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Biodegradable Thermogel as Culture Matrix of Bone Marrow Mesenchymal Stem Cells for Potential Cartilage Tissue Engineering*

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Abstract Poly(lactide-*co*-glycolide)-poly(ethylene glycol)-poly(lactide-*co*-glycolide) (PLGA-PEG-PLGA) triblock copolymer was synthesized through the ring-opening polymerization of LA and GA with PEG as macroinitiator and stannous octoate as catalyst. The amphiphilic copolymer self-assembled into micelles in aqueous solutions, and formed hydrogels as the increase of temperature at relatively high concentrations (> 15 wt%). The favorable degradability of the hydrogel was confirmed by *in vitro* and *in vivo* degradation experiments. The good cellular and tissular compatibilities of the thermogel were demonstrated. The excellent adhesion and proliferation of bone marrow mesenchymal stem cells endowed PLGA-PEG-PLGA thermogelling hydrogel with fascinating prospect for cartilage tissue engineering.

Keywords: Bone marrow mesenchymal stem cell; Cartilage defect repair; Scaffold; Thermogel.

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INTRODUCTION

The treatment of cartilage defects is a difficult question faced by the orthopedic surgeons all over the world. Cartilage defects can cause joint pain and dysfunction, and later develop into osteoarthritis[1]. Bone marrow mesenchymal stem cells (BMMSCs), which can differentiate into chondrocytes, are ideal repair cells for cartilage regeneration[2]. The traditional treatment methods, such as drilling[3] and microfracture[4], aim to introduce BMMSCs from the underlying bone marrow. However, the regenerated cartilage tissues are usually different from the normal hyaline cartilage[5]. It is mainly due to the abnormal chondrogenesis of BMMSCs from the bone marrow *in vivo*.

As far as we know, BMMSCs can be induced to undergo chondrogenesis and secrete cartilage-specific extracellular matrix (ECM) in a variety of three dimensional (3D) hydrogels[2, 6]. In addition, 3D hydrogels can provide a better link between single cells and tissues compared with conventional two dimensional (2D) culture[7]. Therefore, the researchers need to find a suitable matrix to support BMMSCs. In recent years, the

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injectable thermogels as promising biomedical materials have attracted more and more attention. These materials have been widely used in many biomedical fields, such as drug delivery, tissue engineering, wound healing and cell therapy[8–10]. They exhibit many advantages such as simple operation and minimal injury in the application. The thermogels are porous 3D scaffolds, which provide necessary space for the survivals and proliferations of cells. In addition, their injectable performances are extremely convenient for application. Currently, several biodegradable and biocompatible thermogelling hydrogels based on synthetic polymers, such as aliphatic polyesters, have been prepared for cartilage tissue engineering. For instance, the chondrocytes could well adhere and proliferate on the methoxy poly(ethylene glycol)-poly(ε-caprolactone) thermogels[11]. Autogenic chondrocytes loaded with PLGA-PEG thermogels were used to repair rabbit articular cartilage, and the basically satisfied result was obtained[12].

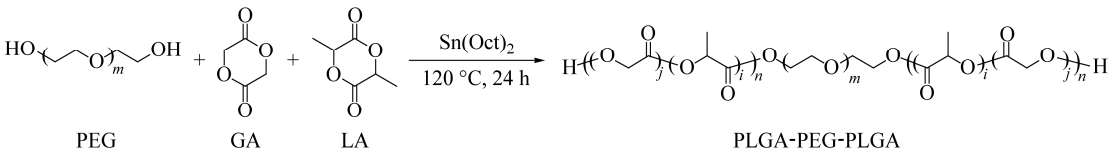
Poly(lactide-*co*-glycolide)-poly(ethylene glycol)-poly(lactide-*co*-glycolide) (PLGA-PEG-PLGA) triblock copolymer is another potential thermogel. It is liquid at a lower temperature (such as 4 °C), while transforms into gel at a higher temperature (such as body temperature, 37 °C)[13]. The gelling mechanism of this copolymer is a delicate balance between hydrophilic and hydrophobic segments due to micelle aggregation[14]. It is a kind of fantabulous biomedical materials and shows a lot of advantages, such as ease of preparation in a large amount, thermo-responsiveness, biocompatibility and biodegradability. In addition, the study about whether BMMSCs can grow well in PLGA-PEG-PLGA thermogel would lay a foundation for the further cartilage repair.

In this study, a PLGA-PEG-PLGA triblock copolymer was synthesized through the ring-opening polymerization (ROP) of LA and GA with PEG as macroinitiator and stannous octoate ($Sn(Oct)_2$) as catalyst. The chemical structure and composition were confirmed by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) and gel permeation chromatography (GPC). The vial of inversion method and rheological experiment studied the properties of gelation. The biodegradability and biocompatibility of the PLGA-PEG-PLGA thermogel were confirmed by *in vitro* and *in vivo* degradation assays, and methyl thiazolyl tetrazolium (MTT) and pathological analyses, respectively. The viabilities and proliferations of BMMSCs in the gel were evaluated by mixing cells with copolymer solution in phosphate-buffered saline (PBS, 0.01 mol·L^{-1}, pH 7.4) (20 wt%) at 4 °C and gelatin at 37 °C. The excellent adhesion and proliferation of BMMSCs in the gel indicated the great prospect of PLGA-PEG-PLGA thermogel for potential cartilage tissue engineering.

