Poloxamer 407 Hydrogels for Intravesical Instillation to Mouse Bladder: Gel-Forming Capacity and Retention Performance

Sang Hyun Kim¹, Sung Rae Kim¹, Ho Yub Yoon¹, In Ho Chang², Young Mi Whang²,
Min Ji Cho², Myeong Joo Kim², Soo Yeon Kim³, Sang Jin Lee³, Young Wook Choi¹

¹Drug Delivery Research Lab, College of Pharmacy, Chung-Ang University, Seoul, Korea

²Department of Urology, College of Medicine, Chung-Ang University, Seoul, Korea

³Immunotherapeutics Branch, Research Institute, National Cancer Center, Goyang, Korea

Purpose: Poloxamer 407 (P407) thermo-sensitive hydrogel formulations were developed to enhance the retention time in the urinary bladder after intravesical instillation.

Materials and Methods: P407 hydrogels (P407Gels) containing 0.2 w/w% fluorescein isothiocyanate dextran (FD, MW 4 kDa) as a fluorescent probe were prepared by the cold method with different concentrations of the polymer (20, 25, and 30 w/w%). The gel-forming capacities were characterized in terms of gelation temperature (G-Temp), gelation time (G-Time), and gel duration (G-Dur). Homogenous dispersion of the probe throughout the hydrogel was observed by using fluorescence microscopy. The *in vitro* bladder simulation model was established to evaluate the retention and drug release properties. P407Gels in the solution state were administered to nude mice via urinary instillation, and the *in vivo* retention behavior of P407Gels was visualized by using an *in vivo* imaging system (IVIS).

Results: P407Gels showed a thermo-reversible phase transition at 4°C (refrigerated; sol) and 37°C (body temperature; gel). The G-Temp, G-Time, and G-Dur of FD-free P407Gels were approximately 10°C–20°C, 12–30 seconds, and 12–35 hours, respectively, and were not altered by the addition of FD. Fluorescence imaging showed that FD was spread homogeneously in the gelled P407 solution. In a bladder simulation model, even after repeated periodic filling-emptying cycles, the hydrogel formulation displayed excellent retention with continuous release of the probe over 8 hours. The FD release from P407Gels and the erosion of the gel, both of which followed zero-order kinetics, had a linear relationship ($r^2=0.988$). IVIS demonstrated that the intravesical retention time of P407Gels was over 4 hours, which was longer than that of the FD solution (<1 hour), even though periodic urination occurred in the mice.

Conclusions: FD release from P407Gels was erosion-controlled. P407Gels represent a promising system to enhance intravesical retention with extended drug delivery. (Korean J Urol Oncol 2017;15:178-186)

Key Words: Poloxamer 407 • Intravesical delivery • Thermo-sensitive hydrogel • Bladder simulation model • *In vivo* imaging system

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Corresponding Author: Young Wook Choi

Drug Delivery Research Lab, College of Pharmacy, Chung-Ang
University, 84 Heukseok-ro, Dongjak-gu, Seoul 06974, Korea
E-mail: ywchoi@cau.ac.kr

Tel: +82-2-820-5609, Fax: +82-2-826-3781

ORCID code: <https://orcid.org/0000-0003-2431-3995>

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INTRODUCTION

Many drugs are used for the treatment of bladder diseases. As they are predominantly administered via the oral route, only a small fraction of the administered drug reaches the bladder. In such cases, the concentration of the drug in the bladder tissue fails to reach the therapeutic range; to overcome this obstacle,

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the dose must be increased, which leads to an increased level of side effects.^{1,2} An alternative, intravesical drug delivery (IDD), involves direct administration of the therapeutic drug into the bladder cavity through catheterization, achieving more concentrated drug exposure and reduced systemic effects. However, there are limitations to IDD: first, the instilled drug is gradually diluted by urine and washed out during periodical urination; second, the bladder permeability barrier of the urothelium acts as an impenetrable barrier, and therefore the instilled drug cannot effectively penetrate to the target bladder tissue.³ Thus, repeated urinary catheterization is required, which is uncomfortable for patients.¹ To overcome these disadvantages, urination prior to drug instillation, to empty the bladder cavity, and the regulation of fluid intake before and after drug instillation, to suppress urine production, were used as supportive strategies. However, these efforts did not result in a considerable increase in the drug residence time in the urinary bladder cavity.⁴

