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A poly(glycerol–sebacate–curcumin) polymer with potential use for brain gliomas

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Abstract: Curcumin has multiple biological and pharmacological activities, including antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, and antitumor activities. However, the clinical use of curcumin is limited because of its poor oral absorption and extremely poor bioavailability. In order to overcome these limitations, we conjugate curcumin chemically into the known biocompatible and biodegradable polymer, poly(glycerol–sebacate), and prepare the unitary poly(glycerol–sebacate–curcumin) polymer. The structure, the *in vitro* degradation, the drug release, and antitumor activity as well as the *in vivo* degradation and tissue biocompatibility of poly(glycerol–sebacate–curcumin) polymer are investigated. The *in vitro* degradation and drug release profile of poly(glycerol–sebacate–curcumin) are in a linear manner. The *in vitro* antitumor assay shows that poly(glycerol–sebacate–curcumin) polymer significantly inhibits human malignant glioma cells, U87 and T98 cells. In view of the cytotoxicity against brain gliomas, local use of this polymer would be a potential method for brain tumors. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 101A: 253–260, 2013.

Key Words: curcumin, tumor, poly(glycerol–sebacate) polymer, poly(glycerol–sebacate–curcumin) polymer, antitumor

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INTRODUCTION

Curcumin, a polyphenolic natural compound from the curry spice turmeric, has a wide range of biological and pharmacological activities, for instance, antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, and antitumor activities.[1] The antitumor activities of curcumin have been extensively studied. It is reported that curcumin modulates growth of tumor cells through regulation of multiple cell signaling pathways including cell proliferation pathway, cell survival pathway, caspase activation pathway, tumor suppressor pathway, death receptor pathway, mitochondrial pathways, and protein kinase pathway (JNK, Akt, and AMPK).[2] In the field of brain tumor study, curcumin has been shown to suppress human glioblastoma cells[3] and human glioma.[4] Purkayastha et al.[5] show that intracerebral injection through a cannula blocks brain tumor formation in mice that have already received an intracerebral bolus of mouse melanoma cells (B16F10), indicating that the *in vivo* effect of curcumin against brain tumor is practicable if the way of curcumin delivery is proper.

Although curcumin shows antitumor activity and has been proved pharmacologically safe in many clinical studies and various animal models, the clinical application of curcumin is restricted because of its poorly oral absorption and extremely poor bioavailability.[6] In order to overcome these limitations, several approaches have been attempted, for instance, curcumin was loaded in liposomes[7,8] or nanoparticles.[9,10] Our previous works find that poly(glycerol–sebacate) (PGS), the biocompatible and biodegradable polymer, is an ideal drug delivery carrier[11] and can modify the drug release pattern.[12,13] Curcumin is a polyphenol; we hypothesize that the phenolic hydroxyl groups in curcumin structure could conjugate chemically with carboxyl groups in sebacate acid, thereby, curcumin, glycerol, and sebacic acid should polycondense to form a unitary polymer. In view of the antibrain tumor activity, this polymer will provide a better treatment for brain tumor when locally used. In the present work, we characterized the poly(glycerol–sebacate–curcumin) polymer and studied the *in vitro* antibrain tumor activity of poly(glycerol–sebacate–curcumin) polymer.

MATERIALS AND METHODS

Preparation of poly(glycerol–sebacate–curcumin) polymer

The starting materials used for reaction were sebacic acid (2*N* purity), glycerol (2*N* purity), and curcumin (95.7%

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TABLE I. Raw Material Composition of the Samples