EXPERIMENTAL

Materials

PEG (M_n = 1000 g·mol^{-1}) was purchased from Sigma-Aldrich (St Louis, MO, USA), PLGA-PEG-PLGA triblock copolymer ($M_{n,\,NMR}$ = 4200 g·mol^{-1}, LA/GA = 75:25, mol/mol) was synthesized *via* the similar approach reported in our previous work (Scheme 1)[15, 16]. MTT and cell counting kit-8 (CCK-8) were purchased from Sigma-Aldrich and Kabuskiki Kaisha (Dojindo, Japan), respectively. Both elastase and proteinase K were obtained from Merck Company (Darmstadt, Germany). All the other reagents and solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and used without further purification.



Scheme 1 Synthesis pathway of PLGA-PEG-PLGA copolymer

Characterizations

^{1}H-NMR spectrum

^{1}H-NMR measurement was performed on a Bruker 400 MHz ^{1}H-NMR spectrometer (Ettlingen, Germany) in deuterated chloroform ($CDCl_3$) to ascertain the chemical structure and composition of copolymer.

Fourier transform infrared (FTIR) spectra

The original copolymer and lyophilized specimen after gelation were characterized by FTIR analysis on a Bio-Rad Win-IR instrument (Cambridge, MA, USA) using a potassium bromide method.

Gel permeation chromatography (GPC)

Number and weight-average molecular weights (M_n and M_w), and molecular weight distribution (polydispersity index, PDI = M_w/M_n) were determined by GPC using a series of linear Styragel columns (HT3 and HT4) and a Waters 515 HPLC pump, with OPTILAB DSP Interferometric Refractometer (Wyatt Technology Corporation, Santa Barbara, CA, USA) as detector. The eluent was chloroform at a flow rate of 1.0 mL·min^{-1} at 40 °C. Monodispersed polystyrene standards were used to generate the calibration curve that were purchased from Waters Co., Ltd. (Milford, MA, USA) with a molecular weight range of 1790 – (4.0×10^5) g·mol^{-1}.

Critical micelle concentration (CMC) measurements

The CMC was determined by fluorescence spectroscopy using pyrene as fluorescence probe on a Photon Technology International (PTI) Fluorescence Master System with software Felix 4.1.0 (PTI, Lawrenceville, NJ, USA, λ_{em} = 390 nm). The CMC was obtained from the intersection of the tangent to the horizontal line of I_{338}/I_{335} with relative constant values and the diagonal line with rapidly increased I_{338}/I_{335} ratio.

Morphology and size of copolymer micelle characterization

Transmission electron microscope (TEM) (JEM-1011, JEOL, Tokyo, Japan) was used to measure the morphology of the copolymer micelle. A drop of copolymer solution in PBS (0.5 wt%) was dripped to the copper net coated with carbon, and then dried at room temperature before measurements. Dynamic laser scattering (DLS) measurements were carried out on a WyattQELS instrument with a vertically polarized He-Ne laser (DAWN EOS, Wyatt Technology Corporation, Santa Barbara, CA, USA). The scattering angle was fixed at 90°.

Sol-Gel Transitions

The sol-gel transition temperature was determined *via* the vial inverting approach. Each sample with a given concentration was prepared by dissolving the copolymer in MilliQ water or PBS in a 2.0 mL vial and stored at 4 °C for about 3 days. With a temperature build-up of 1 K, the sample was regarded as a "gel" if no visual flow within 30 s after inverting the vial. The tests were carried out in triplicate.

Dynamic Mechanical Analysis

Rheological experiments were performed on a US 302 rheometer (Anton Paar Firma, Graz, Austria). The copolymer solution in MilliQ water or PBS (20.0 wt%) was placed between parallel plates of 25 mm diameter and a gap of 0.5 mm. To prevent the evaporation of water, a layer of oil was put around the copolymer samples. The data were collected under a controlled strain of 1% and a frequency of 1.0 rad·s^{-1}. The heating rate is 0.5 K·min^{-1}. The storage modulus (G'), dissipative modulus (G'') and viscosity (η) were obtained in oscillatory shear flow.

In Vitro Gel Duration

PLGA-PEG-PLGA hydrogels were obtained by incubating 0.5 mL copolymer solutions in PBS (20.0 wt%) within different vials (diameter = 16 mm) at 37 °C for 30 min. Tris-HCl buffer solution (0.05 mol·L^{-1}, pH 7.4) containing 0.2 g·L^{-1} elastase, 10.0 mmol·L^{-1} $CaCl_2$ and 0.2 wt% NaN_3 was used as the degradation media, and hydrogels incubated in Tris-HCl buffer containing 0.2 g·L^{-1} proteinase K and PBS were used as controls. The Tris-HCl buffer and PBS (3.0 mL) were added on top of the gel. The whole medium was replaced every two days, and the mass of the remaining gel was measured. The experiments were performed in triplicate.

Animal Procedure

Sprague-Dawley (SD) rats (male, 200 ± 20 g) and Chinese white rabbit (about 2 months) were supplied by the Experimental Animal Center of Jilin University. All the experiments on animals were approved by the Animal Care and Use Committee of Second Hospital, Jilin University.

