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Biodegradation of *In Situ*-Forming Gel of Poly(DLLA-co-CL) *In Vivo*

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ABSTRACT: Poly(DL-lactide-*co*-caprolactone) was dissolved in *N*-methyl-2-pyrolidone (NMP) for preparing blank group formulation, and drug group was prepared by dissolving testosterone undecanoate (TU) as model drug in blank group. These formulations rapidly gelled by solvent diffusion effect after subcutaneous injection. The *in vivo* degradation of copolymer gel in rabbits was investigated after injection. The GPC and ^{1}H NMR results showed that no matter the gel contained drug or not, it had no effect on degradation rate for the P(DLLA-*co*-CL) carrier. Degradation products of copolymer were absorbed or excreted based on metabolism. Copolymer molar composition changed slightly in 3 months. The structures and characteristic of copolymers were characterized via DSC, TGA, and SEM, respectively. Experimental results showed that the copolymer had excellent heat resistance. Crystallinity increased gradually during degradation process. A dense cross-sectional structure formed after 90 days. In addition, it can be obviously found that the degradation process of the copolymer proceeded in two steps. Mechanism of copolymer biodegradation *in vivo* was bulk degradation. The gel could well administrate the release of TU in a sustained way without significant burst features. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 3800–3808, 2013

KEYWORDS: biomedical applications; gels; properties and characterization

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INTRODUCTION

The injectable *in situ*-forming gel is based on the idea that certain biomaterials undergo a simple liquid-to-gel phase transition under physiological conditions.[1,2] The biomaterial solutions can be easily prepared by quantitatively incorporating various drugs through simple mixing and can be injected as liquid, and then it will form a gel *in situ* that acts as the drug depot. Injectable *in situ*-forming gel systems can be divided into four categories according to their formation mechanism: temperature sensitive,[3,4] PH sensitive,[5,6] ionic sensitive,[7] and remove solvent precipitation[8,9] *in situ*-forming gel.

Injectable *in situ*-forming gel systems were developed at the beginning of 1980s as a predominant drug depot system for parenteral sustained/controlled release drug delivery. Recently, injectable *in situ*-forming gels have attracted considerable attention more than ever in the field of sustained/controlled release systems[10–13] because they can localize delivery for a site-specific action, prolong local drug residence time, decrease body drug dosage with concurrent reduction of possible undesirable side effects common to most forms of systemic delivery, improve patient compliance and comfort, lessen the risk of conventional implant migration, overcome the shortcomings of conventional drug formulations by reducing systemic adverse drug reaction, and minimize surgical defects[14] because of the solution–gel state transformation. An aqueous system that undergoes a solution-to-gel transition after being injected has been suggested as one of the most promising implantable administration systems because of its simple fabrication and nonsurgical implanting procedure.[15]

The investigated polymer, poly(DL-lactide-*co*-caprolactone) (P[DLLA-*co*-CL]), is a kind of aliphatic copolymer which is the most extensively studied materials to achieve depots for sustained delivery of many drugs. P(DLLA-*co*-CL) is a promising candidate for *in situ* gelation application, as it is biodegradable *in vivo*, and it is hydrophobic and thus may retain an encapsulated drug for longer periods when used as drug carriers.[16] Degradation products of P(DLLA-*co*-CL) are natural body components. Therefore, P(DLLA-*co*-CL) is promising for use as an injectable local drug delivery system.

In order to achieve parenteral sustained/controlled delivery of drugs, preparation method to remove solvent precipitation *in situ*-forming gel was used in the study. This approach employed the biodegradable polymer dissolved in a pharmaceutically acceptable solvent to which a drug was added that formed a

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solution. After injection of the formulation into the body, the water-miscible organic solvent dissipated and water penetrated into the organic phase. This led to phase separation and precipitation of the polymer at the site of injection. Drugs formulated in polymeric devices were released either by diffusion through the polymer barrier, by erosion of the polymer material, or by a combination of both mechanisms.

The objective of this study was to develop a novel sustained/controlled release formulation using *in situ*-forming gel of P(DLLA-*co*-CL). In this study, we prepared both P(DLLA-*co*-CL) blank group and drug group *in situ*-forming gels and examined whether drug had any effect on degradation rate. In addition, the properties of P(DLLA-*co*-CL) during degradation periods and degradation mechanism were evaluated. The ability to predict and quantify degradation behavior had a direct impact in a variety of biomedical application and was a useful design tool for biodegradable implants. Polymer degradation usually plays a crucial role in drug release from sustained release polyester systems, therefore, in order to elucidate the mechanism governing release, it appears essential to analyze the *in vivo* degradation behavior of these devices.

EXPERIMENTAL

Materials

ε-Caprolactone (CL, Alfa Aesar, Ward Hill, MA) was purified by drying over CaH_2 (Sinopharm Chemical Reagent Co., Ltd, China) and distilled under reduced nitrogen pressure. Polymer grade D,L-lactide (DLLA, Jinan Daigang Biomaterial Co., Ltd, China) was used without further purification. Stannous octoate ($SnOct_2$, Sigma-Aldrich, St. Louis, MO) was used as received. *N*-Methyl-2-pyrolidone (NMP) was purchased from Sinopharm Chemical Reagent Co., Ltd. Testosterone undecanoate (TU) was obtained from Zhejiang Xianju Pharmaceutical Co., Ltd. (China). All other materials were of high performance liquid chromatography (HPLC) or analytical grade.

