In Vitro and in Vivo Release of Albumin Using a Biodegradable MPEG-PCL Diblock Copolymer as an in Situ Gel-Forming Carrier

Hoon Hyun,[†,‡,§] Yu Han Kim,[†,§] In Bum Song,[†,‡] Jung Won Lee,[†,‡] Moon Suk Kim,[*,†]
Gilson Khang,[‡] Kinam Park,[||] and Hai Bang Lee[†]

Medicinal Science Division, Korea Research Institute of Chemical Technology, P.O. Box 107, Yuseong, Daejeon 305-600, Korea, BK-21 Polymer BIN Fusion Research Team, Chonbuk National University, 664-14, Duckjin, Jeonju 561-756, Korea, and Purdue University, Departments of Biomedical Engineering and Pharmaceutics, 206 South Intramural Drive, West Lafayette, Indiana 47907-1791

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An MPEG-PCL diblock copolymer was synthesized as an in situ gel carrier, and its phase transition behavior in aqueous solutions was examined. For comparison, aqueous solutions of Pluronic F-127, a widely used injectable gel-forming solution, were also studied. Both MPEG-PCL copolymer and Pluronic aqueous solutions were sols at room temperature. As the temperature was increased above room temperature, the diblock copolymer and Pluronic solutions underwent a sol-to-gel phase transition, which manifested as an increase in viscosity indicative of the formation of a gel. All of the copolymer solutions became gels at body temperature, although the gel viscosity increased with the increasing concentration of the MPEG-PCL diblock copolymer in the solution. In in vitro experiments, in which the gels were exposed to PBS, the MPEG-PCL gels maintained their structural integrity for more than 28 days, whereas the Pluronic gel disappeared within 2 days. The same results were observed when the polymer solutions were subcutaneously injected into rats. The MPEG-PCL gels maintained their structural integrity longer than 30 days, while the Pluronic gel could not be observed after 2 days. The ability of the gels as drug carriers was studied by measuring the release of fluorescein isothiocyanate-labeled bovine serum albumin (BSA-FITC) from MPEG-PCL diblock copolymer gels in vitro as well as in vivo. In vitro, BSA release was sustained above 20 days, with a greater release at lower diblock copolymer concentration; by contrast, Pluronic gels exhibited almost complete release of BSA-FITC within 1 day. When the BSA-FITC-loaded diblock copolymer and Pluronic solutions were subcutaneously injected into rats, they immediately transformed into a gel. In vivo, sustained release of BSA-FITC over 30 days was observed from the MPEG-PCL gel, whereas BSA-FITC release from the Pluronic gel ceased within 3 days. Collectively, the present findings show that MPEG-PCL diblock copolymer solutions are thermo-responsive and maintain their structural integrity under physiological conditions, indicating that they are suitable for use as injectable drug carriers.

Introduction

During the past decade, injectable polymers have attracted considerable attention as candidate materials for biomedical applications such as polymeric drug carriers, implants, and other medical devices.[1,2] Poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO) block copolymers are the most widely used injectable polymers as commercially available grades such as Poloxamers and Pluronics.[3−7] Aqueous solutions of these block copolymers undergo a reversible sol−gel transition as a function of temperature; this transition can be exploited for applications in which the solution is injected into a human or animal in the sol state to become a gel at body temperature. However, the utility of these block copolymers in clinical applications is limited by some of their characteristics, which include poor mechanical properties, high permeabilities, short residence times, and non-biodegradability.[8,9]

Recently, considerable effort has been devoted to incorporating biodegradable segments into the poly(ethylene glycol) (PEG) block with the aim of creating biodegradable block copolymers. Diblock copolymers of PEG and poly(L-lactic acid) (PLLA) and PEG-poly(lactic-*co*-glycolic acid) (PLGA)-PEG triblock copolymers have been proposed as alternative polymers that could potentially be used as biodegradable and injectable drug delivery systems under physiological conditions.[10,11] However, these copolymers have resulted in an acidic environment. Poly(ϵ-caprolactone) (PCL) is a crystalline polyester that is an attractive synthetic biomaterial due to its good biodegradability and biocompatibility.[12] These polymers have the advantage that their degradation does not result in an acid environment, unlike the degradation of PLLA and PLGA. PEG and PCL block copolymers can be prepared via ring-opening polymerization (ROP) of ϵ-caprolactone (CL) by PEG. Endo et al. and our group investigated the ROP of CL with various alcohol/HCl·Et$_2$O initiator systems via an activated monomer mechanism, which can suppress unfavorable reactions such as back-biting and disproportionation.[13−20]

Previously, we studied methoxy poly(ethylene glycol) (MPEG)-PCL diblock copolymers that underwent a sol-to-gel phase transition as the temperature was varied.[17−20] It was found that MPEG-PCL diblock copolymers with an MPEG chain of molecular weight (MW) 750 and a PCL block with a MW of 1400 to 3000 exhibited sol-to-gel phase transition behavior.

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[*] To whom correspondence should be addressed. E-mail: mskim@krict.re.kr. Tel.: 82-42-860-7095. Fax: 82-42-860-7228.

[†] Korea Research Institute of Chemical Technology.

[‡] Chonbuk National University.

[§] Y.H. Kim and H. Hyun are equal first authors.

[||] Purdue University.

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These block copolymers have an advantage that their degradation does not result in an acidic environment, unlike the degradation of PLLA or PLGA. In addition, these block copolymers were found to maintain their structural integrity in the gel form over time. Thus, they could be considered as potential candidate materials for use in biomedical applications such as drug delivery and tissue engineering via in situ gel formation by simple injection.

In the present study, we prepared an MPEG-PCL diblock copolymer and examined whether aqueous solutions of this copolymer could undergo sol-to-gel phase transitions under physiological conditions. In addition, we compared the structural integrity of the gel formed from an MPEG-PCL diblock copolymer aqueous solution with that of the gel formed from a Pluronic F-127 block copolymer solution. Further, to test the efficacy of the thermo-responsive MPEG-PCL diblock copolymer for use as an in situ injectable gel-forming carrier, we studied the in vitro and in vivo release of a model protein, BSA-FITC, loaded into a MPEG-PCL diblock copolymer carrier.

Experimental Procedures

Materials. MPEG (Aldrich, number-average molecular weight (M_n) = 750) and HCl (Aldrich, 1.0 M solution in diethyl ether) were used as received. ϵ-Caprolactone (CL, Aldrich) was distilled over CaH_2 under reduced pressure. CH_2Cl_2 was distilled sequentially from $CaCl_2$ and CaH_2 under nitrogen before use. Bovine serum albumin−fluorescein isothiocyanate (BSA−FITC, Sigma) was used as received and handled under no light. Pluronic F-127 (BASF) is a polymer of polyoxyethylene (PEO) and polyoxypropylene (PPO) with two 100-unit hydrophilic PEO chains surrounding one 65-unit hydrophobic PPO chain and total weight of 12 600 g/mol and used as received.

