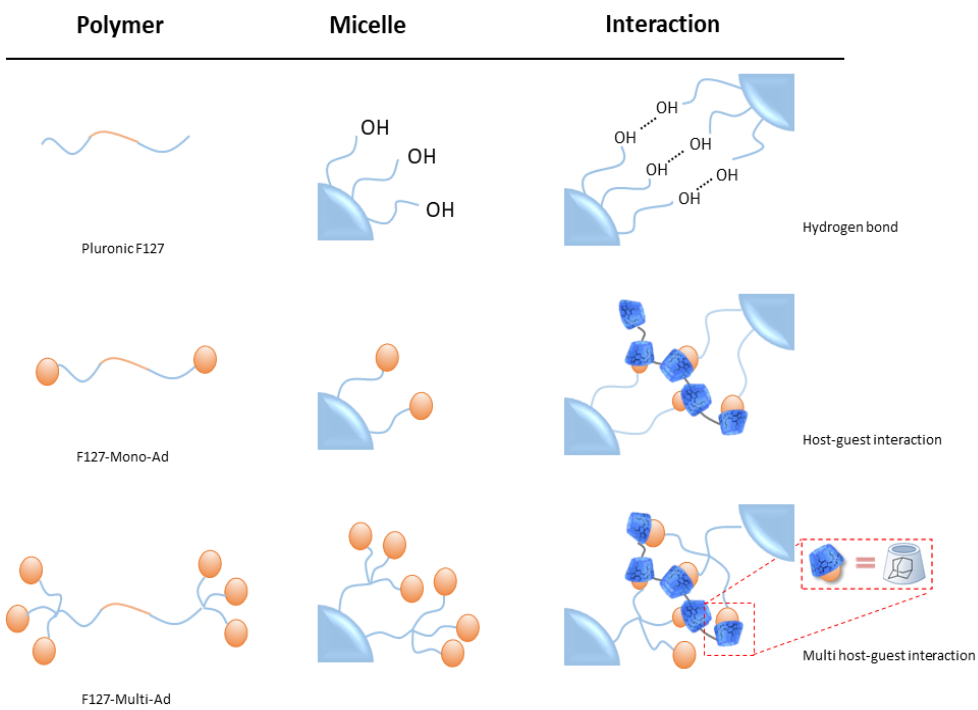


Figure 1. Gelation mechanism of F127 and F127-mono-Ad, F127-multi-Ad / CDP blend hydrogel

2. EXPERIMENTS

2.1 Materials

Pluronic F127 [$(PEO)_{99}$-$(PPO)_{69}$-$(PEO)_{99}$], *p*-nitrophenyl chloroformate (*p*-NPC) (96.0 %), 1-adamantane (Ad) methylamine, β-cyclodextrin (β-CD), trimethylamine (TEA), epichlorohydrin and anhydrous toluene and dimethylformamide (DMF), gelatin from porcine skin (gel strength 300, Type A), recombinant human insulin were obtained from Sigma Aldrich (St. Louis, MO). 2-amino-1,3-propanediol (Serinol) (> 98.0 %) and Tris-hydroxymethyl-aminomethane (Tris) (> 99.0 %) were purchased from Tokyo Chemical Industry (TCI, Japan) and methylene chloride, isopropanol, diethyl ether, hydrochloric acid (HCl), sodium hydroxide (NaOH), sodium chloride (NaCl) and magnesium sulfate were purchased from Daejung (Korea). Methylene chloride and TEA were dried over calcium hydride before use and all other chemicals were used as received.

2.2 Synthetic procedure of multi-guest - Pluronic F127

Synthesis of the F127-Di-Ad and F127-Tri-Ad is a four-step process (1) activation of hydroxyl end group of Pluronic F127 and (2) conjugation of Serinol and Tris at the end and (3) re-activation of multiple hydroxyl groups and (4) conjugation of adamantanes in multi-functional end groups of the triblock copolymer.

2.2.1 Activation of Pluronic F127 with p-NPC (F127-NPC) 15.00 g (1.18 mmol) of Pluronic F127 was placed into a 250 mL two-neck round bottom flask and dried under vacuum at 120 ℃ for 3 h before reaction. After the polymer was clearly dissolved in 150 mL anhydrous methylene chloride, *p*-NPC (0.96 g, 4.77 mmol) and TEA (0.48 g, 4.77 mmol) were introduced and the reaction continued for 24 h at room temperature under nitrogen atmosphere. To the reaction mixture was poured 200 mL brine solution and stirred for additional 1 h. The organic layer was separated and dried over magnesium sulfate. After concentration, the product was precipitated into the 10-fold excess volume of cold diethyl ether and dried under vacuum for 3 days (yield: 90 %). ^{1}H-NMR ($CDCl_3$, δ, ppm) = 4.5 (t, 4 H, -$CH_2CH_2OC(=O)$-), 7.5-8.5 (d, 8 H, Ar of *p*-NPC), 3.2-3.8 (br, 2 H of PEO

and 1 H and 2 H of PPO), 1.0-1.3 (br, 3 H of PPO).

2.2.2 Conjugation of 2-amino-1,3-propanediol (Serinol) with F127-NPC (F127-Di). After F127-NPC (10.02 g, 0.77 mmol) was clearly dissolved in 100 mL anhydrous DMF in a 250 mL two-neck round bottom flask at 40 ° C under a nitrogen atmosphere for 30 min, put 2-amino-1,3-propanediol (0.35 g, 3.87 mmol) to the solution, the reaction was allowed to proceed for 36 h at 40 °C. The mixture was precipitated into cold diethyl ether and dried under vacuum for 1 day. Then the white powder was dissolved in 100 mL methylene chloride and washed with 150 mL brine solution. The organic layer was separated and dried over magnesium sulfate. The product was isolated by precipitating into the 10-fold excess volume of cold diethyl ether and the white powder product was collected and dried under vacuum for 3 days (yield: 79 %). ^{1}H-NMR ($CDCl_3$, δ, ppm) = 4.2 (s, 4 H, -$CH_2C\mathit{H}_2OC$(=O)-), 3.2-3.8 (br, 2 H of PEO and 1 H and 2 H of PPO and 10 H, -NHC$\mathit{H}C_2\mathit{H}_4$-), 1.0-1.3 (br, 3 H of PPO)

2.2.3 Activation of F127-Di with p-NPC (F127-Di-NPC). 8.00 g (0.62 mmol) of F127-Di was dissolved in 80 mL anhydrous methylene chloride in a 100 mL two-neck round bottom equipped with a magnetic stirrer under a nitrogen atmosphere. After the polymer was clearly dissolved, *p*-NPC (1.50 g, 7.40 mmol) and TEA (0.74 g, 7.40 mmol) were put and the reaction continued for 24 h at room temperature. To the reaction mixture was poured 150 mL brine solution and stirred for additional 1 h. The organic layer was separated and dried over magnesium sulfate. After concentration, the product was precipitated into the 10-fold excess volume of cold diethyl ether and dried under vacuum for 3 days (yield: 80 %). ^{1}H-NMR ($CDCl_3$, δ, ppm) = 4.3 (s, 4 H, -CH_2CH_2OC(=O)-), 4.4-4.6 (br, 8 H, -NHCHC_2H_4-), 7.5-8.5 (d, 16 H, Ar of *p*-NPC), 3.2-3.8 (br, 2 H of PEO and 1 H and 2 H of PPO2 H and 2 H, -NHCHC_2H_4-), 1.0-1.3 (br, 3 H of PPO).