In Vivo Gel Degradation and Biocompatibility

SD rats were used for the *in vivo* testing. Rats were anesthetized by inhalation of diethyl ether, and 0.5 mL of PLGA-PEG-PLGA solution in PBS (20.0 wt%) was subcutaneously injected into dorsal area of rat with a 21-gauge needle. At predetermined intervals, rats were sacrificed for the photographs collection.

At each time interval after transplantation, the tissues near the removed gels were immediately collected and fixed with paraformaldehyde. The embedded specimens were sectioned along the longitudinal axis of implant, and the sections were stained with hematoxylin and eosin (H&E) through the standard procedure for the evaluation of the inflammatory responses of hydrogels in rats.

BMMSCs Isolation and Expansion

The intercondylar notch of the left distal femur of rabbit was exposed, and 2.0 mL of blood was aspirated from the bone marrow using an 18-gauge needle attached to a 5 mL syringe containing 0.3 mL of heparin (1000.0 units per mL). The aspirate was washed twice with PBS and centrifuged at 1800 $r \cdot min^{-1}$ for 5 min. The obtained cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL, Grand Island, New York, USA) containing 10% (*V*/*V*) fetal bovine serum, 50.0 $IU \cdot mL^{-1}$ penicillin and 50.0 IU mL^{-1} streptomycin at 37 °C in a 75 cm^2 culture flask with 5% (*V*/*V*) carbon dioxide. The medium was changed on the fourth day of culture and then twice per week.

Cytotoxicity Measurements

The relative cytotoxicity of copolymer was assessed by a MTT viability assay. The rabbit BMMSCs were seeded in 96-well plate at ~7000 cells per well in 100.0 μL of complete DMEM, cultured for 24 h and followed by removing culture medium and adding copolymer solutions (100.0 μL in complete DMEM medium) at different concentrations (0.016 to 1.0 $g \cdot L^{-1}$). The PEI25k was used as positive control. The cells were subjected to MTT assay after being incubated for another 72 h. The absorbances of media were measured at 492 nm on a Bio-Rad 680 microplate reader. Cell viability (%) was calculated according to the following equation: Cell viability (%) = $(A_{sample}/A_{control}) \times 100\%$, where A_{sample} and $A_{control}$ were the absorbances of sample and control wells, respectively. The measurements were performed in triplicate.

Scanning Electron Microscope (SEM)

The network structure of PLGA-PEG-PLGA hydrogels was observed by a SEM (JXA-840, JEOL, Japan). The specimen was prepared through the following procedure: the PLGA-PEG-PLGA copolymer solution in PBS (20.0 wt%) formed into a gel at 37 °C for about 30 min; the gel was frozen at −196 °C in sub-cooled liquid nitrogen and freeze-fractured to remove superficial frosting artefacts; and then the specimen was coated with a conductive layer of sputtered gold. The micrograph was taken at an accelerating voltage of 20 kV in order to ensure a suitable microimage resolution.

Cellular Adhesion and Proliferation Assays

In order to observe the ability of the cellular adhesion, after being sterilized by UV irradiation for 30 min at 4 °C, the copolymer solution in PBS (200.0 μL, 20.0 wt%) was first added into a 24-well plate and formed into gels in the thermostat at 37 °C, and then BMMSCs were seeded to the plate. Tissue culture plastic (TCP) without hydrogel (blank well) was set as positive control.

For the cellular proliferation assays in the hydrogel, the PLGA-PEG-PLGA solution (200.0 μL, 20.0 wt%) was mixed with 5×10^4 harvested cells at 20 °C and maintained at 37 °C for 20 min in 24-well culture plates to form cell-encapsulating hydrogel. DMEM (1.0 mL) was added on the top of hydrogel and the medium was replaced every 3 days by a fresh medium.

The amounts of cells on the surface of or inside the hydrogels were determined using CCK-8. The cellular adhesion and proliferation assays were performed with the same operation as follows: 1) After the predetermined period, the incubation medium was removed and the wells were washed three times with PBS; 2) 1.0 mL of CCK-8 solution in DMEM (10%, *V*/*V*) was added to each well; 3) after incubation for given time, the absorbance value at 450 nm was measured with an ELISA reader (Model 550; Bio-Rad, Hercules, CA, U.S.A.), and the absorbance at 600 nm was used for baseline correction.

RESULTS AND DISCUSSION

Synthesis and Characterization of Triblock Copolymer

PLGA-PEG-PLGA triblock copolymer was synthesized through random ROP of LA and GA using PEG as macroinitiator and $Sn(Oct)_2$ as catalyst. The chemical structure and composition of copolymer were confirmed by ^{1}H-NMR and FTIR spectra, and GPC chromatograms (Fig. S1, Supporting Information). As shown in Fig. S1A (Supporting Information), all signals in the ^{1}H-NMR spectrum were well assigned, which demonstrated the successful synthesis of copolymer. The M_n of PLGA block and LA/GA (mol/mol) were calculated to be 1600 g·mol^{-1} and 75:25, respectively, and were listed in Table 1. The results of FTIR (Fig. S1B, Supporting Information) also demonstrated the generation of PLGA blocks based on the increased strength of carbonyl absorption at 1728 cm^{-1} ($\upsilon_{C=O}$) attributed to ester bond. The waveform of lyophilized thermogel sample was similar with the former copolymer specimen, which indicated that the chemical structure unchanged before and after the gelation. In addition, the PDI of copolymer was tested to be 1.4 by GPC chromatogram (Fig. S1C, Supporting Information), and is listed in Table 1.