Characterization. ^{1}H NMR spectrum for the calculation of molecular weight was measured using a Bruker DPX-300 MHz instrument (Bruker, MA) with $CDCl_3$ in the presence of TMS as an internal standard. Polymer disperse index (PDI) of the MPEG-PCL diblock copolymer was measured by a Futects At-3000 GPC system (Shodex RI-71 detector) using two columns (Shodex K-802 and Shodex Asahipak GF-510). $CHCl_3$ was used as the eluent at a flow rate of 0.8 mL/min.

Synthesis of Methoxy Poly(ethylene glycol)-block-poly(ϵ-caprolactone) Diblock Copolymers (MPEG-PCL).[16−20] MPEG (M_n = 750, PDI = 1.09) (1.5 g, 2 mmol) and toluene (80 mL) were introduced into a flask. The MPEG solution was distilled by azeotropic distillation to remove water. Toluene was then distilled off completely. CH_2Cl_2 (25 mL) was added to MPEG, followed by the addition of CL (4.8 g, 42 mmol) using a syringe. The polymerization was initiated by the addition of a 1.0 M solution of HCl in diethyl ether (4 mL, 4 mmol) at 25 °C. After 24 h, the reaction mixture was poured into *n*-hexane to precipitate a polymer, which was separated from the supernatant by decantation. The obtained polymer was redissolved in CH_2Cl_2 and then filtered. The polymer solution was concentrated by a rotary evaporator and dried in a vacuum to give a colorless polymer of quantitative yield. The molecular weight of the PCL segment in the diblock copolymer was determined by the intensity of the terminal methoxy proton signal of MPEG at $\delta = 3.38$ ppm and the methylene proton signal of PCL at $\delta = 2.31$ ppm in ^{1}H NMR spectroscopy. The polymerization afforded the MPEG-PCL diblock copolymer with a molecular weight of ca. 750−2490 and a PDI of 1.14.

Determination of the Sol-to-Gel Transition. The MPEG-PCL diblock copolymer was dissolved in deionized water at various concentrations in 5 mL vials at 80 °C and then stored at 4 °C for 15 h. After this time, the vials with the polymer aqueous suspensions were immersed in a water bath controlled to the desired temperature. The phase transitions of the polymer solutions were investigated by heating from 10 to 80 °C in increments of 2.5 °C. The vials were kept at each temperature for 10 min, and they were removed from the water bath and then tilted. If the solution flowed, the system was taken to be in the sol state, and if it did not flow, it was taken to be in the gel state. The phase transition temperature was determined from the results of these tilting tests.

Viscosity Measurements. The viscosity was measured using a Brookfield Viscometer DV-III ultra with a programmable Rheometer and circulating baths with a programmable controller (TC-502P). The viscosity measurement in the vessel was performed using a tight cap to prevent the evaporation of water in polymer solution. The viscosity of the polymer solutions was investigated using a T-F spindle at 0.2 rpm from 6 to 70 °C in increments of 1 °C.

Crystallinity Measurements. The 10, 15, and 20 wt % MPEG-PCL diblock copolymer solutions and the 20 wt % Pluronic solution were prepared. The crystallinity of the diblock copolymer solutions at room temperature was measured by X-ray diffraction (XRD; D/MAX-III B). A Ni filter at 35 kV and 15 mA generated the radiation. The samples were placed in a quartz sample holder and scanned from 0 to 60° at a scanning rate of 5°/min. The degree of crystallinity was calculated as the ratio of the crystalline peak areas to the total area under the scattering curve. The heat of fusion (H_m) and melting temperature (T_m) were determined with differential scanning calorimetry (DSC; Q 1000, TA Instruments) from 4 to 80 °C at a heating rate of 5 °C/min under a nitrogen atmosphere.

In Vitro Gel Formation and Protein Release Study. The 10 mL vials containing 5 mL solutions of 1−20 wt % diblock copolymers in PBS were immersed in a water bath at 80 °C to dissolve quickly the block copolymer above the melting temperature of PCL. The solutions were then left overnight in a refrigerator at 4 °C for 15 h. For the protein release experiment, 1 wt % BSA-FITC was added to the diblock copolymer solution at room temperature, and the mixture was gently mixed. A 1 mL aliquot of the diblock copolymer solution containing BSA-FITC was taken from the 5 mL solution and transferred to fresh 5 mL vials. It was incubated at 37 °C for 1 h to form a gel. Then, 4 mL of PBS at 37 °C was added to each gel, and the vial was shaken at 100 rpm and 37 °C. At specified sample collection times, 1 mL of solution was removed from the vial, and 1 mL of fresh PBS at 37 °C was added to the vial. Fluorescence spectroscopy (F-4500, Hitachi) was immediately performed on the removed solution. The amount of cumulatively released BSA was calculated by comparison with a standard calibration curve prepared through measurements of solutions of BSA-FITC in PBS with various known concentrations. Three independent release experiments were performed for each gel composition.

In Vivo Gel Formation and Protein Release Study. To test the in vivo gel formation behavior of the MPEG-PCL diblock copolymer, the diblock copolymer solutions were injected into rats. The MPEG-PCL diblock copolymer was sterilized using ethylene oxide (EO) gas. Twelve Sprague−Dawley (SD) rats (320−350 g, 8 weeks), divided into three groups of four rats each, were used in the release tests. The rats were housed in sterilized cages with sterile food and water and filtered air and were handled in a laminar flow hood following aseptic techniques. All animals were treated in accordance with the Catholic University of Korea Council on Animal Care Guidelines.

Three types of gel formulations were used in the in vivo release experiments: a mixture of 1 mL of 20 wt % MPEG-PCL solution and 1 mg of BSA-FITC; a mixture of 1 mL of 15 wt % MPEG-PCL solution and 1 mg of BSA-FITC; and a mixture of 1 mL of 20 wt % Pluronic solution and 1 mg of BSA-FITC. Each gel formulation was prepared by dissolving the diblock copolymer in PBS and incubating at 4 °C for 1 day. The BSA-FITC was then added, and the resulting solution was mixed. Within 5 min of the addition of BSA-FITC, a 1 cm^3 syringe with a 26-gauge needle was used to inject 0.5 mL of the solution into the subcutaneous dorsum of a rat that had been anesthetized with ethyl ether. The resulting gels were then allowed up to 4 weeks for in vivo release experiments and for biopsy.