Hydrogels are hydrophilic polymer networks that are able to retain plenty of water and maintain gel shape.⁵⁻⁷ As many drugs, proteins, and cells can be easily incorporated into hydrogel formulations, hydrogels are of great interest in biomedical applications. Injectable hydrogels can form a drug reservoir in the target site via an *in situ* sol-gel transition after administration by simple injection. There are many mechanisms involved in the sol-gel phase transition, including pH- and temperature-induced transitions.^{8,9} Thermosensitive polymer hydrogels remain in a 'free-flowing' sol state, with low viscosity at low temperatures, and maintain an 'injectable' state. However, at elevated temperatures, such as body temperature, they rapidly undergo sol-gel transition and form a 'hard-to-flow' gel phase with high viscosity.⁵ Therefore, after the polymer solution is easily injected into the target body site, it forms hydrogel at 37°C, which acts as the drug reservoir. Thermosensitive polymer solutions also have reversible gelling properties, with the ability to convert back into the polymer solution from the gel state at low temperatures. This characteristic enables the easy elimination of the hydrogel from the bladder cavity through simple washing with saline when administered into the urinary bladder.¹ The introduction of an injectable hydrogel via IDD can enhance the retention in the bladder cavity compared to that with a drug solution, even after periodic urine voiding.¹⁰

Poloxamer copolymers are the group of block copolymers comprised of ethylene oxide (EO) and propylene oxide (PO)

monomers. Since their introduction in the late 1950s, they have been used in various pharmaceutical applications.¹¹ The thermo-sensitive gelation of poloxamer copolymers is perfectly reversible and is characterized by a sol-gel transition temperature. As the temperature increases, poloxamer copolymer molecules aggregate and form micelles, thereby micellization is accelerated. When the concentration of micelles is sufficiently continuous, gelation occurs. The sol-gel transition is attributed to micelle packing.¹² Poloxamer 407 (P407), commercially available under the trade name of Kolliphor P 407 (BASF Laboratories, Wyandotte, MI, USA) or Synperonic F127 (ICI Laboratories, Wilton, UK), has triblock EO_x-PO_y-EO_x structure, with x and y values of 95–105 and 54–60, respectively. The polymer has a molecular weight of approximately 12,600 (range, 9,840–14,600) and an hydrophilic-lipophilic balance value of 22 at 22°C.^{13,14} Aqueous solutions of greater than 20 w/w% P407 demonstrate thermo-reversible gelling properties; 20–30 w/w% solutions of P407 are frequently used for hydrogel formulation.^{15,16} The U.S. Food and Drug Administration has presented P407 as an "inactive" material for various types of formulations, and it is generally considered to be nontoxic.^{12,17} Therefore, P407 offers a safe and appropriate thermosensitive gelling agent for intravesical delivery in hydrogel systems.

The intravesical administration of bacillus Calmette-Guerin (BCG) has been used in immunotherapy for superficial transitional cell bladder cancer. It was shown to reduce the number and frequency of recurrent tumors and to prevent disease progression.¹⁸ BCG, the attenuated strain of the bovine tuberculous bacterium, consists of living bacilli, dead microorganisms, and subcellular debris; however, the immunological properties and antibiotic sensitivities of the parent strain are conserved.¹⁹ When BCG contacts the bladder endothelium, it is endocytosed and induces inflammatory reactions with both CD4 and CD8 T-cells and macrophages. There is an increase in the production of cytokines, which also activate the lymphocytes. In clinical practice, as BCG solution is usually administered into the bladder cavity by urinary catheterization, the drugs are diluted by urine and eliminated regularly by periodic urination. Thus, to reduce urine production, water intake is restricted for 4 hours before instillation. It is recommended that patients suppress urination to allow sufficient absorption of BCG, but this is only of limited value.²⁰ Through the introduction of P407 hydrogel to enhance the retention of BCG, patient convenience is improved by the reduced need for re-

peated catheterization.