Table 1. Characterization of PLGA-PEG-PLGA copolymer and micelle

Entry	$M_{n,\ PLGA/PEG/PLGA}$[a]	LA/GA[a]	M_n[b]	PDI[b]	CMC (mg·L^{-1})	R_h (nm)
Copolymer	1600/1000/1600	75/25	7900	1.4	6.6	32.1 ± 6.9

[a] Determined by ^{1}H-NMR; [b] Determined by GPC

Self-assembly Behavior of Block Copolymer

The amphiphilic PLGA-PEG-PLGA copolymer can self-assemble into micellar nanoparticles in aqueous solutions prompted by microphase separation that is induced by the selective solvent[17]. CMC was used to assess the physical property of micelle, and determined by a widely reported pyrene-probe-based fluorescence technique in this study. The CMC was obtained to be 6.6 mg·L^{-1} from the pyrene fluorescence intensity ratio of I_{338}/I_{335} versus logarithmic concentration (lg*c*) of copolymer (Fig. 1a)[18].

The typical TEM micrograph of copolymer micelles in PBS at pH 7.4, 25 °C is showed in Fig. 1(b). It revealed that the micelles took a spherical morphology with the respective average radius of around 20 nm. In addition, the micellar size was quantitatively determined by DLS in PBS at pH 7.4, 25 °C. The results are shown in Fig. 1(c) and Table 1. The hydrodynamic radius (R_h) of micelles was (32.1 ± 6.9) nm. The smaller apparent radius from TEM observation as compared to that determined by DLS should be due to the dehydration of micelles in the process of TEM sample preparation[19, 20].

Macroscopic Phase Behavior of Copolymers and Mechanism Analysis

As shown in Fig. 2, the sol-gel phase diagrams of PLGA-PEG-PLGA copolymer in MilliQ water and PBS at different concentrations were revealed, and the physical statuses of copolymer solution and hydrogel in PBS (20 wt%) were observed at 4 °C and 37 °C, respectively (Inset). When the temperature increased from 4 °C to 70 °C, the copolymer system underwent three stages: solution, opaque hydrogel and solution or suspension. Compared with the system in MilliQ water, the copolymer in PBS presented a lower critical gelation temperature (CGT), which was better for fast gelation after injection. It also indicated that the presence of saline could reduce the CGT. The CGT of copolymer in PBS (20 wt%) was between room temperature (*i.e.*, 20 °C) and body temperature (*i.e.*, 37 °C). Therefore, it was suitable as an injectable hydrogel for tissue engineering, and was used in the following experiments of this study.

Rheological tests were performed to assess the changes of G' and η versus the increase of temperature. Both G' and η of copolymer in PBS were higher than those of copolymer in MilliQ water (Figs. 3a and 3b). The CGTs measured by rheological tests were just slightly lower than those from phase diagrams, therefore the two results were corroborated each other. In addition, the G' of copolymer in PBS is up to about 3.0 kPa as the increase of temperature, which highlighted the potential of this material as an injectable hydrogel for cartilage tissue engineering.

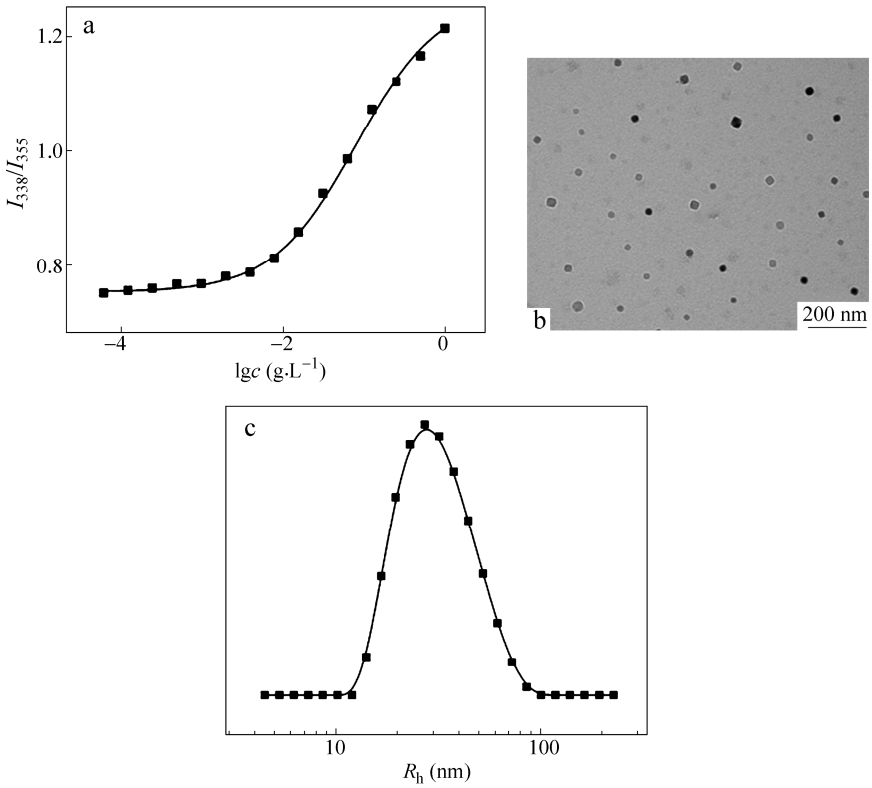


Fig. 1 The intensity ratio (I_{338}/I_{335}) as a function of logarithmic concentration of PLGA-PEG-PLGA copolymer (a); Typical TEM micrograph (b) and R_h (c) of PLGA-PEG-PLGA micelles in PBS (0.5 wt%)