Copolymer Synthesis

P(DLLA-*co*-CL) with 84 mol % of CL was synthesized in our laboratory. Briefly, the polymerization was conducted by ring-opening polymerization in evacuated and sealed glass ampules using $SnOct_2$ as catalyst. Polymerization was carried out for 24 h at 130 ± 2°C. The synthesized polymers were purified by dissolution in chloroform and precipitation into methanol. After being washed in methanol for several times, the polymers were dried under reduced pressure until constant weight.

Preparation of P(DLLA-co-CL) Solution with and Without TU

The P(DLLA-*co*-CL) solution which is called blank group was obtained by dissolving 2 g of P(DLLA-*co*-CL) in 6 mL NMP solvent. Drug group was prepared by dissolving 2 g TU as model drug in blank group. Gas needs to be removed from the prepared solutions by heating to 60°C. The final solutions were then sealed and stored. During the preparation process, aseptic operation was used. Each final liquid solution was homogeneous.

In Vivo Gel Formation

In vivo gel formation and degradation were examined in adult male Japanese rabbits in the animal house of our institution. The rabbits were housed in sterilized cages with sterile food, water, and filtered air. Eight adult male Japanese rabbits, divided into two groups with four rabbits each, were used in the *in vivo* tests of P(DLLA-*co*-CL) blank group and drug group solutions. For injections, each rabbit was anesthetized with ethyl ether. After shaving and disinfection, appropriate solution was administered by dorsal subcutaneous injection using a 2.5 mL syringe with a 21-gauge needle (Scheme 1). At 1, 30, 60, and 90 days after injection, two rabbits were sacrificed and the gels were collected and the morphology of the gels was observed. The copolymer was washed in distilled water and finally dried under reduced pressure until constant weight. The experiments were performed in triplicate.

Histological Evaluation

The specimens including the surrounding tissue were harvested from the surgical sites and the tissue was fixed in 4% buffered formalin. Subsequently, the tissue was embedded in paraffin. The paraffin sections were stained with hematoxylin eosin and investigated with light microscopy. Evaluation of the tissue reaction was mainly based on the presence of foreign body giant cells.

Gel Permeation Chromatography Analysis

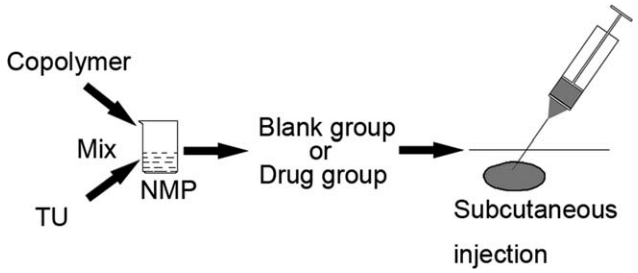
The molecular weight of gels and their distribution (polydispersity index, PDI) were measured by gel permeation chromatography (GPC) using a Waters model 1515 isocratic HPLC pump with a Waters model 2414 refractive index detector, at a flow rate of 1.0 mL min^{-1}(eluent: THF; 35°C). Polystyrene standards (Waters) were used for calibration. GPC spectra were given in supporting information.

Nuclear Magnetic Resonance Analysis

Nuclear magnetic resonance (^{1}H NMR) spectra were recorded on an AV-400 spectrometer (Bruker, Switzerland) at 400 MHz to characterize the chemical composition of the blank group and drug group gel. $CDCl_3$ served as solvent and TMS served as shift reference.

Differential Scanning Calorimetry

Thermal analysis was conducted using differential scanning calorimetry (DSC) 200 F3 Maia thermal analyzer (Netzsch, Germany). The weight of all gels was maintained between 5 and 6 mg. The reference material was a blank aluminum pan. The thermal properties of all the samples were characterized in a temperature of −90 to 110°C under a nitrogen atmosphere at a heating rate of 10°C min^{-1}. T_m was taken as the maximum of the endothermic melting peak from the heating scans. The heat of fusion (ΔH) was obtained from the areas under the melting peak.



Scheme 1. Schematic diagram of preparation and injection of liquid formulation.

Thermogravimetric Analysis

Thermogravimetric analysis (TGA) was performed by TG 209 F3 Tarsus instrument (Netzsch, Germany). The TGA was used to measure the weight change of samples as a function of temperature, at a heating rate of 10°C min^{-1} under an atmosphere of nitrogen.

Scanning Electron Microscopy

Scanning electron microscopy (SEM; Philips XL-30ESEM, FEI, Hillsboro, Oregon) was employed to investigate the morphology of *in situ*-forming gel after injection. Surfaces and cross sections of the gels were coated with gold by means of ion sputter coater. Subsequently, the gels were examined under the SEM.

In Vivo Release in Rats

Male Sprague–Dawley rats weighting about 200 g were used in this study. All the rats were housed in sterilized cages with sterile food, water, and filtered air in the animal house of our institution.