In the present study, P407 hydrogels (P407Gels) were prepared to enhance the retention time in the urinary bladder after intravesical instillation. To visualize the *in vitro* and *in vivo* characteristics of P407Gels, fluorescein isothiocyanate (FITC)-dextran (FD; MW 4 kDa) was used as a model probe. The following physical properties of the P407Gels were evaluated: homogeneity, gelation temperature (G-Temp), gelation time (G-Time), and gel duration (G-Dur). To mimic the physiological environment of the urinary bladder, an *in vitro* bladder simulation model was established, in which drug retention and release was measured. Finally, FD-loaded P407Gel was instilled to nude mice via urinary bladder catheterization, and the retention behavior was observed by using an *in vivo* imaging system (IVIS).

MATERIALS AND METHODS

1. Materials

Poloxamer 407 (Kolliphor P 407) was kindly provided by BASF Laboratories (Wyandotte, MI, USA). Phosphate-buffered saline (PBS) tablets and FITC-dextran (FD, MW 4 kDa) were purchased from Sigma-Aldrich (St. Louis, MO, USA). High-performance liquid chromatography (HPLC)-grade acetonitrile (ACN) was purchased from J.T. Baker (Phillipsburg, NJ, USA). Extrapure grade sodium phosphate dibasic anhydrous and sodium citrate were purchased from Duksan Chemical Co. Ltd. (Seoul, Korea). Nude mice were purchased from Orient-Bio (Seongnam, Korea).

2. Preparation of P407Gels

Thermosensitive P407Gels were prepared by the 'cold' method.²¹ To prepare FD-free P407gels as a control, 20–30 w/w% of P407 was added to a solution of PBS and stirred using magnetic stirrers overnight at 4°C until all the poloxamer granules were completely dissolved and a clear solution of P407 was obtained. Separately, to prepare FD-loaded P407Gels (FD-P407Gels), 0.2 w/w% FD was dissolved in PBS solution, and then P407 was dissolved overnight at 4°C. All samples were prepared on a w/w basis and reported as a weight %. The prepared hydrogels were equilibrated at 4°C and 37°C and tilted to check for 'free-flowing' movement, which enabled the identification of the thermo-reversible sol-gel transition.¹⁷

3. Homogeneity of FD dispersion in FD-P407Gels

Images of the prepared FD-P407Gels were obtained by fluorescence microscopy (Motic, Beijing, China) to evaluate the homogeneity of the FD dispersion in the hydrogel matrix after the sol-gel transition. A 10-μL sample of FD-P407Gel was placed on a microscope slide glass (Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany), and observed by using a fluorescence microscope at ×40 magnification.

4. Measurement of gelation temperature and time

The G-Temp and G-Time of the hydrogel formulation were evaluated by using the test-tube inversion method.^{22,23} The sol phase was defined as a free-flowing liquid state, and the gel phase was defined as a non-flowing gel state when the test tube was inverted. One milliliter of the sample was added into a 5-mL test tube (11-mm inner diameter) at 4°C and gradually heated in a temperature-controlled water bath from 4°C to 37°C at a rate of 1°C/min. The temperature at which the sample did not flow for 30 seconds was recorded as the G-Temp. To determine the G-Time, the samples were injected into a test tube at a constant temperature of 37°C, and the tubes were inverted every 5 seconds; the time at which the samples did not flow for 30 seconds was recorded as the G-Time. G-Temp and G-Time were evaluated in triplicate under equivalent conditions.