2.2.4 Conjugation of 1-adamantane (Ad) methylamine with F127-Di-NPC (F127-Di-Ad). The Ad conjugation of F127-Di-NPC achieved same procedure as F127-Ad synthesis. 4.00 g (0.29 mmol) of F127-Di-NPC was dissolved in 50 mL anhydrous methylene chloride in a 100 mL two-neck round bottom flask. Put weighed 1-adamantane (Ad) methylamine (0.58 g,

3.49 mmol) to the solution, the reaction was allowed to proceed for 24 h at room temperature. The mixture was washed with 80 mL brine solution and the organic layer was dried over magnesium sulfate. The polymer was isolated by precipitating into the 10-fold excess volume of cold diethyl ether and the white powder product was collected and dried under vacuum for 3 days (yield: 73 %). ^{1}H-NMR ($CDCl_3$, δ, ppm) = 4.1-4.3 (br, 12 H, -CH_2CH_2OC(=O)- and 8H, -NHCHC_2H_4-), 4.8 (s, 2 H, -NHC$H$$C_2H_4$-), 2.8 (d, 8 H of Ad-C$H_2$-$NH_2$), 1.4-2.0 (br, 60 H of Ad), 3.2-3.8 (br, 2 H of PEO and 1 H and 2 H of PPO), 1.0-1.3 (br, 3 H of PPO)

2.2.5 Conjugation of Tris-hydroxymethyl-aminomethane (Tris) with F127-NPC (F127-Tri). After F127-NPC (7.12 g, 0.55 mmol) was clearly dissolved in 100 mL anhydrous DMF in a 250 mL two-neck round bottom flask at 40 ˚C under a nitrogen atmosphere for 30 min, put Tris-hydroxymethyl-aminomethane (0.35 g, 3.87 mmol) to the solution, the reaction was allowed to proceed for 36 h at 40 ˚C. The mixture was precipitated into cold diethyl ether and dried under vacuum for 1 day. Then the white powder was dissolved in 100 mL methylene chloride and washed

with 150 mL brine solution. The organic layer was separated and dried over magnesium sulfate. The product was isolated by precipitating into the 10-fold excess volume of cold diethyl ether and the white powder product was collected and dried under vacuum for 3 days (yield: 79 %). ^{1}H-NMR ($CDCl_3$, δ, ppm) = 4.2 (s, 4 H, -CH_2CH_2OC(=O)-), 3.2-3.8 (br, 2 H of PEO and 1 H and 2 H of PPO and 12 H, -NHC_3H_6-), 1.0-1.3 (br, 3 H of PPO)

2.2.6 Activation of F127-Tri with p-NPC (F127-Tri-NPC). .21 g (0.38 mmol) of F127-Tri was dissolved in 60 mL anhydrous methylene chloride in a 100 mL two-neck round bottom equipped with a magnetic stirrer under a nitrogen atmosphere. After the polymer was clearly dissolved, *p*-NPC (2.27 g, 11.26 mmol) and pyridine (0.18 g, 2.25 mmol) were put and the reaction continued for 24 h at room temperature. To the reaction mixture was poured 150 mL brine solution and stirred for additional 1 h. The organic layer was separated and dried over magnesium sulfate. After concentration, the product was precipitated into the 10-fold excess volume of cold diethyl ether and dried under vacuum for 3 days (yield: 75 %). ^{1}H-NMR ($CDCl_3$, δ, ppm) = 4.2 (s, 4 H, -CH_2CH_2OC(=O)-), 4.7 (s, 12 H, -NHC_3H_6-), 7.5-8.5 (d, 24 H, Ar of *p*-NPC), 3.2-3.8 (br, 2 H of PEO and 1 H and 2 H of PPO2 H), 1.0-1.3 (br, 3 H

of PPO).

2.2.7 Conjugation of 1-adamantane (Ad) methylamine with F127-Tri-NPC (F127-Tri-Ad). F127-Tri-Ad is synthesized as same procedure as Ad conjugation of F127-Di-NPC. 4.00 g (0.29 mmol) of F127-Tri-NPC was dissolved in 60 mL anhydrous methylene chloride in a 100 mL two-neck round bottom flask. Put weighed 1-adamantane (Ad) methylamine (1.19 g, 7.23 mmol) to the solution, the reaction was allowed to proceed for 24 h at room temperature. The mixture was washed with 80 mL brine solution and the organic layer was dried over magnesium sulfate. The polymer was isolated by precipitating into the 10-fold excess volume of cold diethyl ether and the white powder product was collected and dried under vacuum for 3 days (yield: 73 %). ^{1}H-NMR ($CDCl_3$, δ, ppm) = 4.2 (s, 4 H, -$CH_2CH_2OC(=O)$-), 4.3-4.4 (br, 12 H, -NHC_3H_6-), 2.8 (d, 12 H of Ad-CH_2-NH_2), 1.4-2.0 (br, 90 H of Ad), 3.2-3.8 (BR, 2 H of PEO and 1 H and 2 H of PPO), 1.0-1.3 (br, 3 H of PPO)

2.3 Synthesis of gelatin-CD and gelatin-Ad (gel-CD, gel-Ad)

To conjugate β-cyclodextrin at Lys residue of gelatin (gel-CD), fully dissolve 2.00 g (0.02 mmol) of gelatin from the porcine skin in 100-fold d.d water at first, Put 2.83 g (2.38 mmol) of mono carboxylated β-CD (CM-β-CD). After 30 min, 0.55 g (2.38 mmol) of 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 0.28 g (2.38 mmol) of N-hydroxysuccinimide (NHS) were introduced and the reaction was carried out for 48 h at 40 ℃. A similar procedure is required to synthesize adamantane conjugated gelatin (gel-Ad). Instead of d.d water, dimethylsulfoxide (DMSO) was used as a solvent and 0.47 g (2.38 mmol) of 1-adamantane acetic acid were used. Both products were isolated by dialysis (MWCO: 3,500) for 5 days and used after lyophilization (yield: 80 %). The degree of substitution (DS) was measured by TNBS (2,4,6-trinitrobenzene sulfonic acid) assay. By quantifying the free amino groups of gelatin which generates a highly chromogenic signal at 335 nm, 56 % of β-CD and 89 % of Ad conjugations were verified.