For the in vivo detection of BSA-FITC, an aliquot of blood was drawn from the tail vein of each rat at specified blood collection times. A 0.3 mL aliquot of blood from the catheterized tail vein was collected in an Eppendorf tube and mixed with 0.2 mL of a 1:499 mixture of heparin and saline, followed by vortexing. To obtain plasma, the blood solution was centrifuged at 10 000 rpm for 5 min at room temperature. To the plasma obtained in this way, DW (100 μL), 66 mM EDTA (300 μL), and 50 mM HEPES (pH 7.4) (400 μL) were added. Plasma samples were stored frozen at −20 °C until assayed. To analyze the state of the probe and to examine the reliability of the method, we recorded spectra of BSA-FITC standard solutions of known concentrations of BSA-FITC in blood. The measurement conditions were an excitation wavelength of 490 nm with a bandwidth at 2.5 nm, emission wavelength of 525 nm with a bandwidth at 2.5 nm, and response time of 2 s. The amount of cumulatively released BSA was calculated by comparison with the standard calibration curves prepared with known concentrations of BSA-FITC. For each group of four rats treated with a particular gel formulation, the release experiment was separately performed on each rat, and the results were averaged.

For detection of the remaining BSA-FITC amount at predetermined times, each gel was individually removed from the sacrificed rat. It was dissolved in methylene chloride (2 mL) and then water (5 mL). The amount of remained BSA-FITC was calculated by comparison with the standard calibration curves prepared with known concentrations of BSA-FITC.

Data Analysis. The areas under individual plasma concentration−time curves for a released time period (AUC_{0-t}) were calculated. Bioavailabilities were determined by dividing the mean AUC value for each gel administration by the mean AUC value for direct administration into the subcutaneous dorsum of rats. Statistical analysis was performed using Student's *t*-test.

SEM Measurements of in Vivo Gel State. Scanning electron microscopy (SEM, S-2250N) was used to examine the morphology of the gel in vivo. After removal from the rat, the gel was immediately mounted on a metal stub pre-cooled in liquid nitrogen. After mounting the gel, the metal stub was quickly immersed in a liquid nitrogen bath to minimize alteration of the gel. Then, the stub was freeze-dried at −75 °C using a freeze-dryer. Once completely dry, the sample on the metal stub was coated with a thin layer of platinum using a plasma-sputtering apparatus (Emitech, K575) under an argon atmosphere.

Results

Phase Transition of the Diblock Copolymer Solution. Aqueous solutions of the MPEG-PCL diblock copolymer were prepared in phosphate buffered saline solution (PBS, pH 7.4) (80 °C) at concentrations of 1−20 wt %. At ambient temperature, the diblock copolymer solution was in the translucent suspension state. As the temperature was increased, the diblock copolymer solution showed a sol-to-gel phase transition, as determined by the tilting method. In viscosity measurements as a function of temperature over a temperature range of 6−70 °C (Figure 1), no change in viscosity was observed for the 1 wt % concentration of diblock copolymer, whereas the viscosities of the 10, 15, and 20 wt % solutions began to increase at 40, 37, and 32 °C, respectively. The viscosities of the 10, 15, and 20 wt % solutions at 37 °C were the viscosity of water, 75 × 10^3, and 625 × 10^3 cP, respectively. By comparison, the viscosity of the 20 wt % Pluronic solution began increasing at 20 °C, reaching a value of 2900 × 10^3 cP at 37 °C. Thus, 15 and 20 wt % MPEG-PCL diblock copolymer solutions were chosen as the most suitable candidates to form instantaneous gels at body temperature.

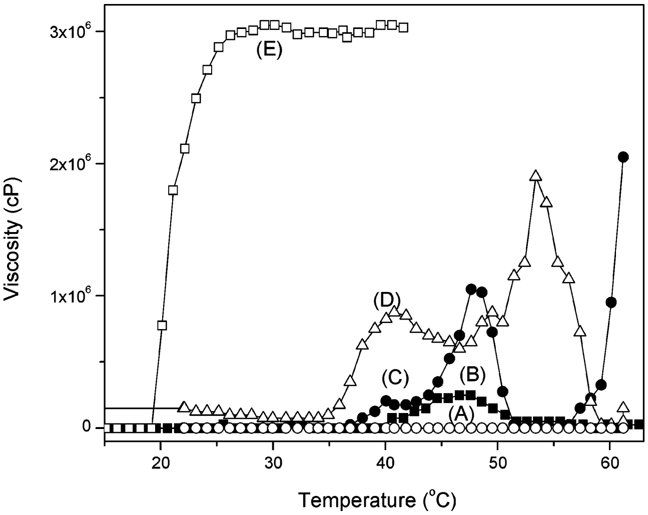

Figure 1. Viscosity as a function of temperature for aqueous solutions of MPEG-PCL copolymer at the concentration of (A) 1 wt % (○), (B) 10 wt % (■), (C) 15 wt % (●), and (D) 20 wt % (△). (E) is for a 20 wt % Pluronic solution (□).

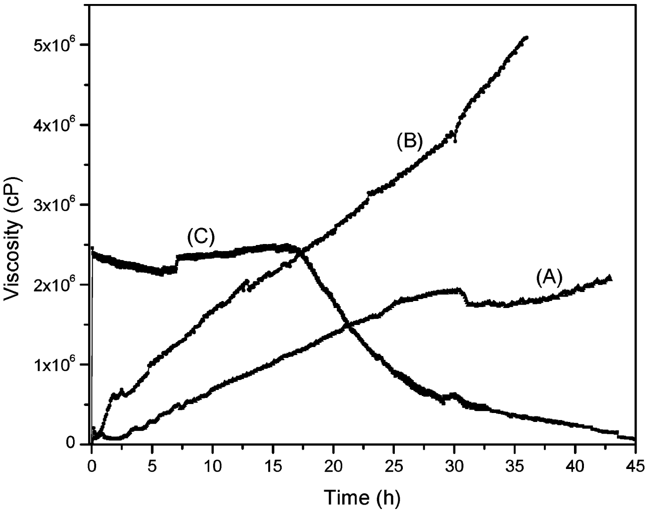

Figure 2. Viscosity as a function of incubation time at 37 °C for aqueous solutions of MPEG-PCL copolymer at the concentration of (A) 15 wt % and (B) 20 wt %. (C) is for a 20 wt % Pluronic solution.