Samples	Composition (Mole Ratio) [(Sebacic Acid)/ (Glycerol)/(Curcumin)]
PGS	1.2:1.0:0
PGS–curcumin-1	1.2:1.0:0.041
PGS–curcumin-2	1.2:1.0:0.082
PGS–curcumin-3	1.2:1.0:0.164
PGS–curcumin-4	1.2:1.0:0.246
PGS–curcumin-5	1.2:1.0:0.328

purity; Hebei Food Additive, China). The prepolymers were prepared by simultaneous addition of the certain amounts of sebacic acid, glycerol, and curcumin monomers into a flask with sebacic acid and glycerol at mole ratio of 1.2:1 and different concentration of curcumin. Under nitrogen surroundings, the reactants in the flask were heated and kept at 185°C for 1 h, and then the reaction pressure was reduced slowly to vacuum, kept the vacuum till the reactant was viscous and no bubble occurred; the cooled prepolymer was placed in vacuum drying chamber at 185°C for 24 h. Then, the poly(sebacic acid–glycerol–curcumin) polymer was produced. All the samples were transparent. For comparison, the PGS was synthesized at 170°C.

Characterization of poly(glycerol–sebacate–curcumin) polymer

Infrared (IR) spectroscopy was obtained with FTIR spectrometer (spectrum 100; Perkin Elmer). X-ray diffraction (XRD) spectra were carried out with a rotating anode x-ray diffractometer (Rigaku-D/max-γb, Rigaku, Japan) at the conditions of Cu Kα, 50 kV, and 40 mA. The surface morphologies of degraded samples were visualized with scanning electron microscopy (SEM; XL30, Philips) after Au spraying treatment on the surface. Water contact angles were measured by the Contact Angle Measurement System (OCA200, Dataphysics, Germany) from the average value of three positions per sample by randomly selection.

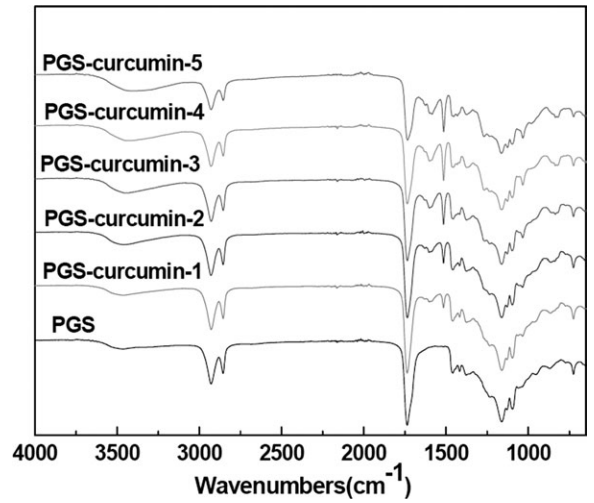

FIGURE 2. The IR spectra of poly(glycerol–sebacate–curcumin) polymers.