5. Measurement of gel strength of P407Gels

The strength of the developed hydrogel was evaluated in terms of G-Dur at body temperature, which was measured by a previously reported gravimetric test tube method.²⁴ A pre-weighed test tube (11-mm inner diameter) containing 0.5 mL of the hydrogel was equilibrated at 37°C, and the weight of the remaining hydrogel was calculated from the difference in the weight of the tube. PBS medium was equilibrated at 37°C, and 0.3 mL was carefully layered over the surface of the gel to avoid mixing. Tubes containing the gels and PBS medium were placed in an incubation chamber (SI-900R, Jeio Tech, Daejeon, Korea) maintained at 37°C. After predetermined time intervals (1 hour), the entire volume of PBS medium was removed and the weight of the test tube was measured. These processes were repeated until significant erosion was found. The percentage of hydrogel erosion was obtained from the weight difference and plotted as a function of time. G-Dur was calculated for a reduction of weight greater than 90% by the

extrapolation of the percentage of decreased weight versus time plot.^{24,25} G-Dur was evaluated in triplicate under equivalent conditions.

6. *In vitro* bladder simulation study

To mimic the human urinary bladder, an *in vitro* bladder simulation model was established from slight modifications of an earlier report.²⁶ Briefly, 12 mL of FD-P407Gel and FD-solution was injected via a pipette into a preweighed empty 250 mL round-bottom flask kept at 37°C. The weight of the flask plus the gel was measured. Then, PBS medium equilibrated at 37°C was added to the flask through the flask wall at a rate of 2 mL/min by using a peristaltic pump (BT100-2J, Longer Precision Pump Co. Ltd., Hebei, China). After peristalsis for 2 hours, the entire volume of PBS medium was poured out. The weight of the flask, in which the partially eroded gel remained, was measured to evaluate the retention behavior in the simulation model. Fresh PBS medium was pumped in again at the same rate and the above process was repeated 4 times. Simultaneously, to evaluate the release of FD from the hydrogel, at predetermined time points, 0.5-mL PBS medium was sampled and an equal volume of fresh PBS was replaced. Each formulation was subjected to the test in triplicate under equivalent conditions. The amount of FD was quantified by HPLC.²⁷ The HPLC system consisted of a separation module (W2695), fluorescence detector (W2475), and a data station (Empower 3, Waters, Milford, MA, USA). A Cosmosil C18 packed column (4.6 ID×150 mm, Nacalai Tesque, Kyoto, Japan) was used and the mobile phase was composed of 0.13 M phosphate buffer with 0.04 M citric acid (pH, 6.0) and ACN (9:1, v/v). The flow rate was 1.0 mL/min and the injection volume was 10 µL. The fluorescence of FD was detected at excitation and emission wavelengths of 495 nm and 520 nm, respectively.

7. *In vivo* intravesical retention study

The retentive ability of FD-P407Gel and FD solution in the urinary bladder was evaluated by using IVIS Lumina XRMS Series III (PerkinElmer Inc., Waltham, MA, USA). A total of 9 mice were randomly divided into three groups (n=3 for each group): group 1, which received saline (control); group 2, which received the FD solution; and group 3, which received the FD-P407Gel. The prepared hydrogel and solution containing 0.2 w/w% FD (50 µL) were instilled into the nude mouse bladder of each group by urinary catheterization. Then, at pre-

determined time points (0, 1, 2, 4, and 6 hours), the mice were anesthetized, and images of FD-derived fluorescence in the mouse bladder were obtained by using the IVIS imaging system at the excitation and emission wavelengths of 460 and 520 nm, respectively. Fluorescence images were processed using Living Image 4.5.5 (PerkinElmer Inc., Waltham, MA, USA).

8. Statistical analysis

All data are expressed as the mean±standard deviation. Significant differences were determined by using Student t-test with the level of significance set at $p < 0.05$.

RESULTS

1. Preparation of P407Gels and dispersion homogeneity of FD

P407Gels at 20, 25, and 30 w/w% were prepared successfully by the cold method. P407 and FD were completely dissolved in PBS to form a transparent solution at 4°C. The thermo-reversible phase transition behavior of FD-free and FD-P407Gels is shown in Fig. 1A. Both preparations formed a gel at 37°C that was resistant to flow, but formed a 'free-flowing' sol phase at 4°C. The top and side views of FD-P407Gels under white and fluorescence field light sources are shown in Fig. 1B. The fluorescence images demonstrated that FD was spread homogeneously when the P407 solution underwent thermo-reversible gelation.