Viscosity Changes at Physiological Temperature. To determine the viscosity behavior of the gels at body temperature, we measured the viscosities of the 15 and 20 wt % diblock copolymer solutions, as well as the 20 wt % Pluronic solution for comparison, at 37 °C for 45 h (Figure 2). In these experiments, solutions in the sol state at 25 °C were immersed in a water bath at 37 °C, and their viscosities were monitored by a Viscometer. At the initial time point (10 min), the viscosities of the 15 and 20 wt % MPEG-PCL gels and Pluronic gel were 0.1 × 10^3, 0.3 × 10^3, and 2500 × 10^3 cP, respectively. As the incubation time at 37 °C increased, the viscosities of the MPEG-PCL gels gradually increased, with the 20 wt % MPEG-PCL system exhibiting faster gelling than the 15 wt % system. By contrast, the viscosity of the Pluronic gel increased up to 2500 × 10^3 cP, remained approximately constant up to 15 h, and then dropped to the viscosity of water.

Crystallinity of the MPEG-PCL Diblock Copolymers. Figure 3 shows the XRD curves for the MPEG-PCL diblock copolymer in bulk and in aqueous solution (10, 15, and 20 wt %) at 25 °C, as well as the corresponding curve for the 20 wt % Pluronic solution. The XRD curves for all of the MPEG-PCL diblock copolymer solutions show peaks assignable to

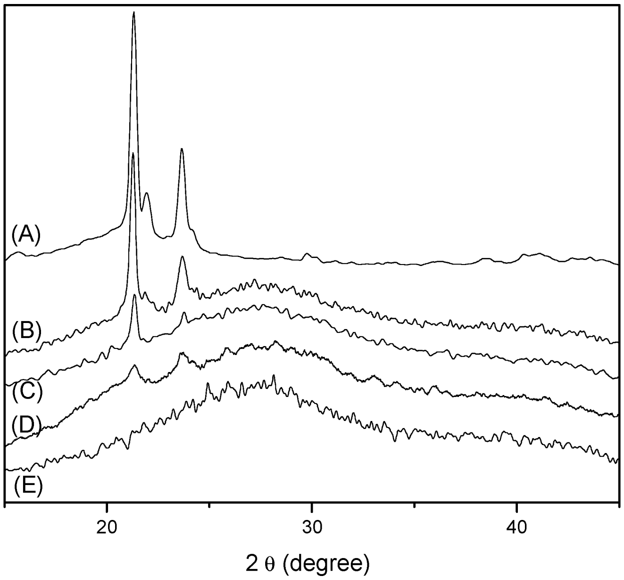

Figure 3. X-ray diffraction diagrams of MPEG-PCL copolymer in (A) the bulk state; (B) 20 wt %, (C) 15 wt %, and (D) 10 wt % aqueous solution; and (E) 20 wt % Pluronic solution.

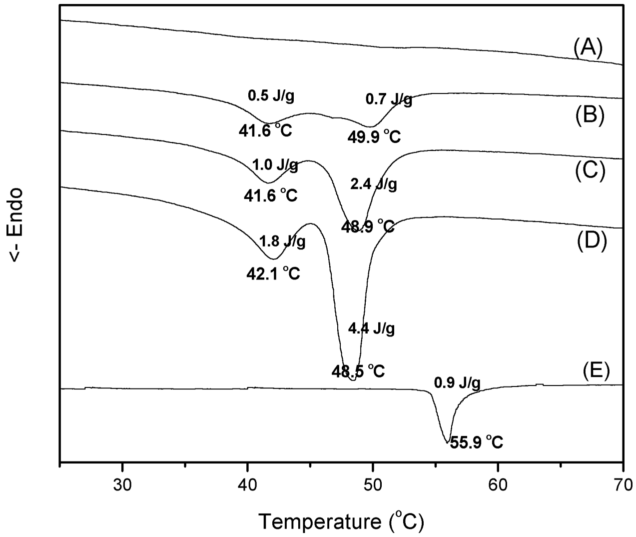

Figure 4. DSC profiles for aqueous solutions with MPEG-PCL copolymer concentrations of (A) 1 wt %, (B) 10 wt %, (C) 15 wt %, and (D) 20 wt %. (E) is for a 20 wt % Pluronic solution.

crystalline regions of PCL blocks. The total crystallinities of the 5, 10, and 20 wt % MPEG-PCL diblock copolymer solutions were 2.5, 10.1, and 16.2%, respectively. Meanwhile, the XRD curve for the Pluronic solution was indicative of the presence of only small amounts of crystallinity.

DSC thermograms (Figure 4) of the 10, 15, and 20 wt % MPEG-PCL diblock copolymer solutions exhibited two endothermic peaks, at around 42 and 46 °C, which can be attributed to the melting of the crystal phases of the MPEG and PCL segments, respectively.[21] The intensities of these peaks increased with increasing copolymer concentration, indicating that more crystalline regions are present at higher diblock copolymer concentrations. The Pluronic solution exhibited a single endothermic peak at around 56 °C, which was attributed to the melting of the crystal phases of the PEG and PPG segments.[22]

In Vitro Gel Maintenance and in Vitro Release. Figure 5 shows the 15 and 20 wt % MPEG-PCL copolymer gels and Pluronic gel before (panels B, D, and G) and after (panels C, E, and H) the addition of PBS (pH 7.4) at 37 °C. The MPEG-PCL gels formed at 37 °C maintained their gel form for 4 weeks, even after the addition of PBS (Figure 5C,E), indicating that the gels maintained their structural gel integrity. By contrast, the Pluronic gel dissolved within 24 h of the addition of PBS (Figure 5H).

When BSA-FITC was added into the MPEG-PCL copolymer and Pluronic solution, the solutions became yellowish. After gelation of the 15 and 20 wt % MPEG-PCL copolymer and Pluronic solution at 37 °C for 1 h (Figure 5B, D, and G), the PBS solution was added to the gel. Figure 6 shows the percent cumulative release profiles of BSA-FITC from the MPEG-PCL copolymer and Pluronic gels. The Pluronic gel exhibited almost complete release of BSA within 1 day, whereas the MPEG-PCL diblock copolymer gels exhibited prolonged release of BSA-FITC above 20 days. BSA was released faster from the 15 wt % than from the 20 wt % MPEG-PCL diblock copolymer gel. Specifically, 40% of the BSA was released from the 20 wt % gel after 20 days, whereas 60% of the BSA was released from the 15 wt % gel in the same time period.