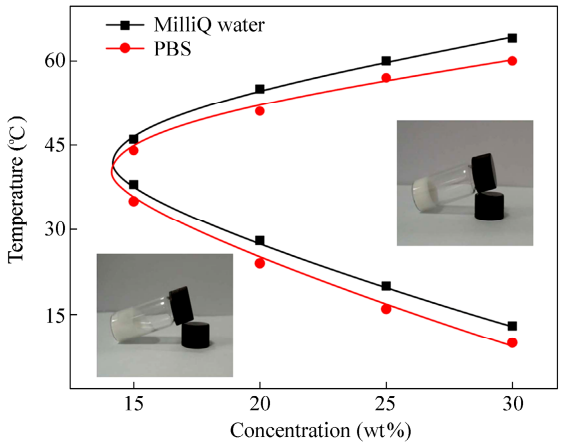


Fig. 2 Sol-gel phase diagrams of PLGA-PEG-PLGA copolymer in MilliQ water and PBS, and photographs of copolymer solution and hydrogel at 20 wt% in PBS (Inset)

The gelation mechanism of PLGA-PEG-PLGA copolymer was confirmed by DLS. In order to investigate the change of copolymer aggregation behavior, the change in micelle radius and its distribution in PBS at 0.5 wt% were revealed to be as a function of temperature (Fig. 4). In a temperature range from 10 °C to 20 °C, the average radius of copolymer micelle was around 30 nm. The micelle radius notably increased to ~147 nm with a broad distribution as the temperature increased to 30 °C, which indicated that the micelle began to form larger aggregation (Scheme 2). At above 40 °C, significant increases in both the radius and its distribution of the

aggregates were observed. The change trends of micelle radius and its distribution were consistent with the results of other relative studies, and indicated that the aggregation between micelles emerged during the sol-gel transition[14].

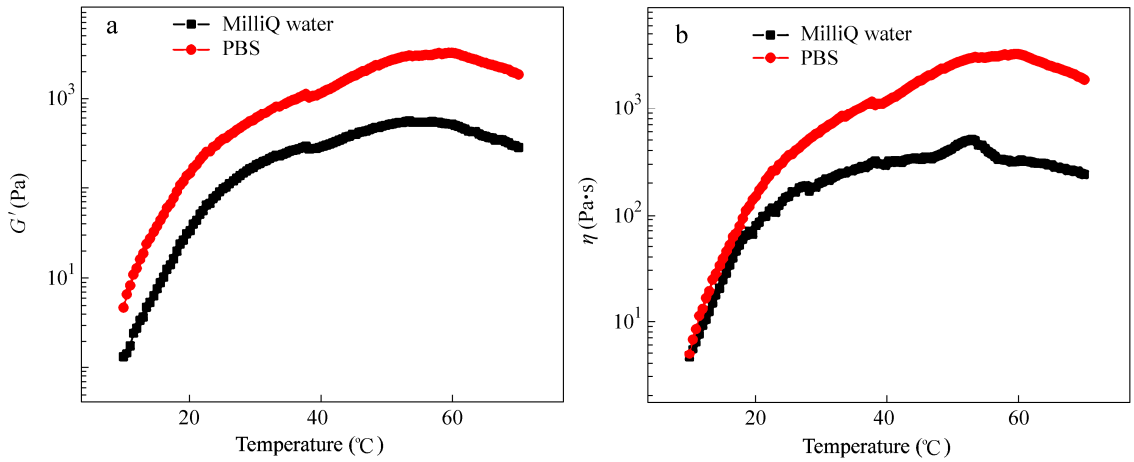


Fig. 3 G' (a) and η (b) of PLGA-PEG-PLGA copolymer in PBS (20 wt%)