2.4 Phase diagram of sol-gel transition

Pluronic F127 and F127-Ad, F127-Di-Ad, F127-Tri-Ad hydrogels were prepared by dissolving in pH 7.4 phosphate buffered saline (PBS, 10 mM, 0.138 M NaCl) solution with various concentrations at 4 ℃. For making homogenous mono and multi-guest molecule conjugated F127 and CDP solution, aqueous CDP solution was introduced to the F127-Mono, Di, Tri-Ad and immersed in a cool water bath for 12 h at 4 ℃. The sol-gel transition was monitored using a test tube tilting method by gradual temperature increase, and the gel state was determined when no fluidity is observed for 1 min [17].

2.5 Size analysis

The micelle size of guest molecules conjugated Pluronic F127s and hydrodynamic size of β- CDP and blended solutions were measured using dynamic light scattering (DLS) with a zetasizer (Malvern Zetasizer Nano Zs. Malvern Instruments, Worcestershire, UK). Samples (1 mL) were prepared in 0.01 w/v% concentration. The size was measured at 25 °C.

2.6 Rheological studies

Viscosity values of Pluronic F127 and F127-Mono, Di, Tri-Ad/CDP blend solution at injection condition (4 ℃) were measured by advanced rheometric extended systems (ARES, The Rheometric Science Inc., NI) with a cone and plate (40 mm diameter plate, 2 ° cone angle and 60 mm diameter, 1 ° cone angle) fixture. The gap between the cone and plate was adjusted to 0.05 mm and the measurement was performed through a continuous ramp test in a temperature controlled environment within 4 ± 0.2 ℃. Viscosity (Pa·s) of each sample was calculated as a function of shear stress (Pa) over shear rate (1/s).

2.7 *In vitro* gel dissolution rate

Pluronic F127 and F127-Mono, Di, Tri-Ad/CDP blend solution were prepared by dissolving each polymer in PBS solution in a cool water bath for 12 h at 4 ℃. 1 mL of each blended solution was placed in 4 mL vials. To give

sufficient gelation time, all samples were incubated in a water bath with 37 ℃ for 3 h, a 3 mL PBS solution was added to the vial with preformed hydrogel and the height of the gel was measured with changing PBS solution at determined periods. The remaining gel volume was calculated by comparing with the initial one.

2.8 *In vitro* Protein Release Profile

The samples were prepared by dissolving F127-Ad, F127-Di, Tri-Ad in various concentration in 1 mL PBS solutions at 4 ℃ and the insulin and CD and Ad conjugated gelatin model proteins were mixed at a final concentration of 2 mg/mL and 5 mg/mL each. After incubating in a water bath with 37 ℃ for 6 h, 3 mL PBS solution was introduced in the hydrogel contained vial. At each determined time interval, the release medium was replaced with fresh PBS solution and the release profile was measured by BCA assay. The amount of released protein was calculated by subtracting from those of protein loaded hydrogels.

2.9 Instruments.

1H-NMR analysis was performed using Bruker Advance 300 MHz spectrometer in $CDCl_3$ and D_2O. Measurement of micelle size was carried out using Malvern zetasizer and the viscosity was analyzed by advanced rheometric extended systems (ARES, The Rheometric Science Inc., NI)