In Vivo Gel Maintenance. When the 20 wt % MPEG-PCL copolymer and 20 wt % Pluronic solutions (0.5 mL) were injected at room temperature into live SD rats, they became gels almost immediately after injection. The injected MPEG-PCL gel was maintained at the injection site for the full 4 week experimental observation period (Figure 7A). The MPEG-PCL gel was found to retain its original shape and remain within the injected site. By contrast, the Pluronic gel had disappeared within 2 days after injection, as shown in Figure 7B.

Morphology of the in Vivo Gel. The 20 wt % MPEG-PCL gel removed from a rat at 2 weeks after injection was frozen in liquid nitrogen, freeze-dried, and observed by SEM. SEM micrographs (Figure 8) of the gel cross-section indicated that it consisted of three layers: a closed surface with cracks; a plate layer-by-layer middle region; and an inner region with an interconnected structure with a pore diameter of 10−30 μm.

In Vivo Release. To study the release of BSA from BSA-FITC-loaded gels in vivo, we made BSA-FITC-loaded 15 and 20 wt % MPEG-PCL copolymer and Pluronic gels in rats and monitored the BSA concentration in the rat plasma as a function of time. Figure 9 shows a plot of the plasma BSA-FITC concentration versus release time. The plasma from rats injected with the BSA-FITC-loaded MPEG-PCL gel showed a significantly higher BSA-FITC concentration at 1 day. The plasma BSA concentration increased rapidly, reached a maximum within 7.5 h, and exhibited a sustained release profile for 6−10 days depending on the copolymer concentration in the gel. BSA was detected in the plasma for up to 6−10 days post-injection, after which the BSA-FITC concentration was not detectable by fluorescence spectroscopy. For the rats injected with the BSA-FITC-loaded Pluronic gel, the plasma BSA concentration reached a maximum at 18 h post-injection, after which the concentration rapidly declined, reaching near zero levels within 3 days.

The AUC$_{0-t}$ values and absolute bioavailability determined from the plasma concentration profiles are summarized in Table 1. The bioavailability of the 15 and 20 wt % MPEG-PCL gels was 60 and 31%, respectively, whereas that of the Pluronic gel was 60%. The estimated bioavailability of the 15 wt % MPEG-PCL gels at 10 days is similar to the Pluronic gel at 3 days.

Figure 10 shows a fluorescence image of the 20 wt % MPEG-PCL gel removed from a rat after 14 days, when the plasma BSA-FITC concentration dropped below the detection limits

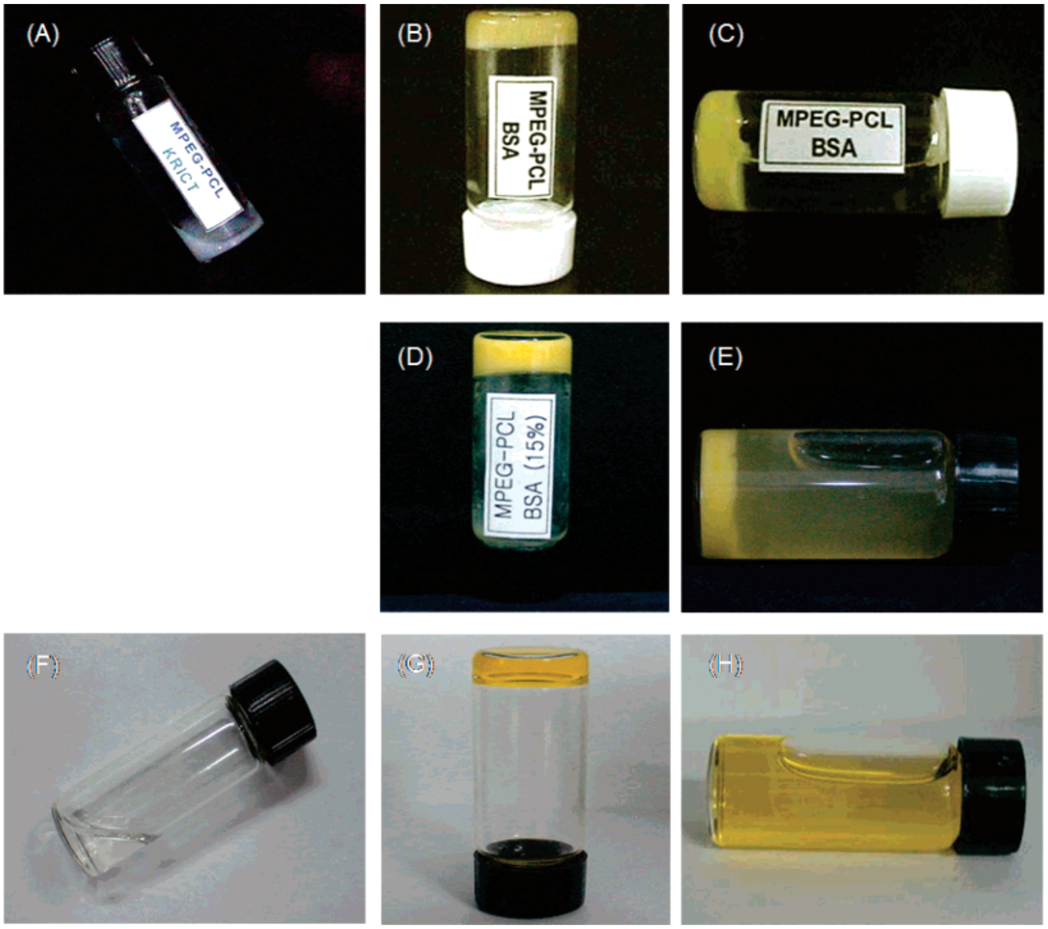

Figure 5. Images of (A−E) MPEG-PCL diblock copolymer and (F−H) Pluronic gels. (A) MPEG-PCL diblock copolymer solution at room temperature. (B and C) BSA-loaded 20 wt % MPEG-PCL copolymer gel at 37 °C before and after BSA release, respectively. (D and E) BSA-loaded 15 wt % MPEG-PCL copolymer gel at 37 °C before and after BSA release, respectively. (F) Pluronic solution at room temperature. (G and H) BSA-loaded 20 wt % Pluronic gel before and after release of BSA at 37 °C.