0.3 g TU-loaded gels was injected subcutaneously at the back of the rats. The rats were sacrificed at the predetermined time intervals: 3, 7, 14, 28, 42, 63, and 90 days. For each designated time, three male rats were sacrificed under anesthesia (isoflurane) and the gels were recovered. Methanol was applied to extract the TU from PLGA with sonication. The solution obtained was filtered for HPLC analysis. The release amount at each time was determined by subtracting the residual drug content from the initial content. All cumulative release values were presented as mean ± SD.

RESULTS AND DISCUSSION

Macroscopic Observations and Histological Examination

All the *in situ*-forming gels had firmly localized *in vivo* 1 day after injection. The state of *in situ* forming gels was semi-hard and flexible for both rabbit groups. At each evaluation point, in tissue surrounding the gels, tissue necrosis, abscess formation, or acute inflammation were not observed. Because of the solvent diffusion, the gels were surrounded by a tissue capsule, with a thin tissue layer (Figure 1). It was found that the foreign body reaction of P(DLLA-*co*-CL) was very mild. Throughout the experimental period, the gels maintained their gel-like morphology, but their size became smaller over time because of gel degradation.

Fibrous hyperplasia was already present from day 1 as shown in Figure 2. At 1 and 30 days, mild inflammatory reaction of the tissue occurred, followed by a normal foreign body reaction. During the first 30 days, the gels were surrounded by fibrous tissue containing numerous giant cells. After 60 days, no giant cell infiltration occurred around the gels. There had been the fibrous capsules around the gels. The tendency of fibrous hyperplasia was getting weaker. At last, there was no significant inflammatory reaction observed in the surrounding tissue around the gels. Gels *in vivo* have good tissue compatibility.

These results of macroscopic observations and histological evaluation indicated that P(DLLA-*co*-CL) gel was biocompatible and biodegradable *in vivo* and was suitable for use as carrier of sustained/controlled release system.

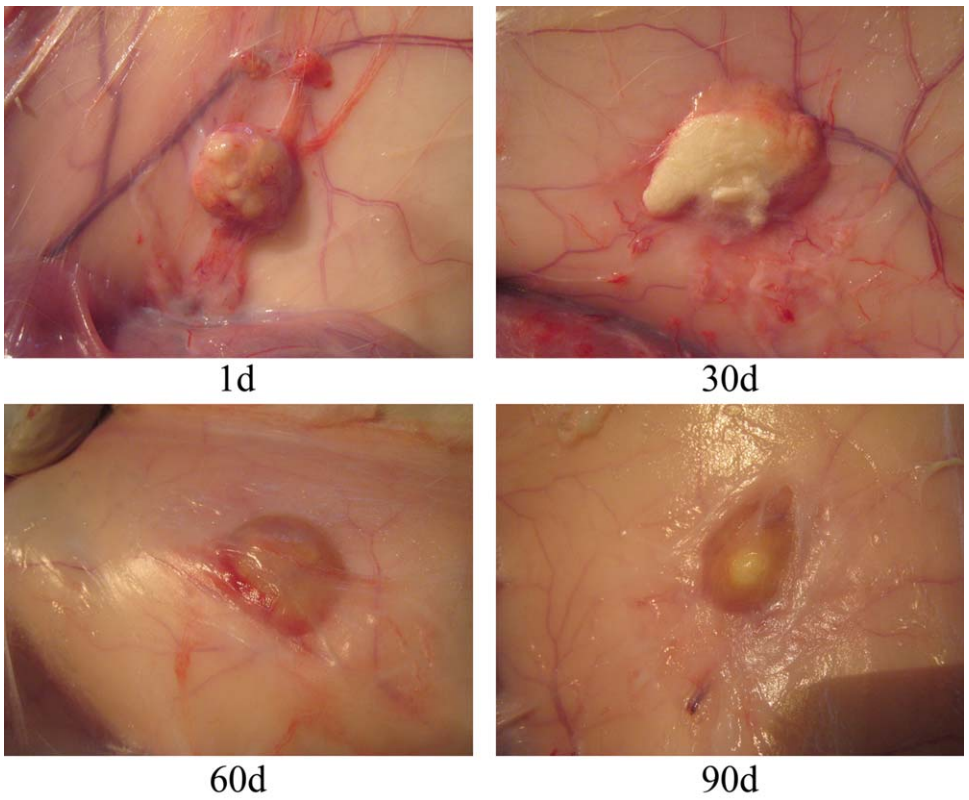


Figure 1. Morphology of *in situ*-forming gel after injection. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