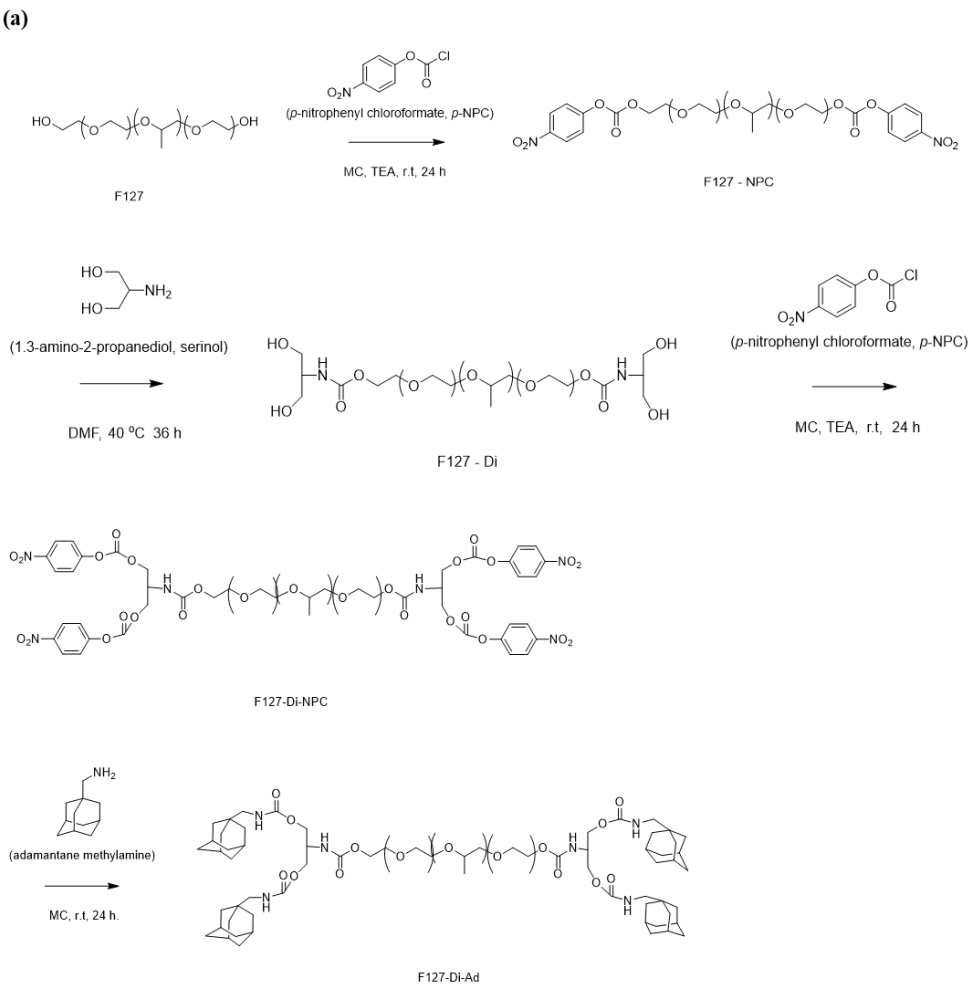
3. RESULTS AND DISCUSSION

3.1 Synthesis and characterization of multi-guest conjugated Pluronic F127 (F127-Di-Ad and F127-Tri-Ad)

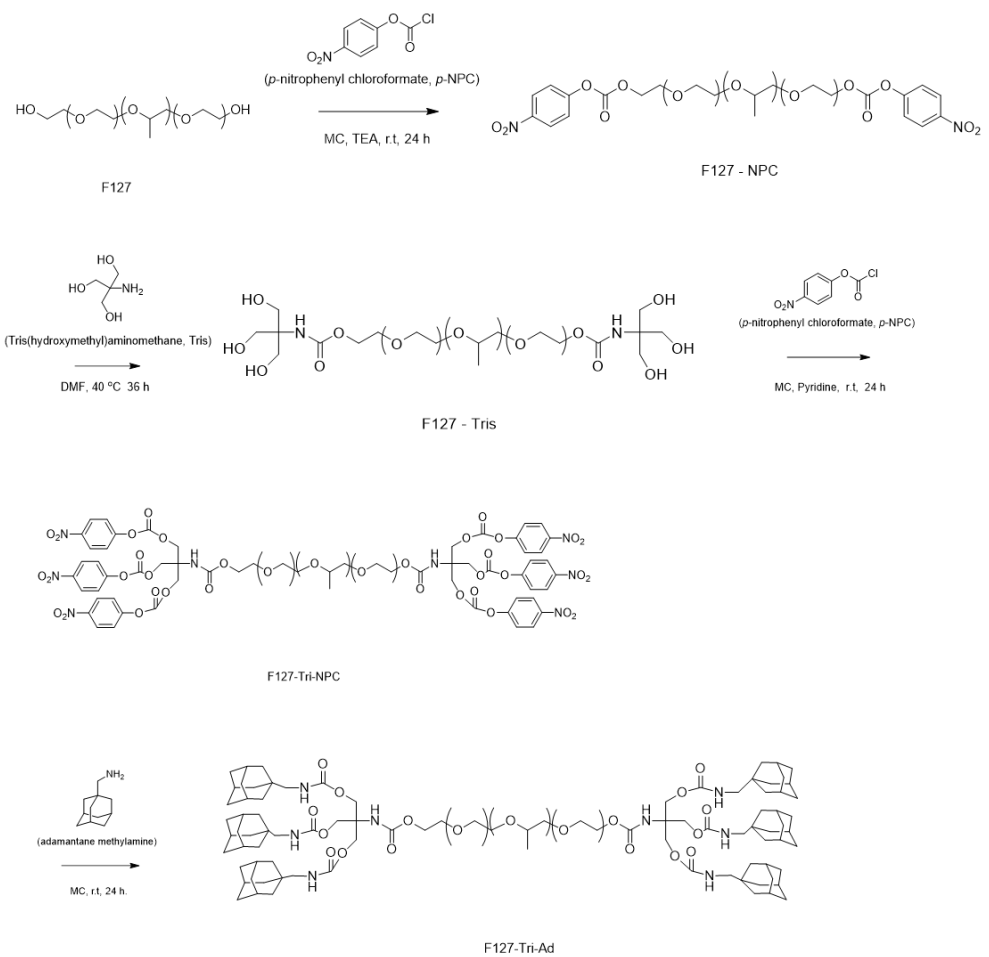
To reduce critical gelation concentration (CGC) of host-guest interaction mediated enhanced micelle packing system, multi Ad groups were introduced into two distal chain ends of Pluronic F127. Synthetic schemes of F127-Di-Ad and F127-Tri-Ad are illustrated in the scheme (1–(a) and 1-(b)). To conjugate multi Ad groups at each end, two hydroxyl groups of Pluronic F127 were firstly activated by *p*-NPC and followed by reacting with 2-amino-1,3-propanediol (Serinol) to synthesize F127-Di and Tris-hydroxymethyl-aminomethane (Tris) for F127-Tri. F127-Di with four hydroxyl groups and F127-Tri with six hydroxyl groups were re-activated by *p*-NPC and followed by reacting with 1-adamantane (Ad) methylamine. The resultant products were successfully modified with Ad groups *via* carbamate linkage and the degree of functionalization was around 100 % when confirmed by ^{1}H NMR (Figure 2). The peak at 2.8 ppm and peaks from 1.5 to 2.0 ppm and 4.0 to 4.2 ppm indicate that the Ad groups were successfully conjugated at the end of Pluronic F127 and undesired nitrophenyl chloroformate and nitrophenol peaks were completely disappeared. β-cyclodextrin polymer (CDP) and mono

Ad conjugated Pluronic F127 (F127-Ad) were synthesized as same procedure as the previous experiment [18].

(a)



(b)