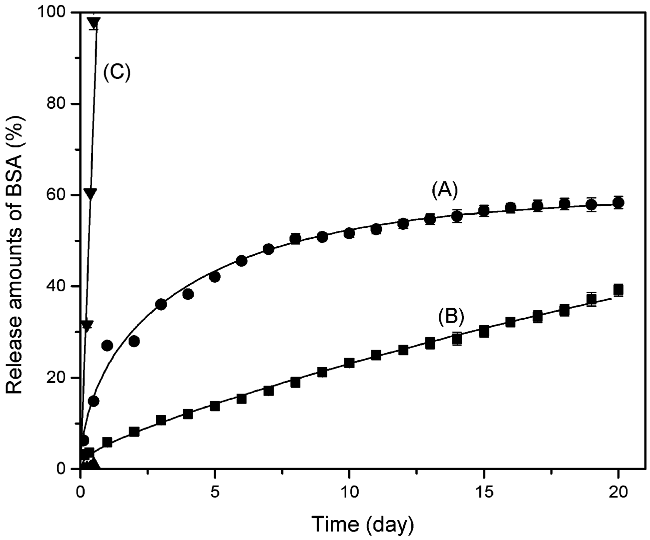

Figure 6. In vitro release of BSA from gels with MPEG-PCL concentrations of (A) 15 wt % and (B) 20 wt %. (C) is for a 20 wt % Pluronic gel.

of fluorescence spectroscopy. A homogeneous distribution of fluorescence was observed throughout the gel. The BSA-FITC contents remaining inside the 15 and 20 wt % gels after 14 days implanted in the rats were about 75 and 89%, respectively. After 30 days, the remaining amount of BSA inside the 15 and 20 wt % gels decreased to about 45 and 61%, respectively.

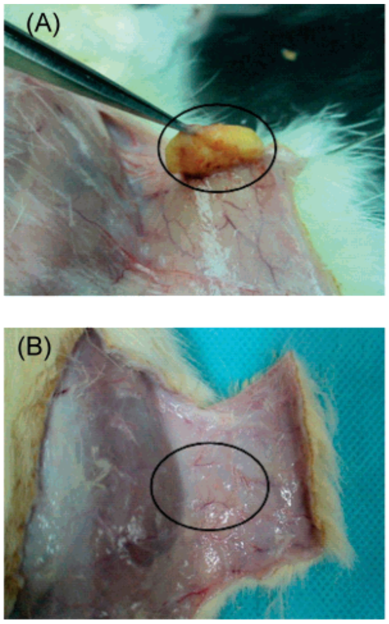

Figure 7. (A) 20 wt % MPEG-PCL copolymer gel (show circle to mark the gel) formed in a rat, 4 weeks after injection, and (B) Pluronic 20 wt % gel after 2 days.

Discussion

Currently, aqueous solutions of PEO and PPO block copolymers (e.g., Pluronic F-127) are widely used as injectable gels

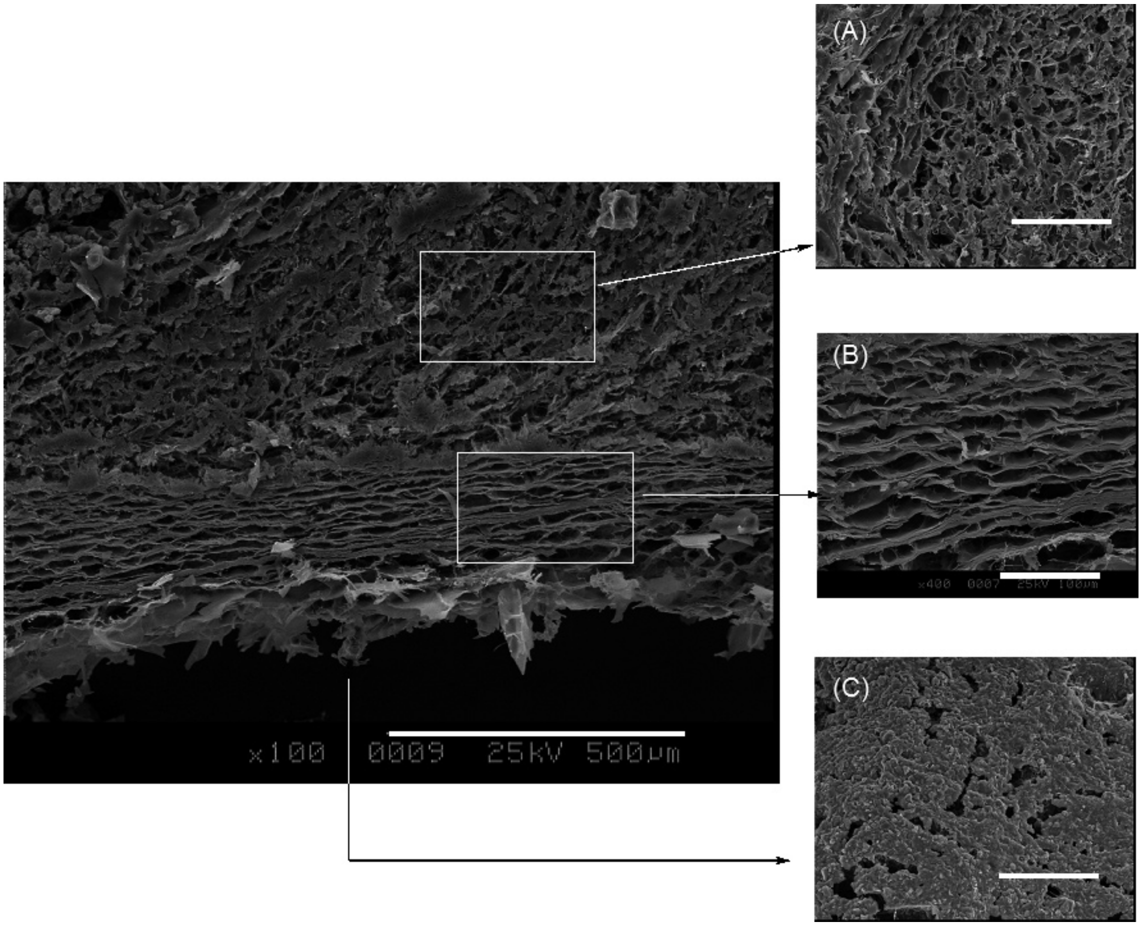

Figure 8. SEM micrographs of a 20 wt % MPEG-PCL gel removed from a rat after 14 days. (Magnification is 100× for the image on the left and 400× for panels A−C; scale bars represent 500 and 100 μm, respectively).

because they undergo a temperature-induced sol−gel transition.[3−8,23] However, the gel achieved by these block copolymers is mechanically weak and displays limited stability, short residence times, and high permeabilities. These drawbacks rendered these systems clearly unsuitable for most biomedical applications.[9] The aim of the present study was to develop thermosensitive MPEG-PCL diblock copolymer gels that maintain an adequate structural integrity over longer periods in vivo in comparison with Pluronic gels.