2. Characterization of gel-forming capacities

To evaluate the gel-forming capacities and select the appropriate concentration of P407, G-Temp and G-Time were measured by a test tube-inversion method, and G-Dur was measured by a gravimetric method. As shown in Table 1, for FD-free P407Gels, when the concentration of P407 increased from 20 w/w% to 30 w/w%, G-Temp decreased from 21°C to 10°C, G-Time was shortened from 30.3 to 12.7 seconds, and G-Dur was extended from 12 to 35 hours. The values for these parameters were not significantly altered by the addition of FD.

3. Retention of FD-P407Gels in the bladder simulation model

The *in vitro* experimental set-up for the bladder simulation model is illustrated in Fig. 2A. The round-bottom flask and PBS medium were placed in a water bath at 37°C. The peri-

Fig. 1. Observation of P407Gels. (A) Thermo-sensitive phase transition of FD-free and FD-loaded P407Gels. (B) Fluorescence images of FD-P407Gel. P407Gel: poloxamer 407 hydrogels, FD: fluorescein isothiocyanate dextran.

Table 1. Gel-forming capacities of P407Gels

P407 concentration (w/w%)	G-Temp (°C)	G-Time (sec)	G-Dur (hr)
FD-free P407Gel			
20	21	30.3±0.3	11.81±0.54
25	16	23.1±0.1	18.83±0.87
30	10	12.7±0.7	35.18±2.51
FD-loaded P407Gel			
20	21	39.1±1.9	11.29±0.52
25	16	25.4±0.6	19.58±0.80
30	10	13.4±0.5	34.04±4.39

Values are presented as mean±standard deviation (n=3).

P407Gel: poloxamer 407 hydrogels, FD: fluorescein isothiocyanate dextran, G-Temp: gelation temperature, G-Time: gelation time, G-Dur: gel duration.

static pump was used to continuously infuse PBS medium to the flask at the rate of 2 mL/min. The infusion hose was stuck closely to the flask wall to allow the medium flow through it. The process for periodic filling and emptying consisted of 4 repeated cycles, as depicted in Fig. 2B. The hydrogel instilled by simple injection in the flask underwent rapid gelation at body temperature. The remaining fraction of P407Gel (%) was calculated by the equation: $(W_t/W_0) \times 100$ (%), where W_0 and W_t are the weights of flask at the initial time (gel-loaded, before filling) and after 2 hours (partial gel-remaining, after pouring out), respectively. The plot of hydrogel retention (%) over 4 repeated cycles is shown in Fig. 2C. The remaining fraction of the P407Gel decreased by approximately 15% at every pouring point, with a reduction of approximately 60% at 8 hours—

that is, the remaining fraction of the hydrogel decreased proportionally over time, which suggested that the erosion followed zero-order kinetics.

4. Drug release behavior of FD-P407Gels in bladder simulation model

The release behaviors of FD-P407Gel and FD-solution are presented in Fig. 2D. The release condition met the sink conditions. In case of the FD-solution, approximately 100% of FD was released immediately after the instillation. Then, the released FD in the artificial bladder fell to nearly 0% as soon as the medium was poured out. In contrast, the FD-P407Gel demonstrated the sustained release of FD, with approximately 10%–13% released into the buffer every 2 hours. The sustained release pattern was maintained, even after repeated periodic filling and emptying of the medium over 8 hours. The FD release from FD-P407Gel was approximately 50% over the entire 8-hour period.

5. In vivo intra-vesical retention of FD-P407Gels

The retention behavior of FD-P407Gel was investigated by fluorescence imaging after intra-vesical instillation in the bladder of nude mice. Representative fluorescence images of different treatments are shown in Fig. 3A. The fluorescence of FD appeared as a bright red-to-yellow color at the site of the mouse bladder. Moreover, the relative fluorescence intensity (RFI) profiles of FD-P407Gel versus a FD-solution are presented in Fig. 3B. RFI was expressed by $[(FI \text{ at time } t)/(FI \text{ at time } 0)]$. The group administered NS showed no significant fluorescence at