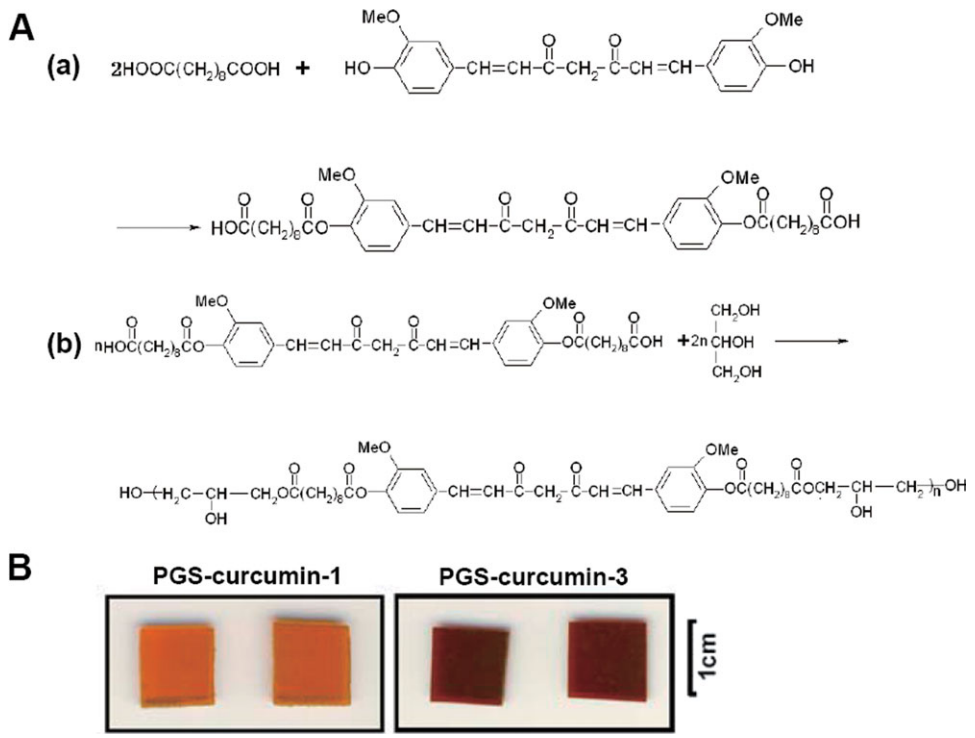

FIGURE 1. A: The chemical reaction formula for synthesis of poly(glycerol–sebacate–curcumin) polymer. B: The representative photographs of poly(glycerol–sebacate–curcumin) polymers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

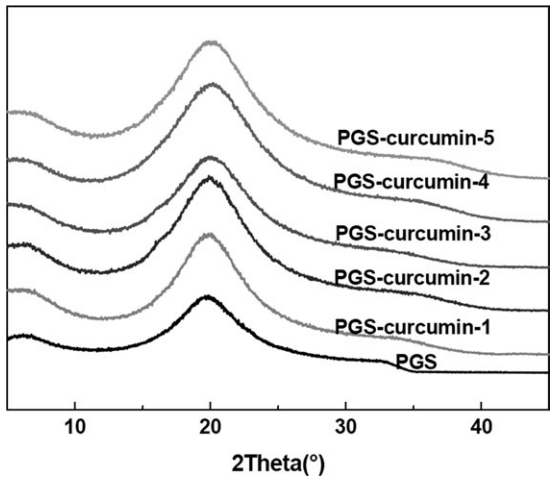

FIGURE 3. The XRD spectra of poly(glycerol–sebacate–curcumin) polymers.

Water absorption test

The sample wafers ($\Phi 10 \times 2$ mm^2) weighed as M_1 were dipped in deionized water for 24 h. Then, the sample weighed as M_2 after the water on the sample surfaces was absorbed, and the increased weigh percent $[(M_2 - M_1)/M_1 \times 100\%]$ was calculated as water absorption. All the data were obtained from the average value of three samples.

Sol contents and swelling capacities test

The sample wafers ($\Phi 10 \times 2$ mm^2) weighed as M_1 were dipped in tetrahydrofuran for 24 h. The sample weighed as M_2 after the tetrahydrofuran on the sample surfaces was absorbed, then the samples were dried in vacuum at 37°C to stable weigh M_3, the sol contents were calculated as $(M_1 - M_3)/M_1 \times 100\%$, and swelling capacities of the gel were calculated as $(M_2 - M_3)/M_3 \times 100\%$. All the data were obtained from the average value of three samples.

Mechanical properties

The tensile tests were conducted using electromechanical universal testing machine (Instron-3365; Instron) with a loading rate of 10 mm/min. Dumbbell shaped samples have effective dimensions of $16 \times 4 \times 0.3$ mm^3. All the experimental data were obtained from the average value of three samples.

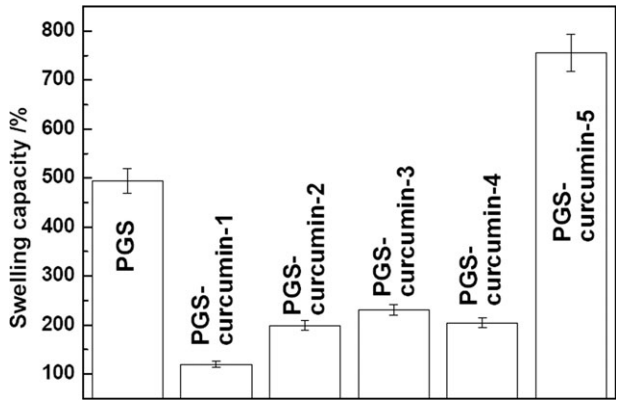

FIGURE 5. The gel swelling capacities of poly(glycerol–sebacate–curcumin) polymers.