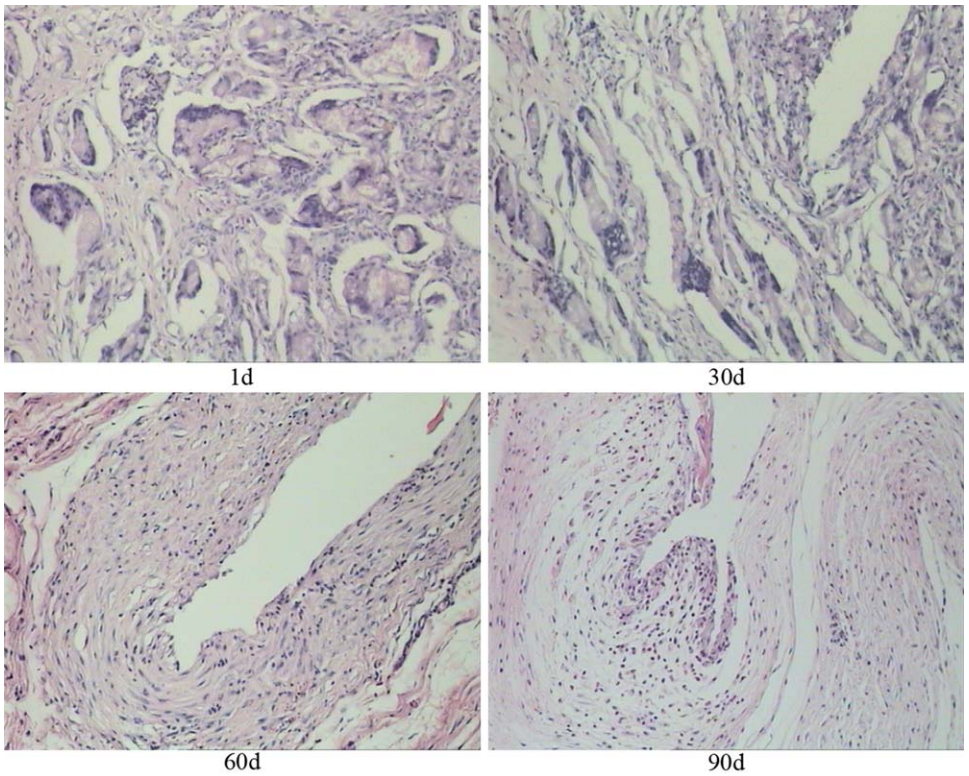


Figure 2. Light micrographs of P(DLLA-*co*-CL) gel at 1, 30, 60, and 90 days (×100). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Molecular Weight and Distribution Analysis by GPC

Results of molecular weight measurement of degraded gels were presented in Figure 3. The initial molecular weight (number average, M_n) of P(DLLA-*co*-CL) was 5.0×10^4 g/mol.

The plots (Figure 3) revealed a rapid decrease of the number average molecular weight of gels from 1 to 90 days for both groups. The M_n of all the gels started decreasing from the injection time, and decreased to half of its initial value after 90 days. At each evaluation time, no differences were observed between blank group and drug group. The results indicated that no matter the gel contains drug or not, it had no effect on degradation rate for the P(DLLA-*co*-CL) carrier.

As seen in Figure 4, PDI of the two groups showed almost exactly the same tendency from 0 to 90 days. For both blank group and drug group, PDI decreased over the degradation time.

PDI was defined as the ratio of the weight average molecular weight, M_w, to the number average molecular weight, M_n. High

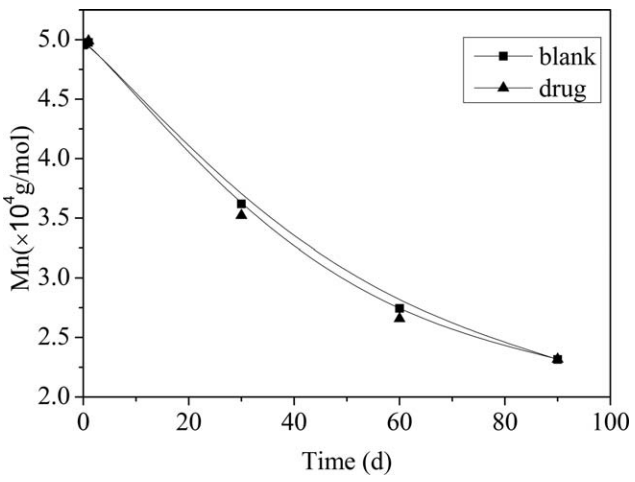


Figure 3. Plot of M_n as a function of degradation time for blank group and drug group.

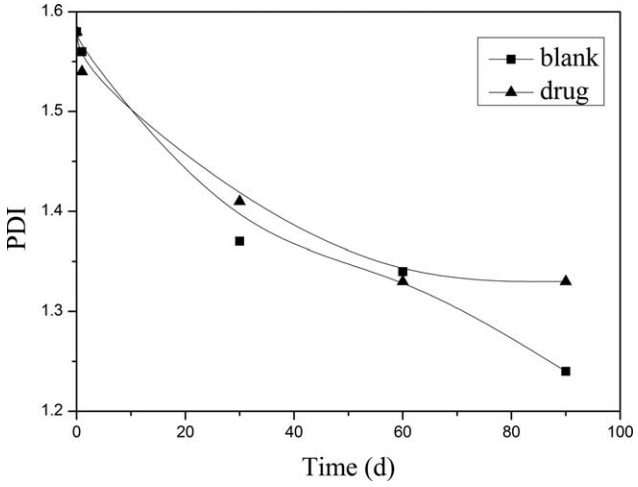


Figure 4. Plot of PDI as a function of degradation time for blank group and drug group.

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PDI values correspond to a conglomerate of chains spanning a wide range of molecular weights.[17] The decrease in PDI indicated a very homogeneous degradation. Therefore, no aggregation of low molecular substances produced by degradation was proved. Rapid absorption and excretion of the low molecular substances occurred in rabbits. In other words, data obtained from PDI indicated that all the gels underwent surface erosion and bulk degradation at the same time.