Scheme 1. Synthetic scheme of (a) F127-Di-Ad and (b) F127-Tri-Ad

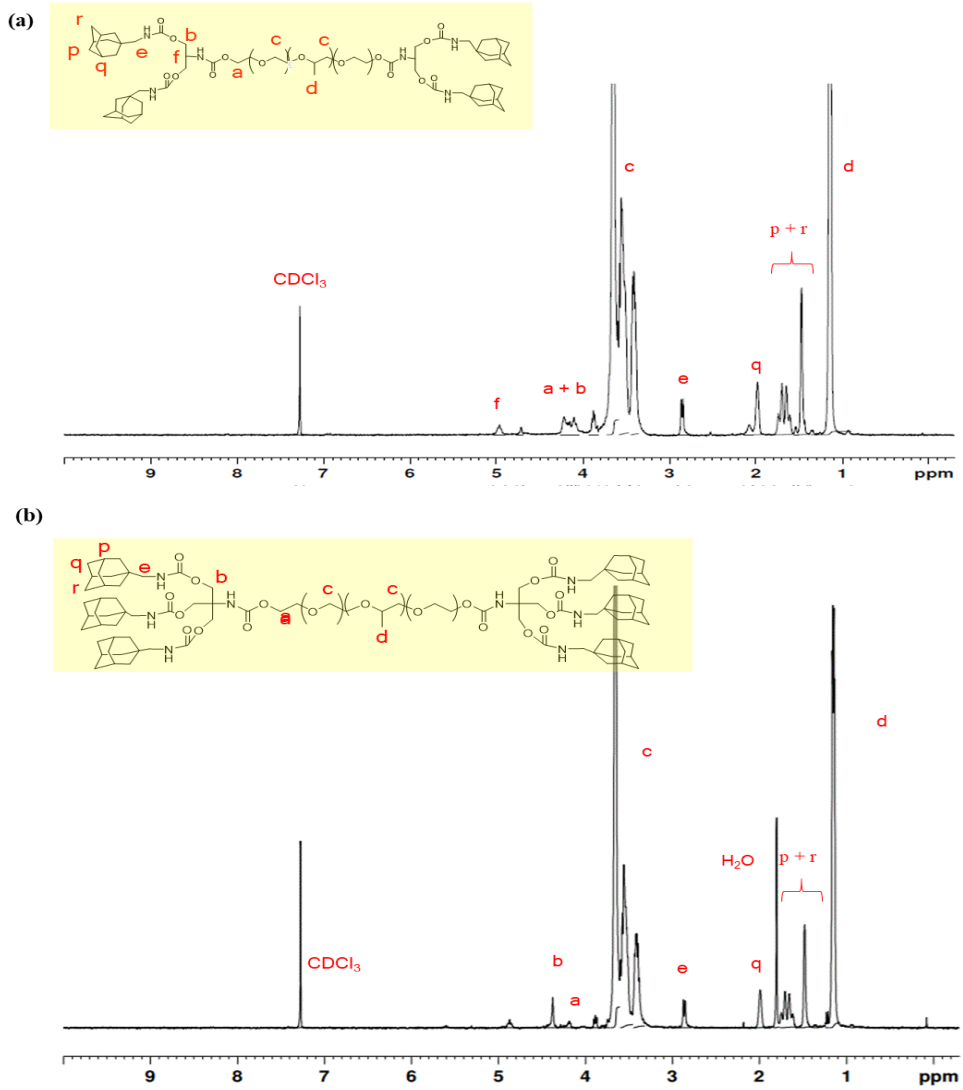


Figure 2. [1]H-NMR spectrum of synthesized (a) F127-Di-Ad and (b) F127-Tri-Ad in $CDCl^3$

3.2 Phase diagram of sol-gel transition

Hydrogels were prepared by dissolving each polymer in pH 7.4 PBS solution with various concentrations at 4 ℃ water bath for 1 day and sol-gel phase transition behaviors were observed using vial converting method (Figure 3). All the samples exhibited reversible sol-gel transition behavior as temperature increases above critical gelation concentration. When the four and six hydroxyl groups of Pluronic F127 were all modified with Ad groups and in the presence of CDP, the critical gelation concentrations were significantly decreased compared with F127-Ad/CDP blended solution and unmodified Pluronic F127 hydrogel as shown in Figure 4. Reduction of gel formation concentration is related to the enhanced packing of micelles due to host-guest interaction. As already reported, Pluronic F127 is composed of PEO-PPO-PEO triblock copolymer and the property of hydrophilic PEO shell is a key factor in forming micelle packing structure for gelation. The hydroxyl groups in Pluronic F127 which induce hydrogen bonding between micelles can facilitate tightly packed structure. In the case of Ad conjugated Pluronic F127 (F127-Ad) and CDP blended system, the host-guest interaction between β-CD and Ad molecules significantly increase interaction between micelles, resulting in much lowered critical gelation concentration. However, this

condition is too viscous for injection. To achieve good injection ability as well as high stability, multiple guest molecules were conjugated at the end of Pluronic F127 to reduce required gel formation concentration. Resulting F127-Di-Ad has two adamantane groups and F127-Tri-Ad has three adamantane molecules at the ends. Due to maintained host-guest complexes by multiple guest molecules, it was possible to achieve stable hydrogel in reduced concentration. In Ad modified F127 and CDP blended solution system, the ratio between host molecules and guest molecules is important for gel formation. It appears that too much CDP concentration hinders host-guest interaction mediated tightly packed structure because the number of F127-mono-Ad and F127-multi-Ad micelles interacting with one CDP chain decreases, thereby showing higher critical gelation concentration.

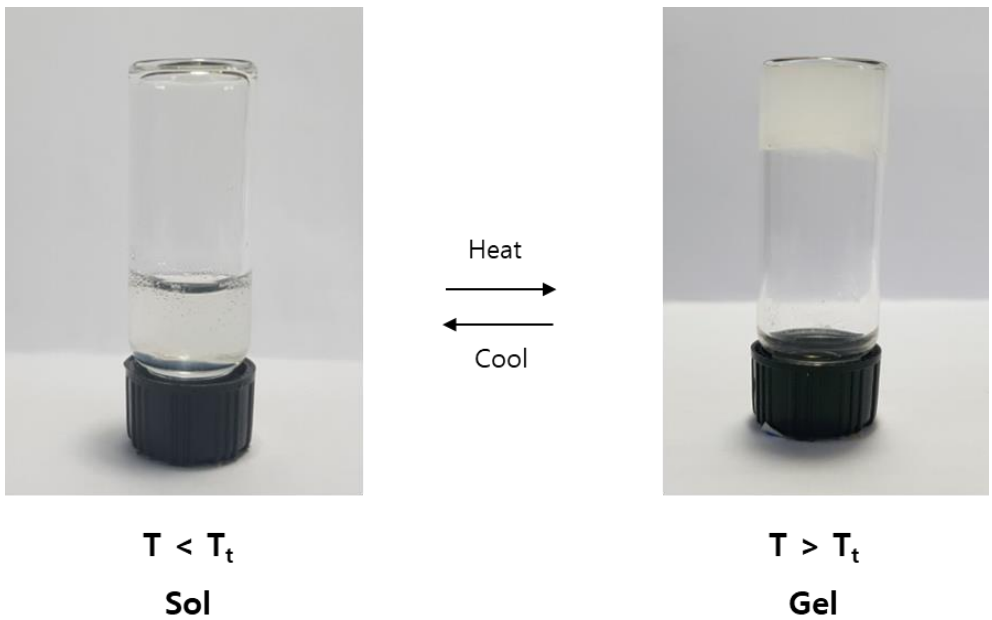


Figure 3. Photographs of gelation of F127-multi-Ad / CDP blend hydrogel by temperature change

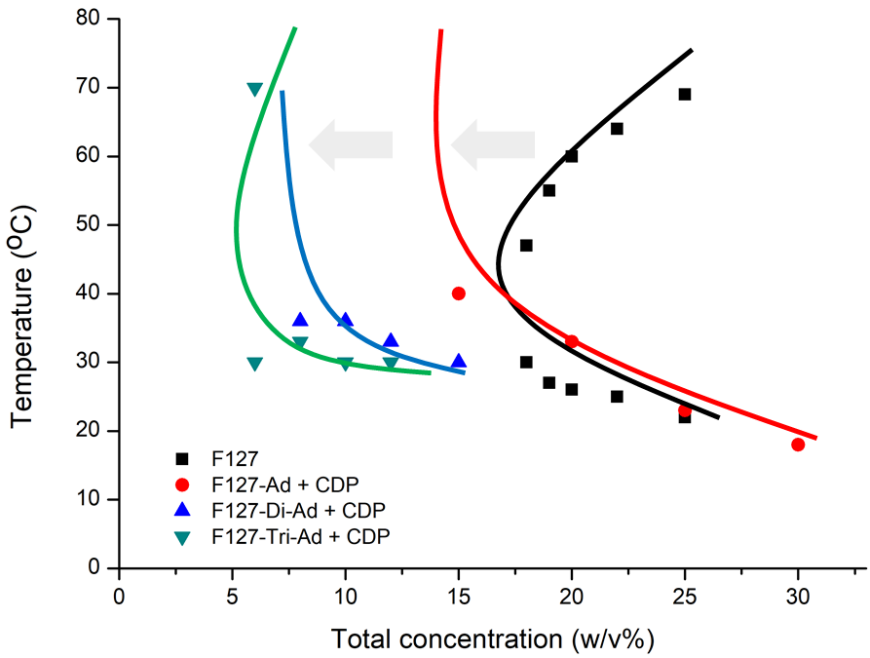


Figure 4. Sol-gel phase diagram of F127, F127-Ad, F127-Di-Ad, F127-Tri-Ad / CDP blend hydrogel