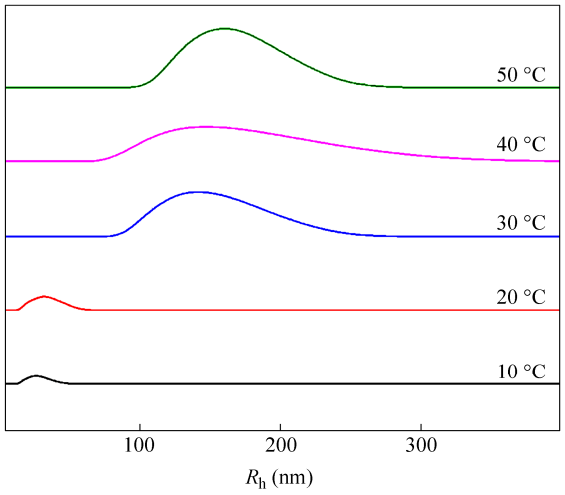
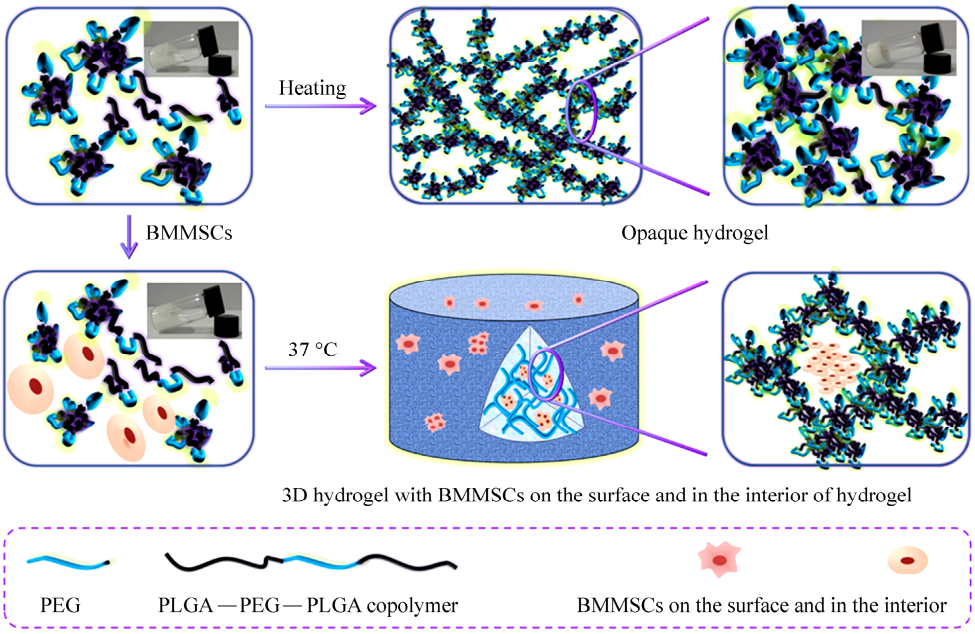


Fig. 4 Distributions of R_h of PLGA-PEG-PLGA micelles in PBS (0.5 wt%) at different temperatures

Degradation and Biocompatibility of PLGA-PEG-PLGA Hydrogel

The degradation of material is one of the important factors for biomedical applications. Therefore, the *in vitro* and *in vivo* degradation tests were performed to investigate the degradation behavior of the hydrogel in this work. Tris-HCl buffer containing 0.2 $g \cdot L^{-1}$ elastase was used to simulate the *in vivo* microenvironment, and Tris-HCl buffer containing 0.2 $g \cdot L^{-1}$ proteinase K and PBS were used as controls. The degradation rate of copolymer hydrogel exposed to proteinase K was faster (complete degradation in 16 days) than that in elastase medium (complete degradation in 52 days) as proteinase K could cause degradation of ester bond of polyester efficiently (Fig. 5)[21]. The degradation rate of copolymer in PBS is the lowest, which still remain more than 70% after 70 days.



Scheme 2 Schematic presentation of a spontaneous thermogelling mechanism of PLGA-PEG-PLGA copolymer in water through a micelle network, and the preparation of 3D hydrogel with BMMSCs on the surface or in the interior of hydrogel

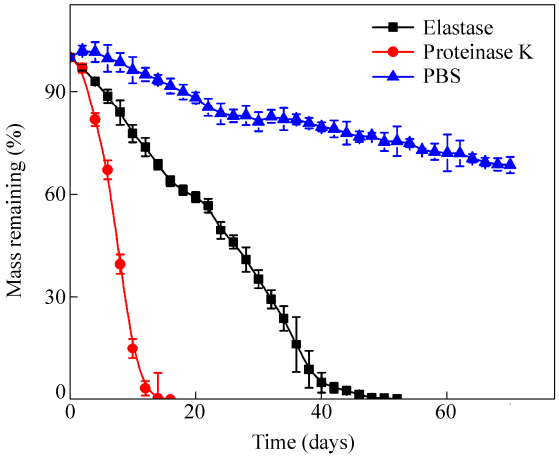


Fig. 5 *In vitro* mass loss profiles of PLGA-PEG-PLGA hydrogel (20 wt%) at 37 °C soaked in 3.0 mL of Tris-HCl buffer containing 0.2 $g \cdot L^{-1}$ elastase or proteinase K, PBS (Data were presented as mean ± standard deviation (n = 3).)

As shown in Fig. 6, the photographs recorded the shape and amount of hydrogel at the predetermined time. For *in vivo* degradation, the hydrogel little reduced until 14 days, and it was almost eliminated at 42 days. The *in vivo* degradation rate of hydrogel was slightly faster than that in elastase medium for *in vitro* degradation. It suggested that the *in vivo* microenvironment is complex, there are other enzymes or factors that accelerate the degradation besides elastase[22].