Degradation test *in vitro*

The poly(sebacic acid–glycerol–curcumin) wafers accurately weighed were immersed in 10 mL phosphate buffer solution (PBS, pH 7.4, 0.01*M*) and thermostated at 37°C. The PBS was withdrawn and renewed at a certain incubation time; meanwhile, the wafers were taken out, washed with distilled water, and dried under vacuum at room temperature until constant weight was obtained. The dried samples were weighed and the mass loss was presented as initial weight-subtracted normalized to initial weight.

Curcumin release test by ultraviolet-visible spectrophotometer

The concentrations of the curcumin solutions prepared for the standard curve are 5, 10, 20, 40, 60, 80, and 100 μ*M* by UV spectrophotometer (Shimadzu UV-2550, Japan). The standard linear equation is $y = 261.79x + 26.54$, $r = 0.99641$, at the absorption wavelength of 425 nm. The released curcumin amount was calculated according to the standard curve.

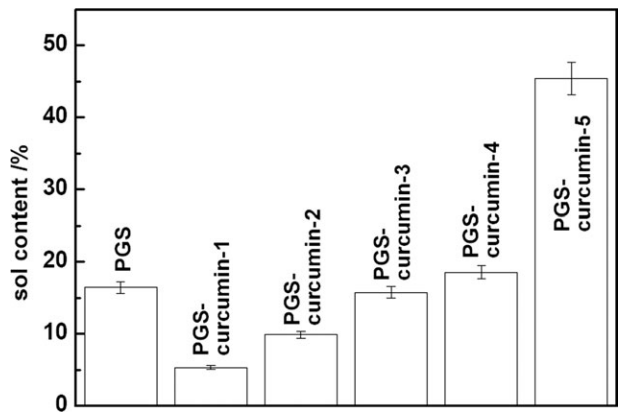

FIGURE 4. The sol content of poly(glycerol–sebacate–curcumin) polymers.

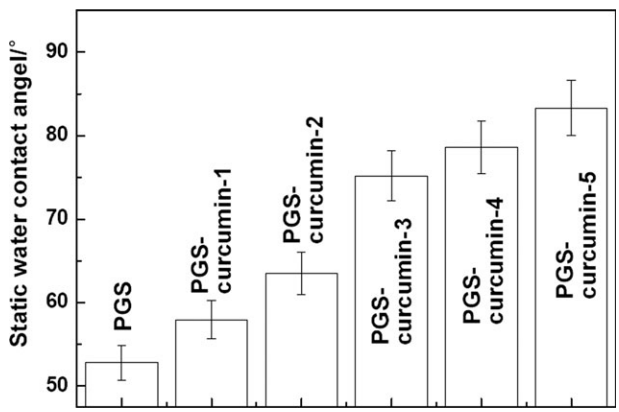

FIGURE 6. The static water contact angles of poly(glycerol–sebacate–curcumin) polymers in air.

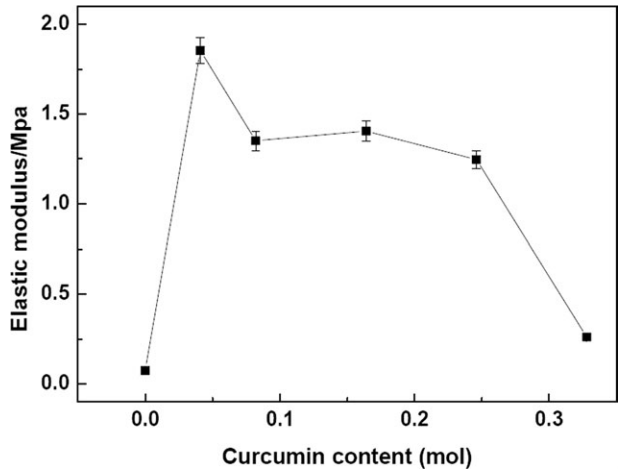

FIGURE 7. The elastic modulus of poly(glycerol–sebacate–curcumin) polymers.