These degradation behaviors meant that mechanism of copolymer biodegradation *in vivo* was bulk degradation.

Composition Analysis Using ^{1}H NMR

The ^{1}H NMR spectrum of copolymer was recorded in Figure 5. The [CL]/([CL]+[DLLA]) molar ratio percentage was calculated from the intergrations of signal a of PCL at 4.1 ppm and signal f of PDLLA at 5.1 ppm.[18] The initial copolymer used in this study was composed of 84% CL according to the calculation method.

CL ratio of all the gels was investigated. For the two groups, similar tendencies were observed. Composition changes of the two groups were nearly the same. It was concluded that the presence of TU had no effect on degradation rates.

As seen in Figure 6, whether with or without drug, CL ratio of gels increased over degradation time. It was demonstrated that the area of DLLA in the copolymer degraded faster than that of CL. Amorphous area degraded first, which would lead to the result that crystallinity of the copolymer carriers would be increased. For the two groups, the CL ratio increased less than 5% during the degradation time.

Thermo Properties of Blank Group Gels

As seen in Figures 7 and 8, melting temperature decreased initially and then increased over degradation time, which was caused by the low molecular substances produced by degradation and increasing crystallinity.

In the beginning, low molecular substances played the role of plasticizer. The existence of plasticizer would make the melting temperature decrease. Melting temperature of P(DLLA-*co*-CL) was decreased by the presence of low molecular substances as a kind of plasticizer. After 30 days, crystallinity increased because

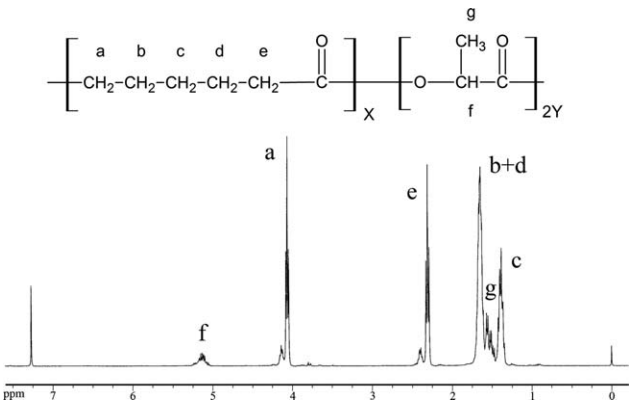

Figure 5. ^{1}H NMR spectrum of P(DLLA-*co*-CL) copolymers.

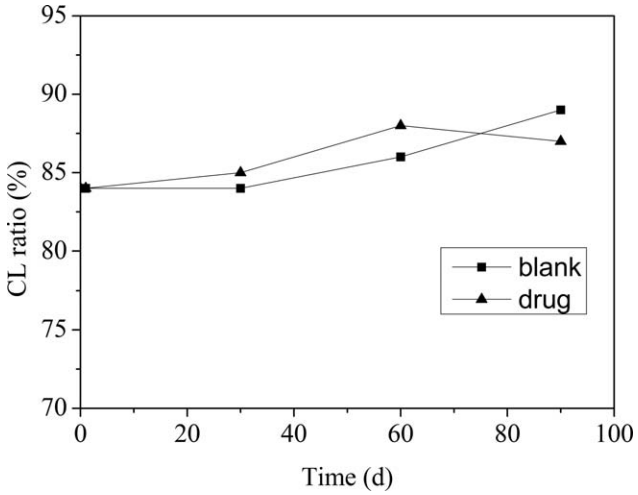

Figure 6. Composition changes of blank group and drug group vs. degradation time.

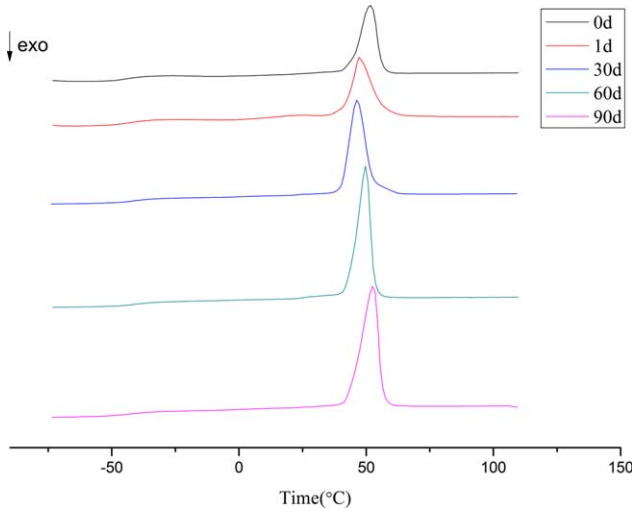

Figure 7. DSC curves for blank group at different time points. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