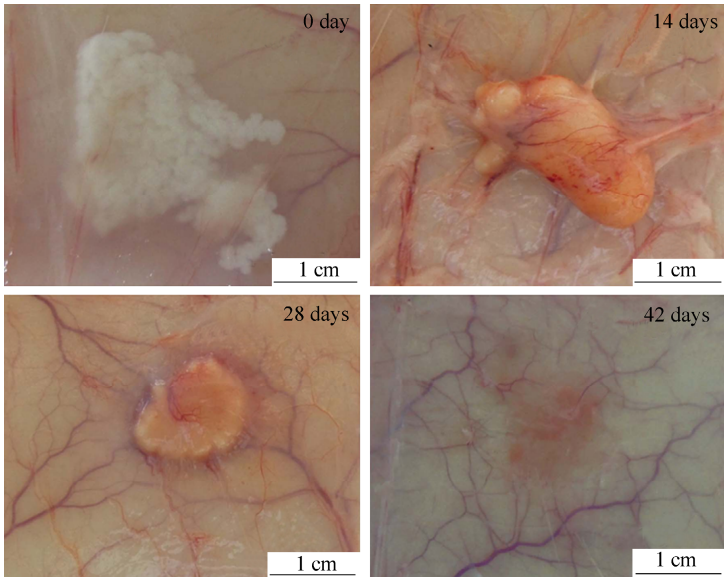


Fig. 6 *In vivo* gel maintenance for *in situ* formed PLGA-PEG-PLGA hydrogel after subcutaneous injection of copolymer solutions into rats (20 wt%) (Photographs were taken at 0 (30 min), 14, 28 and 42 days, respectively.)

A standard MTT assay was performed to assess the *in vitro* cytotoxicity of the copolymer. The viabilities of BMMSCs (Fig. 7) toward PLGA-PEG-PLGA copolymer at different concentrations remained more than 90% up to 0.5 $g \cdot L^{-1}$ after incubation for 72 h. The cytotoxicity of copolymer was remarkably lower than that of positive control (PEI25k), which revealed a good biocompatibility.

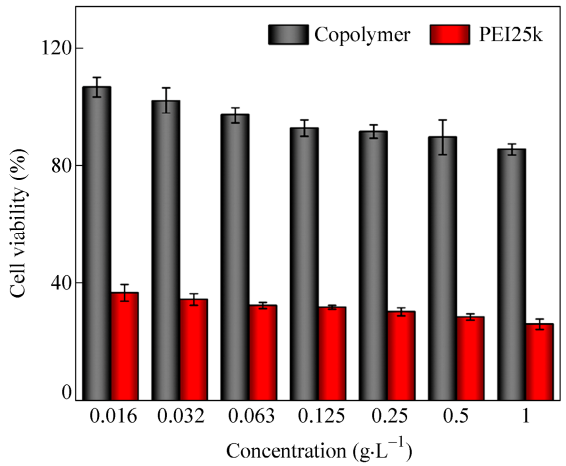


Fig. 7 *In vitro* cytotoxicities of BMMSCs toward the PLGA-PEG-PLGA copolymer solutions at different concentrations (0.016–1.0 $g \cdot L^{-1}$) after incubation for 72 h (Data were presented as mean ± standard deviation ($n = 3$).)

To observe the *in vivo* biocompatibility and the inflammatory response, the H&E-stained subcutaneous tissue surrounding the gel was observed after injection for 0 (30 min), 7, 14 and 42 days. As shown in Fig. 8, an acute inflammation around the surrounding tissue reached a peak at 7 days, and the visual field was filled with granulocytes. The granulocytes significantly decreased, and the macrophages and lymphocytes increased at 14 days. It indicated that the acute inflammatory reaction turned into chronic inflammation. The macrophages and lymphocytes also decreased at 42 days, which revealed the excellent *in vivo* biocompatibility of hydrogel.

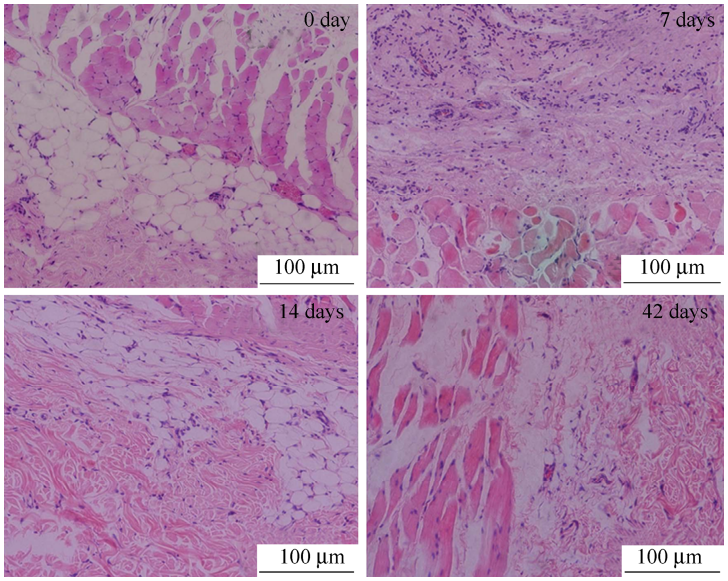


Fig. 8 Tissue biocompatibility of *in situ* formed PLGA-PEG-PLGA hydrogel after subcutaneous injection of copolymer solutions for 0 (30 min), 7, 14 and 42 days

Cellular Adhesion and Proliferation toward PLGA-PEG-PLGA Hydrogel

The surface and internal morphology of PLGA-PEG-PLGA hydrogel was observed by SEM (Fig. 9a). It illustrated that the hydrogel had an interconnected porous network structure. The cellular adhesion assays were conducted to determine whether the cells could adhere on the surface of hydrogel or not. In this study, the adhesions of BMMSCs on the surface of PLGA-PEG-PLGA hydrogel were investigated at 6, 12 and 24 h after being seeded on the hydrogel (Fig. 9b). The relative adhesion rates of BMMSCs were above 50%. The result indicated that PLGA-PEG-PLGA hydrogel could be a good substrate to support the culture of BMMSCs[23], and it showed a good adhesion and biocompatibility toward BMMSCs.