Cell culture and cell viability assay

U87 cells and T98 cells were grown as a monolayer culture at 37°C in a humidified atmosphere of 5% CO_2 and 95% air in standard culture medium, consisting of Dulbecco's Modified Eagle's Medium (Hyclone products) supplemented with 10% fetal bovine serum. Viability of cells cultured in the 96-well culture plates was assessed by measuring mitochondrial dehydrogenase activity, using the colorimetric MTT assay, based on the fact that viable cells (but not dead cells) can reduce MTT.

Live and dead cells staining

U87 cells and T98 cells were seeded in 35 mm × 10 mm cell culture dishes and incubated at 37°C overnight. Cells were then incubated with a 10 × 5 × 2 mm^3 poly(sebacic acid–glycerol–curcumin) polymer for 48 h. The cells were washed three times with PBS and stained with calcein-AM and ethidium bromide to assess cell viability, according to the manufacturer's instruction (Molecular Probes). The live and dead cells were stained green and red, respectively.

In vivo tissue response

The detail information was described in our previous work.[11] Briefly, Wistar rats (200–250 g) were anesthetized with pentobarbital and implanted with poly(sebacic acid–glycerol–curcumin) polymer (10 × 5 × 2 mm^3) samples intramuscularly on one side of the backbone. A sham operation on the other side of the backbone was performed as control. Rats were sacrificed on postoperative days 5 and 20, and the poly(sebacic acid–glycerol–curcumin) polymer samples were explanted along with the surrounding muscle tissues. The tissues were fixed in 10% formalin and then stained using the standard protocol for hematoxylin and eosin histological analysis. All the experimental procedures were approved by the Institutional Animal Care and Use Committee of Harbin Medical University, China, for animal experiments.

RESULTS

Preparation of poly(glycerol–sebacate–curcumin) polymer

The reactant compositions for preparing poly(glycerol–sebacate–curcumin) polymers were as shown in Table I. The reaction equation and the photographs of PGS–curcumin-1 and PGS–curcumin-3 samples were as shown in Figure 1.

Characterizations of poly(glycerol–sebacate–curcumin) polymers

Comparing the IR spectra of the samples undoped and doped with curcumin (Fig. 2), there are two new absorptions at 1510 and 1600 cm^{-1} in the samples doped with curcumin, and with the doped content of curcumin increasing, the absorption intensities increased. The absorption peaks at 1510 and 1600 cm^{-1} are the stretch libration absorption of carbonyl in phenolic ester, and the increase of the intensities was attributed to the content increase of phenolic ester in the samples.

As shown in Figure 3, the samples undoped and doped with curcumin showed similar XRD spectra, which could be attributed to the similar microstructure of amorphous states. The doped curcumin and the concentration of doped curcumin had unobvious influence on the microstructure of the poly(sebacic acid–glycerol–curcumin) samples.