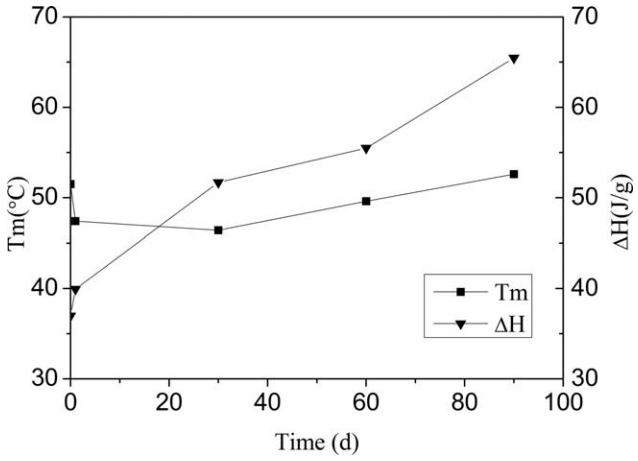

Figure 8. Thermo properties of blank group as a function of degradation time.

the amorphous area was degraded and absorbed first. Subsequently, melting temperature increased. The results showed that the crystallinity was playing a leading role at the later stage and restrained the effect of the low molecular substances. At the same time, crystal structure was better and thickness of lamellae was increased, which caused the expulsion of the plasticizer from crystalline region of copolymer carrier.

Furthermore, the peak area in DSC curve attributing to melting enthalpy (ΔH) increased, which proved that crystallinity was increased gradually.

Low molecular substances and crystallinity played a part in degradation process simultaneously. It was found that, factors playing the leading role were different at different stages of degradation.

For the glass transition temperature (T_g), opposite tendencies to the melting temperature were observed. The results were depicted in Figure 9. The value of T_g increased slightly at early stage and then decreased over degradation. The phenomenon of decrease occurred because of the internal plastication effect of low molecular substances. With the low molecular substances increased, macromolecular chain presented better flexibility and mobility, and intermolecular interaction decreased. With all the structural factors above, T_g decreased after 30 days.

We anticipated that degradation of P(DLLA-*co*-CL) was divided into two stages, respectively, according to the analysis of ^{1}H NMR and DSC. At the initial stage, degradation occurred mainly in the amorphous area because of the inability of water and enzyme to penetrate the crystal zone of the copolymer. At this stage, the random scission of the ester bonds by hydrolysis and enzymatic degradation caused the reduction of the molecular weight. Smaller chains were produced, which were more eager to dissolve and diffuse through the copolymer carriers leading to macromolecule segment rearrangement and increase of crystallinity. Therefore, there would be more free moving space of segments. In the second phase, degradation took place from the edge to the core of the crystal zone while mostly amorphous degraded. However, with higher crystallinity, degradation rate of crystal zone was slower than that of amorphous area.

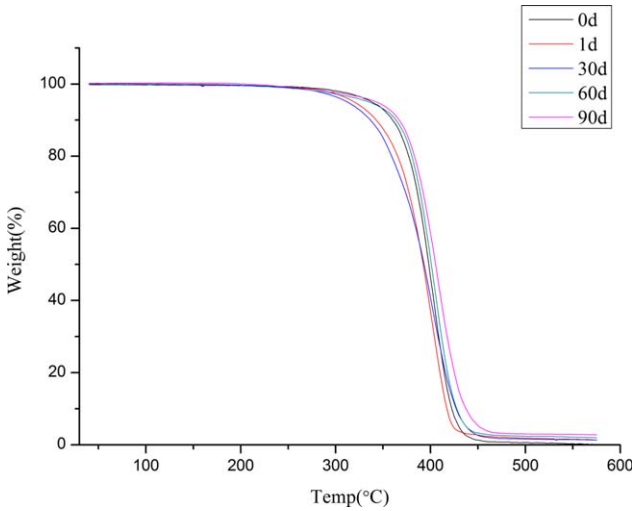

Figure 10. TGA thermograms for blank group at different time points. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Thermogravimetric Analysis of Blank Group Gels

The development of thermal decomposition temperature (T_d) was shown in Figures 10 and 11. It was easier for the macromolecule chain to move when intermolecular interaction was weak, resulted from the presence of small molecules. Hence, thermal decomposition temperature decreased in early days. While crystallinity increased, regular crystal morphology was formed, resulting in thermal decomposition temperature increased in later days. The data were in good accordance with that measured by DSC. According to the results, it was demonstrated that the P(DLLA-*co*-CL) gels had high thermal resistance.

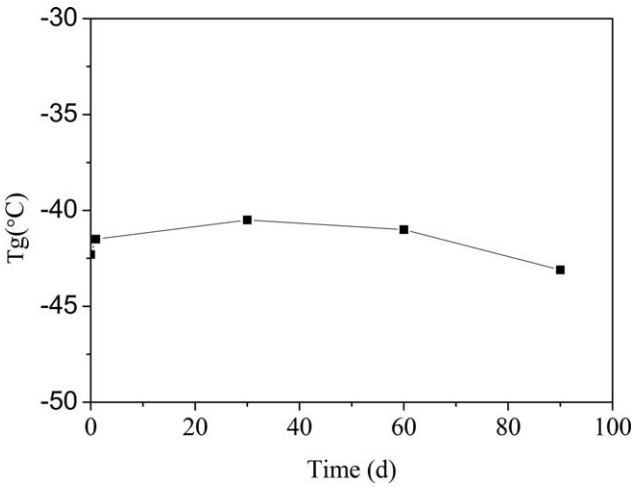

Figure 9. Glass transition temperatures of blank group as a function of degradation time.