3.3 Size analysis

The interactions between F127-multi-Ad and CDP were confirmed by measuring each micelle size to support the proposed gelation mechanism. As shown in Figure 4, the DLS results revealed that the micelle size of unmodified Pluronic F127 was measured at 15 nm and the size increased about slightly after conjugated with Ad molecules at the chain ends. After the introduction of CDP in the solution of F127-multi-Ad, the peak shifted to right indicating the size over 500 nm. This result shows that the host-guest interactions between F127-multi-Ad and CDP caused large aggregates.

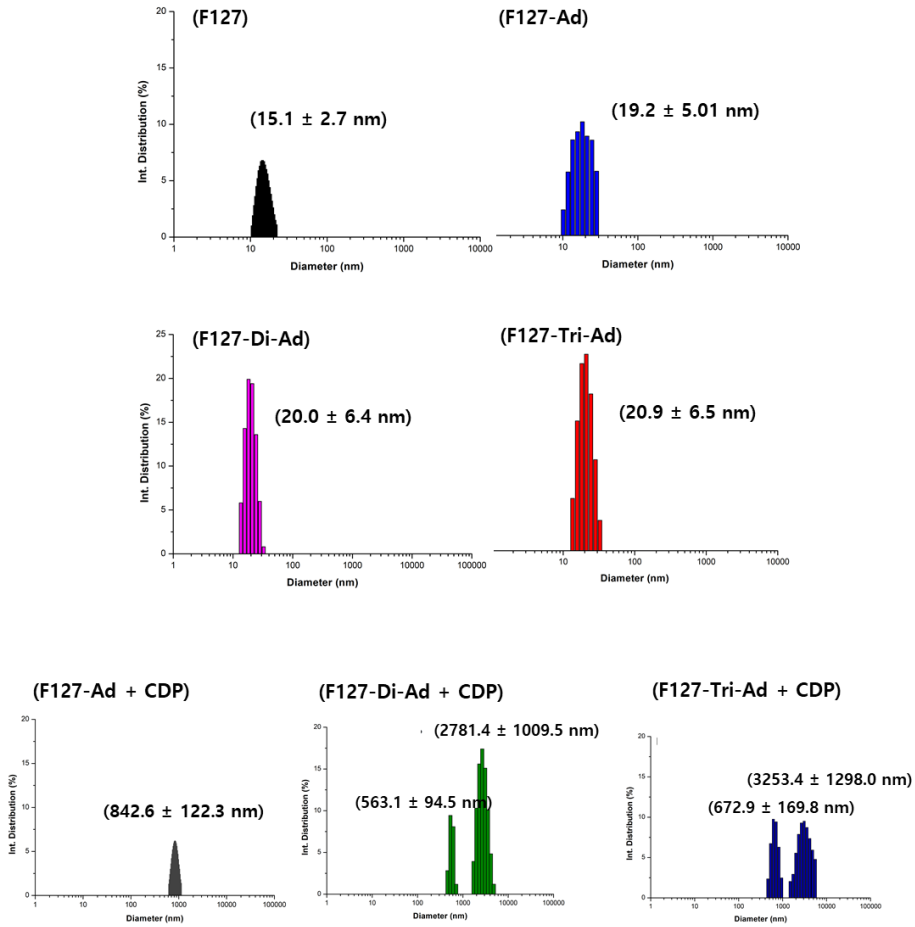


Figure 5. DLS analysis of F127, F127-Ad, F127-Di,Tri-Ad and CDP blended solution (0.1 w/v%)

3.4 Viscosity comparison

Reason for reducing gel formation concentration of host-guest interaction mediated Pluronic F127 system was to achieve low viscosity for fine injectability. The high viscosity of the system is due to the strong host-guest interaction between β-CD and Ad even under injection condition (at 4 °C) where no micelle is formed. Thus multiple guests conjugated Pluronic F127 (F127-Di-Ad, F127-Tri-Ad)/CDP system reduced CGC while maintaining a number of host-guest complexes. The blended solution was subjected to continuous flow experiments and the viscosity change was monitored. As shown in Fig 6, F127-Di, Tri-Ad/CDP blended solution showed almost 7 times less viscosity compared with F127-Ad/CDP solution at the lowest gelation concentration that is similar to sECM based hydrogel which was used to deliver cells for treatment. Interestingly, F127-Di-Ad 7 w/v% with CDP 5 w/v% solution has lower viscosity compared with F127-Tri-Ad 5 w/v% with CDP 3 w/v% as shown in Table 3 even F127-Di-Ad blended solution has lower total concentration. Reason for the difference is due to the absolute number of host-guest complexes. F127-Tri-Ad 5 w/v% has more guest molecules than F127-Di-Ad 7 w/v%. In both cases, β-CDs

are always larger enough to make a complex with adamantane molecules.