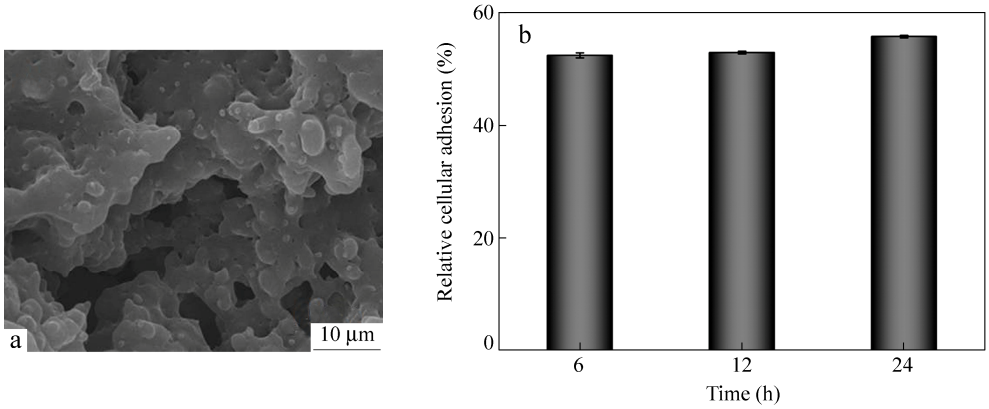


Fig. 9 (a) Surface morphology of PLGA-PEG-PLGA hydrogel (20 wt%); (b) Adhesions of BMMSCs on the surface of PLGA-PEG-PLGA hydrogel with TCP as control (Data were presented as mean ± standard deviation (n = 3).)

To explore whether the PLGA-PEG-PLGA-based porous hydrogel could provide the space for cellular growth, the cellular proliferation ability test was performed. BMMSCs were embedded in PLGA-PEG-PLGA hydrogel and incubated for 1, 7, 14 and 21 days. The cells exhibited a good gradual proliferation in PLGA-PEG-PLGA hydrogel up to 21 days (Fig. 10). At the beginning 7 days, the relative proliferations of BMMSCs in thermogel were relatively slow, and showed less than 1 times increase of cellular number in relative to that of the

first days, while the cellular proliferations of control group were as high as 6 to 7 times. The reason is that fewer cells are directly attached to the fiber surfaces in hydrogel[24]. From 14 days, the cells in the hydrogel presented rapid proliferation. However, at 21 days, the cellular proliferations of the cells in PLGA-PEG-PLGA hydrogel were higher than those of control group. It was because that the PLGA-PEG-PLGA hydrogel provide a suitable 3D scaffold for the growth of BMMSCs. This study aimed at the synthesis and characterization of PLGA-PEG-PLGA triblock copolymer that could be used as an injectable and biodegradable material, and the BMMSCs could adhere on its surface and proliferate in it. The preliminary results obtained in this work provided the important data for the potential cartilage tissue engineering application of PLGA-PEG-PLGA hydrogel in the next stage. The long-term cartilage repair results will be reported in the near future.

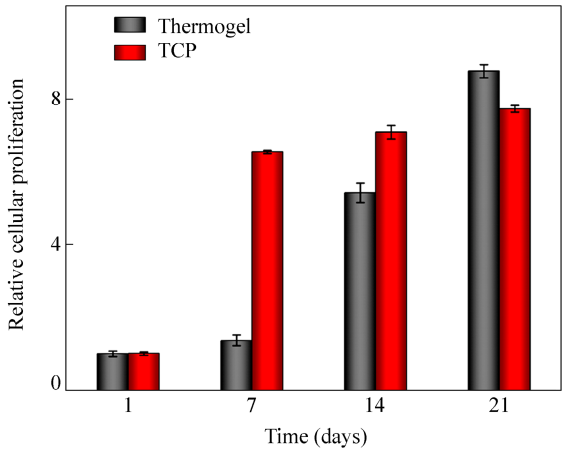


Fig. 10 Relative proliferations of BMMSCs in the interior of PLGA-PEG-PLGA hydrogel cultured *in vitro* for 1, 7, 14 and 21 days with TCP as control (Data were presented as mean ± standard deviation (n = 3).)

CONCLUSIONS

The PLGA-PEG-PLGA triblock copolymer with exact chemical structure was synthesized through the random ROP of LA and GA using PEG as macroinitiator and $Sn(Oct)_2$ as catalyst. The amphiphilic copolymer self-assembled into spherical micellar nanoparticles in PBS with R_h at (32.1 ± 6.9) nm. The copolymer in aqueous solution with an appropriate concentration (>15 wt%) could form hydrogel as the increase of temperature. The CGT of copolymer in PBS (20 wt%) was between 20 °C and 37 °C (body temperature), and the G' of that is up to about 3.0 kPa, both of which indicated its good prospect as an injectable hydrogel for tissue engineering. The thermogel exhibited appropriate *in vitro* (~52 days) and *in vivo* biodegradability (~42 days), and good biocompatible properties toward cells and tissues. In this work, the thermogelling PLGA-PEG-PLGA hydrogel was firstly employed to support the excellent adhesion and proliferation of BMMSCs. Therefore, PLGA-PEG-PLGA thermogel can be used as a potential injectable biomaterials used in tissue engineering, such as cartilage defect repair.

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