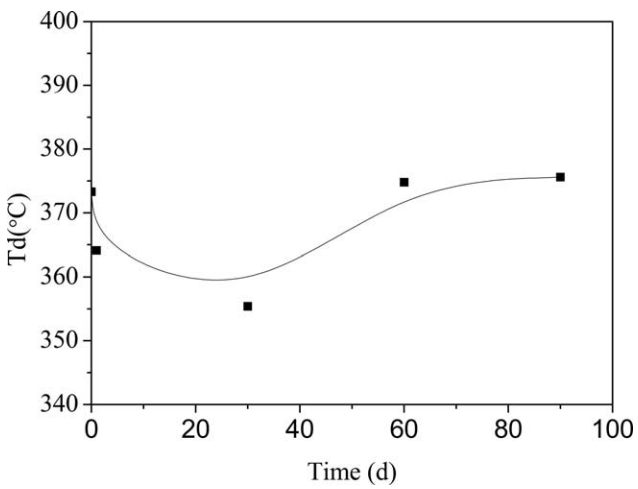

Figure 11. Thermo degradation temperature of blank group against degradation time.

Morphology of Blank Group Gels

Cross section and surface morphology changes were followed by SEM, which was a technique of choice to monitor copolymer degradation. Figure 12 shows the cross-sectional SEM micrographs of blank group gels at predetermined points. One day after injection, multilayer structures were observed in the cross-sectional micrographs. Porous layers structure and some holes were present in the cross-sectional morphology. Degradation process occurred among multilayers. Owing to localizing in the subcutaneous tissue, the gels were enwrapped by a fiber capsule, which can gradually attenuate, and the fiber tissue had the trend of inosculation with the gels. In addition, the gels had undergone solvent-exchanged effect. Therefore, uniform and dense structures were formed, as seen in the 90 days micrograph in Figure 12. Multilayer structures disappeared at last.

Actually, the formation of dense structures was related to the increased crystallinity. Increased crystallinity led to the reduction of degradation rate, so it was easy to form the dense structures.

Surface morphology micrographs were shown in Figure 13. Owing to the penetration and degradation effects of water and lipase, gels took the structure of drape with a few holes in them on the surface. With time elapsing, drape and holes disappeared gradually, and the surface became regular and compact, which indicated that the surface was eroded continuously.

Using cross-sectional morphology analysis in combination with surface morphology analysis, it was confirmed that the surface and inner degradation of copolymers took place simultaneously. Thus, it was revealed that mechanism of copolymer biodegradation *in vivo* was bulk degradation.

In Vivo Release

In vivo release profile of TU from the gels was depicted in Figure 14. The initial release of the third day was very low, and the cumulative release amount was only 2.48%. Figure 14 reveals the release plot from day 3 to day 90 were nearly in line ($R = 0.97$). The initial burst of the gels was very low that could be neglected. The cumulative release amount as shown in Figure 14 was increased from 2.48% to 99% over the period of day 3 to day 90. After 60 days, the release rate became slower.

CONCLUSIONS

In this study, an injectable *in situ*-forming gel was developed by dissolving P(DLLA-*co*-CL) in nontoxic solvent NMP. Gels with porous structures were formed when the prepared solution was injected and came into contact with aqueous body fluid.

The finding that injected gels with or without drugs resulted in no evident inflammation reaction indicated that the degradation products led to minimal inflammatory or none and P(DLLA-*co*-CL) solution can be injected into body as carriers of drug release system.

The gels degraded *in vivo* lost up to 50% in molecular weight in 90 days. Based on the PDI analysis, it can be concluded that no aggregation phenomenon of small molecules took place. These degraded products can be absorbed and excreted by body. Compositional ratio changed 5% for CL. Copolymers had