On the basis of previous work,[17−20] we identified the MPEG-PCL diblock copolymer with an MPEG chain of MW 750 and a PCL block of MW 2490 as the most suitable candidate materials for use as a drug carrier via in situ gel formation after subcutaneous injection into rats. When this MPEG-PCL diblock copolymer was placed in PBS, the hydrophobic PCL segments acted as associative crosslinkers, while the hydrophilic MPEG segments would favor dissolution of the copolymer molecule.[16] At room temperature, the MPEG-PCL diblock copolymer solution was a translucent emulsion, whereas the Pluronic solution was a clear solution. Both solutions flowed easily. As the temperature of the polymer solution was increased from room temperature, both the MPEG-PCL diblock copolymer and the Pluronic solutions underwent a sol-to-gel phase transition. The MPEG-PCL solutions with copolymer concentrations of 15 and 20 wt % were gels at 37 °C. The typical transition time to form a gel at 37 °C was about 25, 10, and 5 sec for the 15 and 20 wt % MPEG-PCL and Pluronic solutions, respectively. The Pluronic solution became a gel faster at 37 °C than those of the MPEG-PCL solution, probably due to the high molecular weight of the hydrophobic PPG block (3600 g/mol) as compared to the PCL block (2490 g/mol). The gels reverted back to the sol state when the temperature dropped to below room temperature.

The sol−gel phase transition as a function of temperature should manifest a substantial change in viscosity[18,24,25] due to the formation of a structured network of polymer solutions. We found that as the copolymer concentration increased, the viscosity increased and the onset temperature for gelation decreased. Increasing the concentration of the MPEG-PCL copolymer in aqueous solution should eventually lead to the ordering or aggregation of the PCL hydrophobic domains.[20,26−28] The formation of such PCL aggregates was indicated by the appearance of crystalline XRD peaks and peaks in the DSC thermograms corresponding to the melting of PCL blocks, even though the copolymers were dissolved in aqueous medium. The viscosity of the gel state increased with increasing diblock copolymer concentration due to the formation of a more tightly aggregated gel with more extensive crystalline regions in systems containing higher copolymer concentrations.

In aqueous medium, the viscosity of the Pluronic gel at 37 °C was found to be 5 times that of the MPEG-PCL gel at 20 wt % concentration of MPEG-PCL. However, the Pluronic gel maintained its structural integrity for less than 15 h and had completely dissolved within 24 h, whereas the MPEG-PCL gel maintained its integrity for a period of 4 weeks. These findings indicate that the MPEG-PCL copolymer in aqueous solution forms a strong and stable gel via strong hydrophobic interactions of the PCL blocks. Further, given that Pluronic and MPEG-PCL copolymers both contain PEG segments, although Pluronic contains PPG blocks whereas MPEG-PCL contains

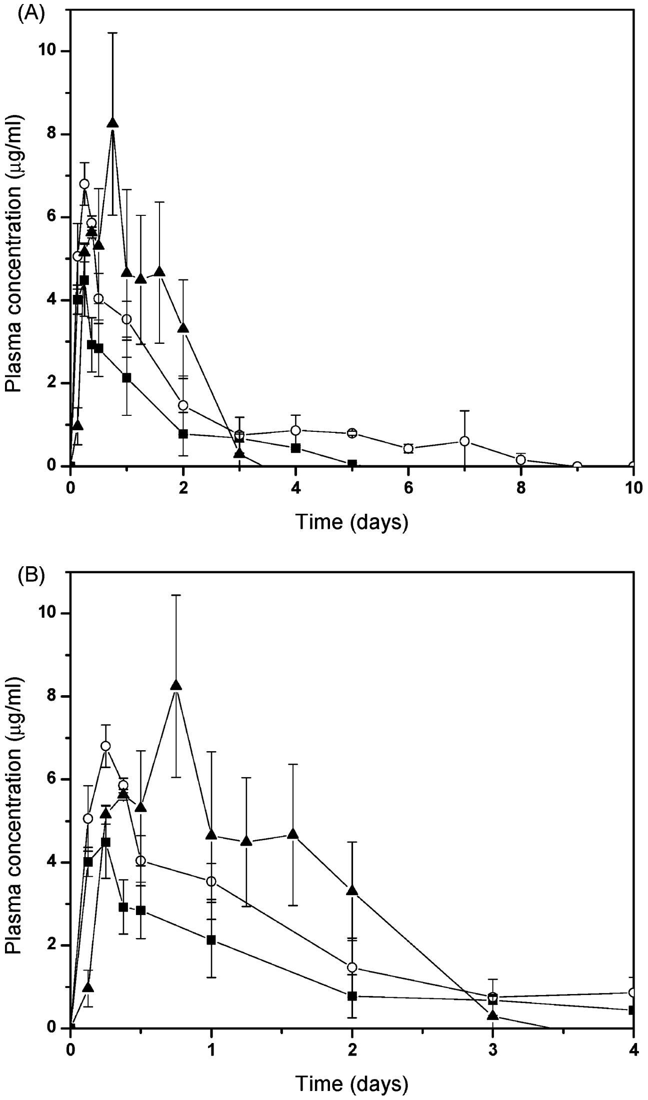

Figure 9. Plasma BSA concentration profiles in SD rats ($n = 4$). In vivo release of BSA from MPEG-PCL (○) 15 wt % and (■) 20 wt % gels and (▲) Pluronic 20 wt % gel for (A) 10 days and (B) at enlarged scale for 0−4 days.

PCL, the present results suggest that the ability of maintaining the aggregation degree and strength of the PPG segment is lower than that of PCL even though PCL has a lower molecular weight than PPG.

We found that both MPEG-PCL copolymer and Pluronic solutions became gels almost immediately after injection into rats. The Pluronic gel lost its original shape within 1 day of injection, whereas the MPEG-PCL gel maintained its original form at the injection site for the full experimental period of 4 weeks. This indicates that the MPEG-PCL gel can maintain sufficient structural integrity in vivo. Furthermore, SEM images of gels removed from rats and then freeze-dried showed a porous structure that could provide useful cavities for the penetration of biological medium into the gel. The interior structure of the MPEG-PCL gel seemed to form an interconnected network. The surface was found to have a closed morphology with large cracks, probably due to it being in direct contact with the surrounding tissue. The middle region showed a multilayered structure. Although the freeze-drying of samples for SEM experiments may have led to structural artifacts in the gel specimens, the SEM images should still reflect the intrinsic in vivo nature of the MPEG-PCL gels.