Fig. 2. *In vitro* bladder simulation study. (A) Illustration of experimental set-up for the *in vitro* bladder simulation model. (B) Schematic process for repeated periodic filling and emptying. (C) Plot of hydrogel retention (%) over 4 repeated cycles. (D) Plot of amount of FD released (%) versus time. FD: fluorescein isothiocyanate dextran, P407Gel: poloxamer 407 hydrogels, PBS: phosphate-buffered saline, FD-Sol: FD-solution-treated, FD-P407Gel: FD-loaded P407Gel-treated.

the bladder site over the entire period. Intravesical instillation of the FD-solution showed fluorescence right after instillation into bladder, but resulted in a drastic decrease in fluorescence levels within 1 hour. In contrast, the FD-P407Gel exhibited significantly higher fluorescence intensity, which extended over 4 h, longer than that by the FD-solution (<1 hour).

DISCUSSION

We developed a thermosensitive hydrogel formulation for enhanced retention in the bladder. This formulation resulted in the sustained release of drug as an intravesical retentive drug reservoir. P407 is a biocompatible polymer that is widely used in pharmaceutical and medical applications because of its low toxicity. Moreover, P407 formulations have simple preparations and requires no organic solvents. For these reasons, P407 was selected for the preparation of the thermosensitive hydrogels. FD is a fluorescent molecule with excitation and emission

wavelengths of approximately 495 and 520 nm, respectively. It can be easily solubilized in a hydrogel, with hydrophilicity similar to that of BCG; furthermore, BCG can be labelled with FD by using a simple incubation procedure.^{28,29} Thus, throughout this experiment, FD was used as a fluorescent probe because of its hydrophilicity and ability to label BCG.

Three main parameters were evaluated to ensure rapid gel formation and long-term retention *in vivo*. First, the G-Temp was the minimum temperature at which the thermosensitive hydrogel forms. To enable the phase transition into a gel in the bladder cavity after instillation, G-temp should be lower than body temperature (37°C). Simultaneously, it should not be below room temperature, to avoid a gelation that would complicate instillation. As all the G-Temp of all preparations (20–30 w/w%) were below body temperature, they were expected to undergo gelation after intra-vesical instillation. P407Gel with 20 w/w% was thought to be adequate, because the G-Temp was slightly higher than room temperature. Second, the G-Time is

Fig. 3. *In vivo* imaging system observation after intravesical instillation to nude mice. (A) Representative fluorescence images of different treatments: Control, normal saline-treated; FD-Sol, FD-solution-treated; FD-P407Gel, FD-loaded P407Gel-treated. (B) Changes of relative fluorescence intensity in FD-sol-treated and FD-P407Gel-treated groups (n=3 for each group). FD: fluorescein isothiocyanate dextran, P407Gel: poloxamer 407 hydrogels, RFI: relative fluorescence intensity.

the time required for the gelation of polymer solution. This should be estimated to be in the order of seconds, to allow rapid gelation without unwanted dilution by urine; however, it should not be too rapid and obstruct the catheter needle during intra-vesical instillation. All preparations resulted in rapid gelation within 1 min. The G-Time was dependent on the concentration of P407: P407Gels with 30 and 25 w/w% resulted in fast gelation, but P407Gel with 20 w/w% resulted in the longest G-Time (over 30 seconds), which was satisfactory for intravesical catheterization. Third, the G-Dur should be longer than 2 hours as the urination-restricted period in conventional clinical BCG therapy is generally 2 hours. The G-Dur for all preparations was greater than 10 hours, which was too long to for erosion and excretion in the urine. However, the gravimetric test tube method used in this study is limitation because the gel is eroded only from the contact of the upper surface with PBS. Thus, it was necessary to develop a model simulation to mimic the physiological environment of the urinary bladder. After accounting for all these parameters, 20 w/w% P407Gel

was selected for further experiments.