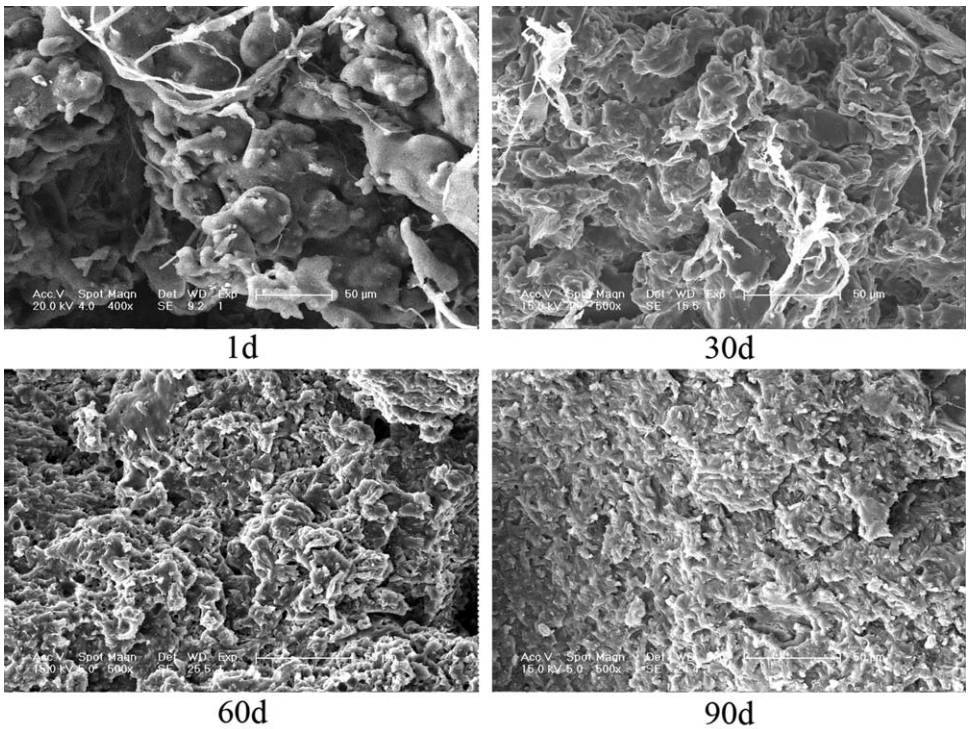

Figure 12. SEM micrographs of cross section of blank group during degradation time.

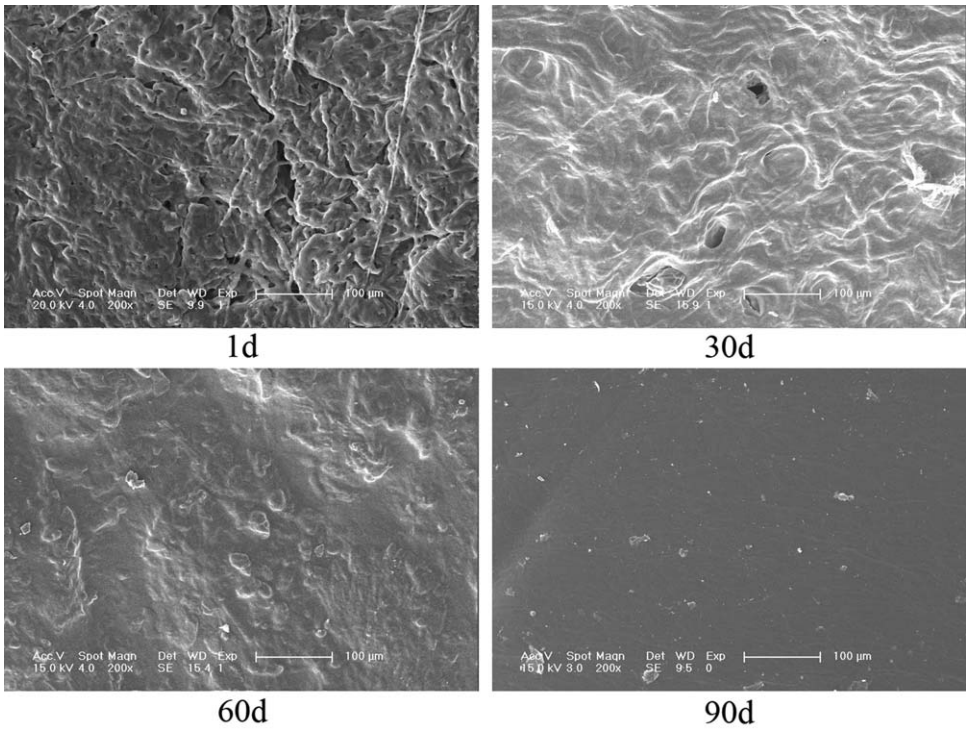

Figure 13. SEM micrographs of surface of blank group during degradation time.

excellent heat resistance. All the analysis of measurements in this study was consistent with each other.

Recognizing that degradation occurred in the surface and cross section, we suggested that mechanism of copolymer biodegradation *in vivo* was bulk degradation. Moreover, with the increasing crystallinity, copolymer degraded slowly, which could greatly eliminate the burst release of drug and was a favor to sustained/controlled release system. Finally, no significant differences were observed between blank group and drug group during degradation process.

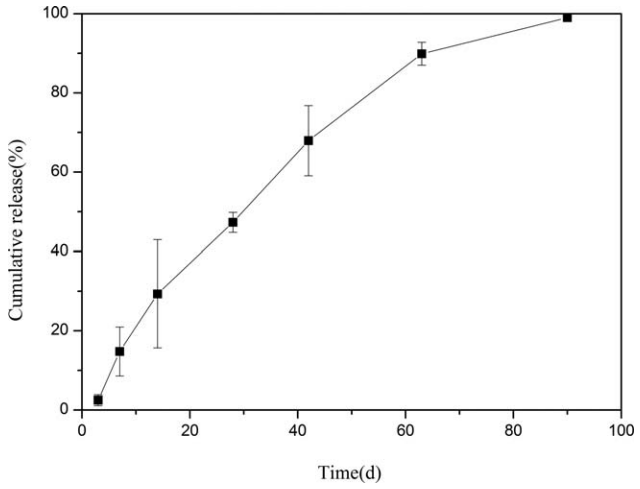

Figure 14. *In vivo* release of TU from P(DLLA-*co*-CL) gels (data represent mean ± SD; $n = 3$).

In addition, the gel could well administrate the release of TU in a sustained way without significant burst features. From our work, the potential prospect of the gel, as a new kind of drug delivery system, can be highly expected.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

Chong Zhang: designer of the experiment, experimenter, paper author, and reviser. Xiaowei Zhang, Ping Wang: experimenter. Dan Yang: experimenter and corrector of the paper.

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