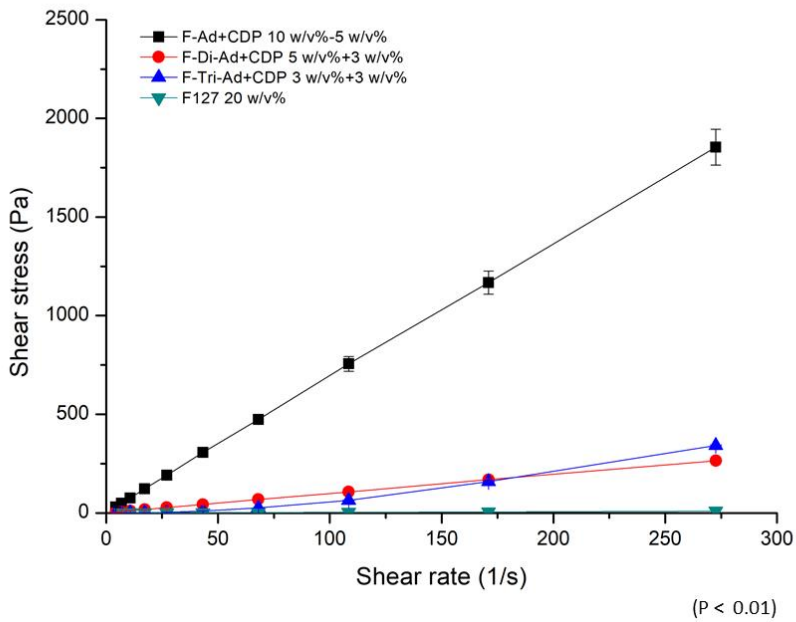


Figure 6. Viscosity measurement of F127 and F127-Ad, F127-Di,Tri-Ad / CDP blended solution at the lowest gelation concentration (at 4 °C)

Material	Viscosity (Pa·s)	*Material*	Viscosity (Pa·s)
F127-Di-Ad + CDP (5 w/v% + 3 w/v%)	1.00	F127-Tri-Ad + CDP (3 w/v% + 3 w/v%)	1.56
F127-Di-Ad + CDP (5 w/v% + 5 w/v%)	1.40		
F127-Di-Ad + CDP (7 w/v% + 5 w/v%)	3.33	F127-Tri-Ad + CDP (5 w/v% + 3 w/v%)	4.90
F127-Ad + CDP (10 w/v% + 5 w/v%)	7.02		

Table 1. Viscosity value of F127-Mono,Di,Tri-Ad / CDP blend hydrogel

3.5 *In vitro* gel dissolution rate

To confirm the stability of F127-Di, Tri-Ad/CDP blended hydrogel, swelling and erosion behaviors of the hydrogel were observed with different concentrations at 37 ℃ in pH 7.4 PBS solution (Figure 7). The complete erosion of prepared blend samples was not observed over 60 days like F127-Ad/CDP blended hydrogel except F127-Tri-Ad 3 w/v% and CDP 3 w/v% hydrogel. Despite the total concentration of hydrogel decreased, the stability of hydrogel maintained because of the increased number of host-guest complexes. However, F127-Tri-Ad 3w/v% and CDP 3w/v% showed complete erosion within 15 days and the reason of the fast erosion seems scarcity of the Pluronic F127 that makes up the micelles based hydrogel network.

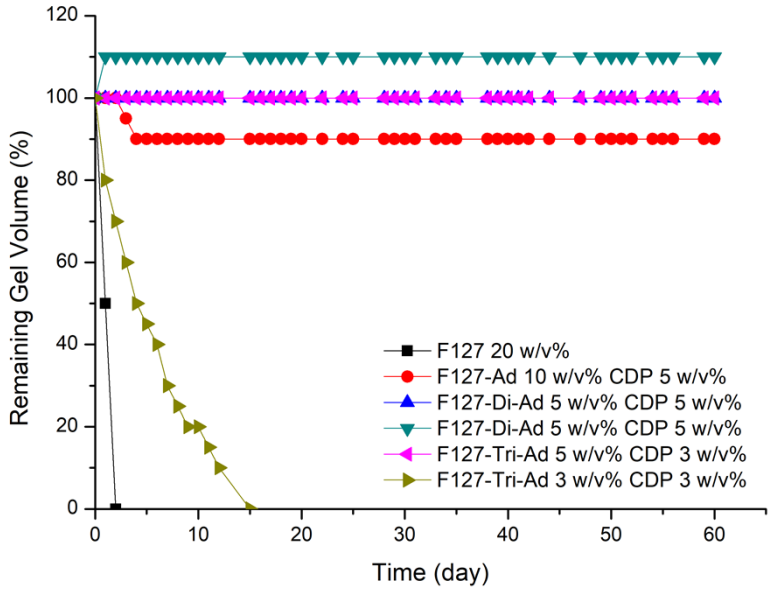


Figure 7. *In vitro* gel dissolution rate of F127, F127-Ad, F127-Di-Ad, F127-Tri-Ad / CDP blend hydrogel

3.6 *In vitro* Protein Release Profile

Abundant host (CD) and guest (Ad) molecules in this hydrogel system are expected to be able to function for the affinity-based sustained protein release when host or guest molecule modified therapeutic proteins are physically loaded into the gel matrix by strong host-guest interaction and specific protein like insulin which makes a complex with β-CD by Van der Waals interaction. The gelatin from porcine skin was used as model protein and absolute number of guest (Ad) units are abundant in the order of F127-Tri-Ad 7 w/v% + CDP 5 w/v%, F127-Di-Ad 7 w/v% + CDP 5 w/v% and F127-Ad 10 w/v% + CDP 5 w/v% hydrogels. Release behaviors of CD and Ad modified gelatin (gel-CD, gel-Ad) and unmodified gelatin were observed in the host-guest hydrogel systems. It showed that gel-CD and gel-Ad showed sustained release behavior compared to unmodified gelatin and gel-CD showed slower release rate and gel-Ad showed faster release profile when Ad units are abundant. The release profile of Insulin was also observed in the F127-Ad / CDP blended hydrogel over 80 days. It showed sustained release during the period without any specific burst release.