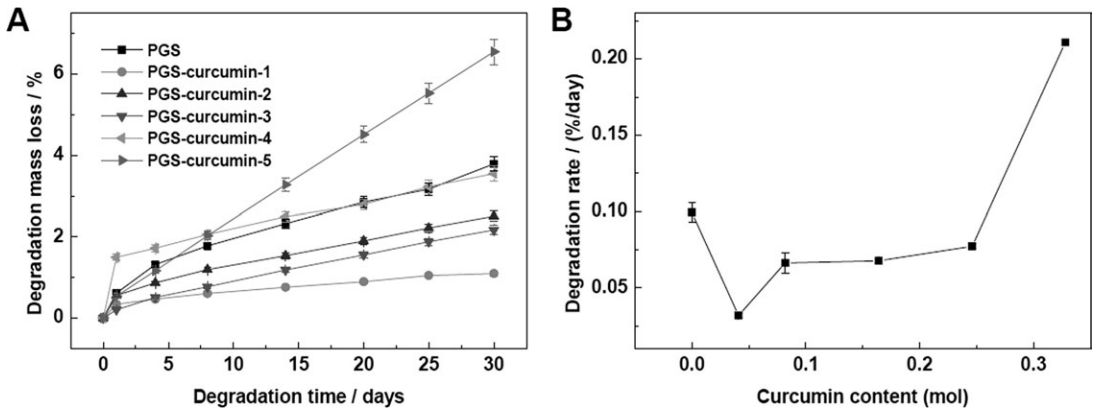

FIGURE 8. The degradation of poly(glycerol–sebacate–curcumin) polymers *in vitro*. A: The time course of degradation of poly(glycerol–sebacate–curcumin) polymers. B: The degradation rate of poly(glycerol–sebacate–curcumin) polymers.

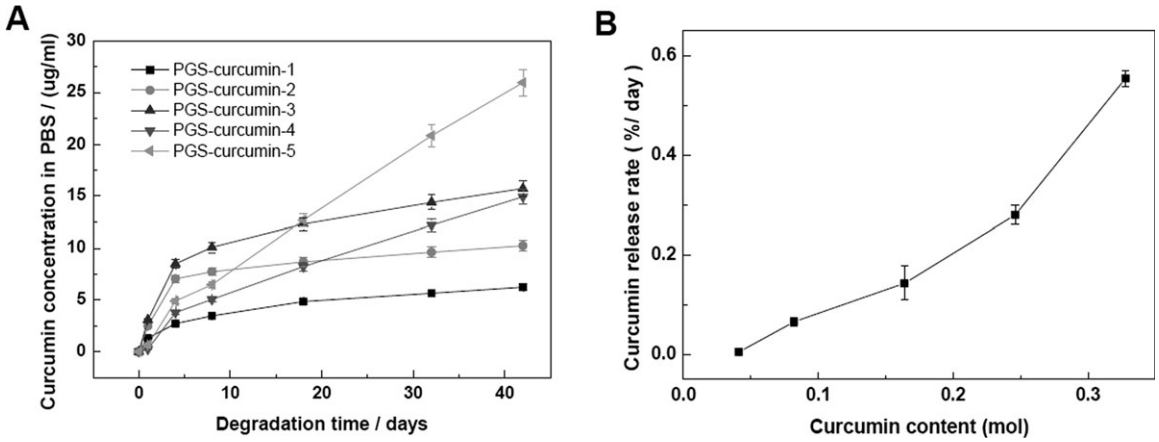

FIGURE 9. Curcumin release of poly(glycerol–sebacate–curcumin) polymers *in vitro*. A: The released curcumin concentration in PBS during degradation process. B: The curcumin release rate of poly(glycerol–sebacate–curcumin) polymers.

As shown in Figures 4 and 5, the less doped content of curcumin samples, PGS–curcumin-1, PGS–curcumin-2, and PGS–curcumin-3, contained less sol content and showed lower swelling capacities than undoped sample of PGS; the possible reason was the higher reaction temperature for the samples doped with curcumin than for the undoped sample of PGS, and the higher reaction temperature made the degree of polymerization increase. For samples doped with curcumin, with the doping content increasing, the sol content and swelling capacities tended to increase. It could be attributed to the chemical structure of the curcumin, which had two functional group of phenol hydroxide radical reacting with sebacic acid. Relative to three functional groups of hydroxide radical in glycerol reacting with sebacic acid, two functional groups would decrease the crosslink densities; moreover, relative to glycerol, the molecular structure of curcumin ($C_{21}H_{20}O_6$) was much larger and more complicated than that of glycerol. Hence, curcumin molecular structure could hinder from esterification reaction. The swelling capacity of PGS–curcumin-4 was lower than that of PGS–curcumin-3, the possible reason being the reaction condition changed by accident, which would be studied further.