If a gel is to be successfully used as an in situ drug depot such as a protein drug in vivo, the gel should exhibit a desirable release profile of the drug under physiological conditions.[1,2,23] To test this, we loaded the copolymer and Pluronic solutions with BSA-FITC and tested the release of this protein from the resulting gels in vitro and in vivo. The BSA-loaded copolymer solutions were easily prepared and formed a sol at room temperature. In vitro, the BSA-loaded Pluronic gels lost their structural integrity over time, whereas the BSA-loaded MPEG-PCL diblock copolymer maintained adequate structural integrity for 4 weeks, consistent with the results obtained in the absence of BSA. Prolonged release of BSA-FITC was observed from the MPEG-PCL diblock copolymer gels above 20 days, with the 15 wt % MPEG-PCL copolymer gel exhibiting a greater release of BSA than the 20 wt % copolymer gel. Given that these experiments were performed by adding release media to the already formed gel, the greater release of BSA from the 15 wt % gel may be explained by its lower viscosity.

Simple subcutaneous injection of the MPEG-PCL copolymer and Pluronic solutions at room temperature into rats afforded a gel almost immediately. These in situ gel-forming properties strongly suggest that the MPEG-PCL copolymer is suitable for use as a drug carrier. When the BSA-loaded MPEG-PCL copolymer and Pluronic solutions were injected into rats, BSA-FITC release from the MPEG-PCL gels was sustained for 6−10 days, whereas that from the Pluronic gel lasted for only 3 days. This behavior can be attributed to the fact that the MPEG-PCL gels maintained their structural integrity for longer than the Pluronic gel. Both gels showed an initial burst in the release profile. This initial burst was possibly due to the high BSA concentration gradient between the gel surface and the surrounding tissue at the initial stage after injection since the concentration gradient after gelation is the driving force for BSA diffusion.

After the initial burst, the plasma BSA concentration decreased gradually up to 6−10 days in rats injected with the MPEG-PCL copolymer solutions. Although the BSA release seemed to cease after about 10 days due to the detection limits of the plasma BSA-FITC concentration in fluorescence spectroscopy, the sustained release of BSA from the copolymer gel for 30 days was confirmed by a change in the BSA concentration remaining inside gel.

The in vivo release of BSA from the 15 wt % copolymer gel was sustained for longer than that of the 20 wt % copolymer gel, indicating that the former gel has a higher bioavailability. This in vivo behavior may be associated with our observation that when these gels were incubated at 37 °C, their viscosity increased with time, with the 20 wt % gel showing a faster viscosity increase than the 15 wt % gel. These observations suggest that in vivo, the gels may become more tightly packed with time. This tighter packing, which is expected to be more pronounced for the 20 wt % gel, may retard the interactions between the biologic media and BSA or increase the interactions between the BSA and the gel network, thereby hindering BSA release. This explains a higher bioavailability of the 15 wt % gel than that of the 20 wt % gel. The hypothesis that BSA release is hindered by tighter packing is also supported by fluorescence images of the removed gels, which showed a homogeneous distribution of fluorescence in the gel, indicating incomplete BSA-FITC release due to the presence of a smaller amount of biologic fluid near the injected site and a higher fluorescence intensity for the 20 wt % gel than for the 15 wt % gel.

Table 1. Bioavailabilities of BSA-FITC after Subcutaneous Injection of MPEG-PCL Copolymer and Pluronic Solution Containing 1 mg of BSA-FITC[a]

			BSA amount remaining in gel (%)	
formulation	AUC_{0-t} (μg/mL day)	bioavailability[b] (%)	at 14 days	at 30 days
MPEG-PCL 15 wt % gel	11.20 ± 0.80	60.70 ± 2.75[c]	75.5 ± 6.1	44.8 ± 5.2
MPEG-PCL 20 wt % gel	5.87 ± 1.62	31.3 ± 8.62[c]	88.7 ± 5.4	61.2 ± 3.9
Pluronic gel	11.18 ± 2.53	59.5 ± 13.3[c]		
Administration of BSA-FITC solution only	18.78 ± 2.33	100		

[a] Data represent the mean ± SD ($n = 4$). Statistical analysis was performed using Student's *t*-test. [b] Bioavailability = (mean AUC value for each gel administration)/(mean AUC value for direct administration). [c] $P < 0.05$ vs BSA-FITC solution only.

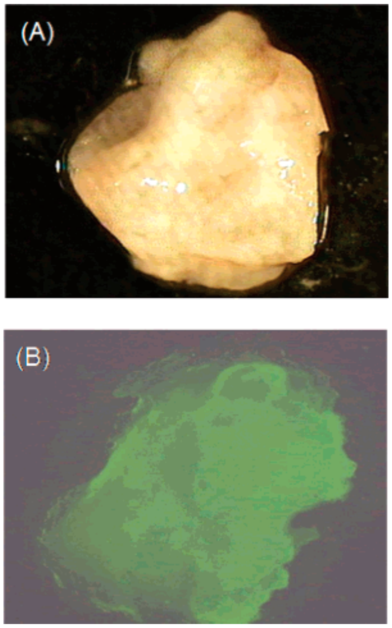

Figure 10. (A) 20 wt % MPEG-PCL gel removed from a rat after 14 days and (B) its fluorescence image.

Conclusion

We prepared a MPEG-PCL diblock copolymer and examined its behavior in aqueous solution. At room temperature, solutions of this diblock copolymer and of Pluronic, which was studied for comparison, formed a sol. As the temperature was increased above room temperature, the diblock copolymer solutions underwent a sol-to-gel phase transition, with the transition temperature decreasing with increasing diblock copolymer concentration. The transition from the sol to the gel state manifested a viscosity increase, which is attributed to the aggregation of the hydrophobic PCL segments. XRD and DSC analysis showed that the diblock copolymers were arranged into crystalline domains in aqueous solution, presumably because the aqueous environment favored the tight aggregation and strong packing interactions between PCL blocks. Experiments examining the release of BSA-FITC from MPEG-PCL diblock copolymer gels under in vitro conditions showed that the protein was released above 20 days, with the release rate decreasing with increasing diblock copolymer concentration. By contrast, the Pluronic gel exhibited almost complete release of BSA within 1 day. When BSA-loaded diblock copolymer solutions at room temperature were subcutaneously injected into rats, they immediately became gels. In vivo, sustained release of BSA-FITC from the MPEG-PCL diblock copolymer gels was observed for 30 days. By contrast, BSA release from the Pluronic gel in vivo quickly declined, reaching zero within 2−3 days. Collectively, the present findings show that MPEG-PCL diblock copolymer solutions are thermo-responsive materials that maintain their structural integrity under physiological conditions, indicating that they are suitable for use as drug carriers.

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