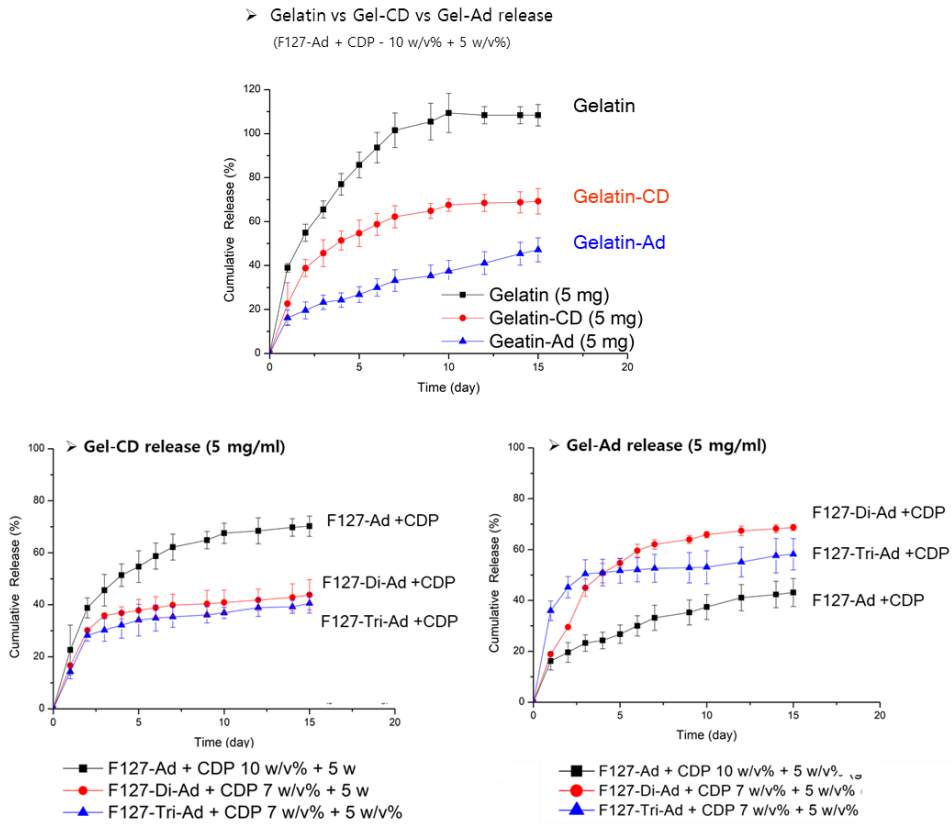


Figure 8. *In vitro* gelatin, gelatin-CD, gelatin-Ad release profile comparison of F127-mono-Ad, F127-multi-Ad / CDP blend hydrogel

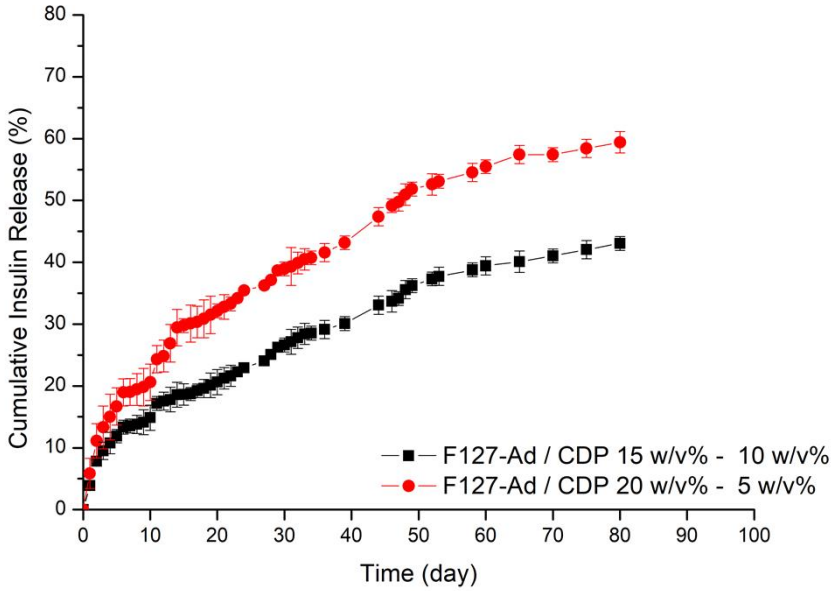


Figure 9. *In vitro* insulin release profile of F127-Ad / CDP blend hydrogel

4. CONCLUSION

In conclusion, the viscosity of host-guest interaction based Pluronic F127 solution significantly decreased by modifying the hydroxyl groups of Pluronic F127 into multiple Ad groups and introducing CDP for blend system. The F127-multi-Ad/CDP hydrogel exhibited much less viscosity at injection condition (at 4 ˚C) compared with the lowest concentration of F127-Ad/CDP blended solution. A number of host-guest complexes maintained in reduced total concentration enabled low viscosity as well as stable hydrogel formation. In addition, this host-guest interaction based hydrogel enabled affinity-based drug release. Insulin, and host or guest molecules conjugated gelatin showed sustained release profile due to Van der Waals interaction between host and guest molecules which present in the system. Multiple guest conjugated Pluronic F127 hydrogel system that shows both good injection ability and improved mechanical property can be applied to various therapeutic agents delivery system.

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국문 요약

폴리에틸렌글리콜-폴리프로필렌글리콜-폴리에틸렌글리콜 공중합체로 이루어진 플루로닉 고분자는 마이셀 패킹 메커니즘에 의해 일정 농도 이상의 수용액 상에서 온도 변화에 따라 가역적으로 졸 젤 거동을 변화하는 물질로 알려져 있습니다. 마이셀 구조는 15도 이상에서 형성되기 시작하며 온도가 올라갈수록 더 많은 마이셀이 형성되는데 이러한 구조 변화의 이유는 공중합체를 이루는 고분자들이 서로 다른 하한 임계 용액 온도를 가지고 있기 때문입니다. 이러한 온도 민감성 하이드로젤은 체내로 주사 가능하며, 세포 외기질과 같은 많은 수분 함량 및 부드러운 재질로 인해 단백질 및 세포 치료제 전달을 위한 전달체로서 생체 의료용 분야에 많은 관심을 끌어왔지만, 체내 환경에서 낮은 물성으로 인하여 임상으로의 응용에는 제약이 있습니다.

플루로닉 고분자의 단점을 극복하기위해 호스트 게스트 상호작용을 시스템을 마이셀 패킹 매커니즘과 접목하는 시도를 하였습니다. 강한 호스트-게스트 결합력을 통해 기존의 낮은 채네 안정성을 극복하였지만 주사 조건에서의 (4 ℃) 높은 점도로 인하여 단백질이나 세포 치료제를 주사하기에는 한계가 있었습니다.

체내 안정성을 유지하면서 점도를 낮추기 위해 플루로닉 고

분자의 양 말단에 다중의 게스트 작용기를 도입하여 호스트-게스트 상호작용을 통한 플루로닉 고분자의 마이셀 패킹을 더 견고히 하는 시도를 하였습니다. 플루로닉 고분자가 줄어들었음에도 증가한 호스트-게스트 복합체로 인하여 기존 플루로닉 F127 젤 또는 단일 게스트가 도입된 F127 젤 시스템에 비하여 젤을 형성하는 최소 농도가 감소하였습니다. 그 결과 주사 가능한 조건에서의 점도는 기존보다 약 7배가량 감소하였고 동시에 체내 환경에서 높은 안정성을 유지하는 결과를 확인하였습니다. 또한 호스트-게스트 결합을 기반으로 한 젤 시스템은 결합력 기반의 약물 단백질 방출을 가능하게 합니다. 호스트 분자가 연결된 단백질은 이 시스템안에서 서방성 방출 효과를 보였습니다. 이러한 기존의 장점을 유지하면서 한계점을 개선한 플루로닉 기반의 주사 가능한 하이드로젤은 다양한 생체 의료 분야에 사용될 수 있을 것으로 기대하고 있습니다.

주요어 : 플루로닉, 수화젤, 호스트-게스트 결합, 주사 가능한 시스템, 마이셀 패킹 메커니즘, 단백질 전달 시스템, 세포 전달 시스템

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