As shown in Figure 6, the static water contact angles of the samples ranged from 57° to 86°, and with the increasing of curcumin content, the water contact angle increased. The increased water contact angle could be attributed to the increased hydrophobic group contents of benzene ring and ethylene linkage in curcumin.

As shown in Figure 7, relative to undoped sample PGS, the elastic modulus of the samples doped with curcumin were higher than PGS, and with doped curcumin increasing, the elastic modulus decreased. As described before, the samples doped with curcumin were with higher crosslink densities, which made the elastic modulus of the doped curcumin samples be higher; with the doped curcumin content increased, the crosslink densities decreased, which made the elastic modulus to decrease. In addition, the change of chemical bond from alcohol ester bond to phenol ester bond also had influence on the elastic modulus.

The degradation and curcumin release of poly(glycerol–sebacate–curcumin) polymers *in vitro*

The degradation curves of poly(sebacic acid–glycerol–curcumin) samples were as shown in Figure 8(A). The degradation rate at 0–3-day period is fastest in the 30-day degradation period, and then, the degradation rate almost kept constant. The faster early degradation rate could be attributed to part of unreacted reactant monomer mixed in the polymer samples, which would be solved and diffused at the first degradation period. The degradation rate in 4–30 days period is shown in Figure 8(B). As we know, the lower crosslink density of PGS made the degradation rate increase; with the curcumin content increasing, the decreased crosslink density made the degradation rate to increase.

The curcumin concentration in PBS during degradation process of poly(sebacic acid–glycerol–curcumin) samples was as shown in Figure 9(A). The curcumin releasing rate during 0–3-day period was the maximum in the 42-day degradation, and then, the curcumin releasing rate almost kept constant. The faster early curcumin releasing rate could be attributed to part of unreacted curcumin mixed in the polymer samples, which would be solved and diffused at the first degradation period. The curcumin releasing rate during 4–42 days period is shown in Figure 9(B). With the curcumin content increasing, the curcumin releasing rate increased significantly.

The SEM observation

As shown in Figure 10, with the curcumin content increasing, the corrosion pits on the surface tended to be more serious. The possible reason was that the molecule structure of curcumin was larger and more complex than sebacic acid and glycerol, which could hinder the esterification during the reaction process. With the curcumin content increasing, the unreacted reactant (sebacic acid, glycerol, and curcumin) was more and began to agglomerate in the polymers. At the beginning of degradation, the agglomerated unreacted reactant dissolved and diffused, which made the more corrosion pits, and with the degradation time increasing, the pits degraded and became more larger. Moreover,