We established an *in vitro* bladder simulation model for the evaluation of the intravesical formulations, which represented a close approximation of human bladder conditions. The round-bottom flask was used to mimic the round shape of the bladder. The PBS medium was chosen as the simulated urine, with the peristaltic pump used to simulate the urine production in the bladder (2 mL/min).²⁶ After the medium was infused for 2 hours, it was poured out to mimic the urine voiding. This was thought to be reasonable as the sensation of urination occurs at a volume of approximately 200 mL in humans,¹ and the BCG instillation treatment requires the patients to suppress urine voiding for 2 hours. After the instillation of FD-solution into this simulated model, it dispersed into, and was diluted with the buffer to form a homogenous solution. The release curve suggested that all FD was diluted instantly into the aqueous medium. Thus, after the emptying process, no more FD was retained in the flask. In contrast, FD-P407Gel continued the sustained release of FD by virtue of the enhanced retention of

the hydrogel, even after repeated emptying. Moreover, as shown in Fig. 2D, the cumulative amount of FD increased linearly ($r^2 > 0.99$) with the time, which indicated that the *in vitro* release of FD from FD-P407Gel followed zero-order kinetics in the simulated bladder conditions.²⁵ To interpret the drug release behavior of P407Gels in the bladder simulation model, the relationship between the cumulative amount of FD released and the erosion of the gels was analyzed further. The erosion of FD-P407Gels (%) was calculated from the equation $[(W_0 - W_t)/W_0] \times 100$ (%), where W_0 and W_t are the weight of gel-loaded flask at the initial time and after 2 hours, respectively, as defined above. As shown in Fig. 4, excellent linearity was found ($y = 0.799x + 2.797$, $r^2 = 0.988$). Thus, we can conclude that the release of FD from the P407Gel follows erosion-controlled release kinetics.²⁵

The extended retention of FD-P407Gel in the mouse bladder was observed in an *in vivo* nude mouse model through the detection of the fluorescence responses after intravesical instillation. As shown in Fig. 3, the response in the FD-loaded P407Gel-treated group was stronger and broader at 1 hour than in the other groups, which indicated that a sufficient amount of FD was released from the gels, followed by quenching of the bright signal. In contrast, the FD-solution-treated group was rapidly quenched, even though the initial response of FD-Sol was similar to that of FD-P407Gel. However, in both treatments, no further fluorescence was detected after 6 hours, which indicated that all formulations were diluted and eliminated by urination. Meanwhile, from the perspective of the retention time

Fig. 4. Linear relationship between the cumulative amount of FD released (%) and the erosion of FD-P407Gels (%). FD: fluorescein isothiocyanate dextran, P407Gel: poloxamer 407 hydrogels, FD-P407Gel, FD-loaded P407Gel-treated.

of FD-P407Gel, this *in vivo* result was not in agreement with the data obtained from the *in vitro* bladder simulation model, which showed 50% retention after 6 hours (3 cycles). This discrepancy might be attributable to the differences in the volume of the hydrogel and the medium. In the case of the *in vitro* study, 12 mL of hydrogel was subjected to 240 mL of release medium in total for every cycle (2 hours). However, in the *in vivo* mouse model, 50- μ L hydrogel was instilled into the bladder cavity, as the maximum capacity of the bladder of nude mice is limited to approximately 100 μ L, following which urination is stimulated.³⁰ Moreover, the free movement of the mouse could affect the *in vivo* residence time compared with the *in vitro* model, which had no stirring process. Nevertheless, it was clear that FD-P407Gel showed extended retention in mouse bladder compared with the FD-solution, even after periodic urination.

CONCLUSIONS

A thermo-sensitive hydrogel system, P407Gels, was successfully developed for extended IDD. The system could be instilled by urinary catheterization without obstruction, and underwent thermo-sensitive gelation to yield enhanced retention. We also established a novel bladder simulation model to mimic the human bladder environment, in which a periodic filling-emptying process over 2 hours was repeated for 4 cycles to evaluate the retention capability. The FD release from P407Gels and the erosion of the gel, both of which followed zero-order kinetics, demonstrated a proportional relationship. Finally, the *in vivo* study of intravesical instillation proved the excellence of P407Gels in terms of their retention performance in the bladder of nude mice. Thus, we conclude that P407Gels represent a promising system of extended drug delivery for the treatment of urinary bladder diseases.

CONFLICT OF INTEREST

The authors claim no conflicts of interest.

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