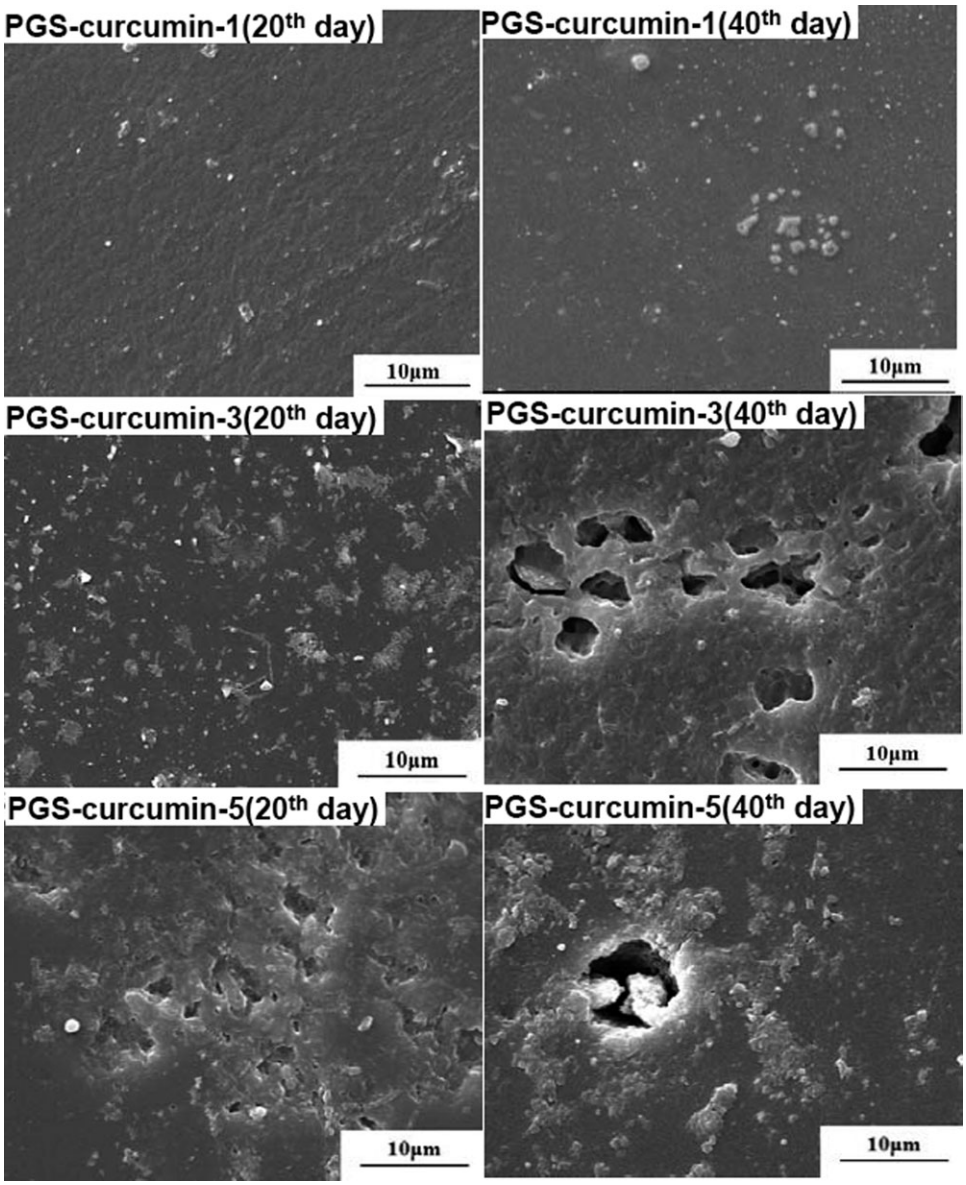

FIGURE 10. The SEM observations of poly(glycerol–sebacate–curcumin) polymers surfaces at different degradation time points.

the increased degradation rate and curcumin releasing rate also made the corrosion pits be more serious.

Antitumor activity of poly(glycerol–sebacate–curcumin) polymers

The evaluation of cytotoxicity of curcumin on human malignant glioma cells T98 and U87 cells is given in Table II. The *in vitro* cytotoxic results for PGS–curcumin-3 sample are shown in Figure 11. Sterilized PGS or PGS–curcumin-3 sample samples ($10 \times 5 \times 2$ mm^3) were floated in the medium for 48 h as described in our previous work.[14] The live cells were stained in green and the dead cells were stained in red. PGS–curcumin-3 significantly inhibited both T98 and U87 cells. The negative control PGS showed no cytotoxic effect on 98 and U87 cells, but the tumor cells were killed by PGS–curcumin-3 (Fig. 11).

Tissue response *in vivo*

In order to evaluate the tissue response *in vivo*, all PGS–curcumin samples were sterilized by Co-60 radiation before implantation. PGS–curcumin samples had similar tissue responses during the implantation period. A typical histological analysis of sham and PGS–curcumin-3 on days 5 and 20 after implantation is shown in Figure 12 (the polymer is indicated with arrow). The tissues surrounding the PGS–curcumin implants were mildly infiltrated by lymphocytic cells.

TABLE II. The Cytotoxicity of Curcumin on Human Malignant Glioma Cells, T98 and U87 Cells

	IC_{50} (48 h) (μg/mL)	Maximal Inhibition (%) (48 h)
T98 cells	20.2	87
U87 cells	23.2	80

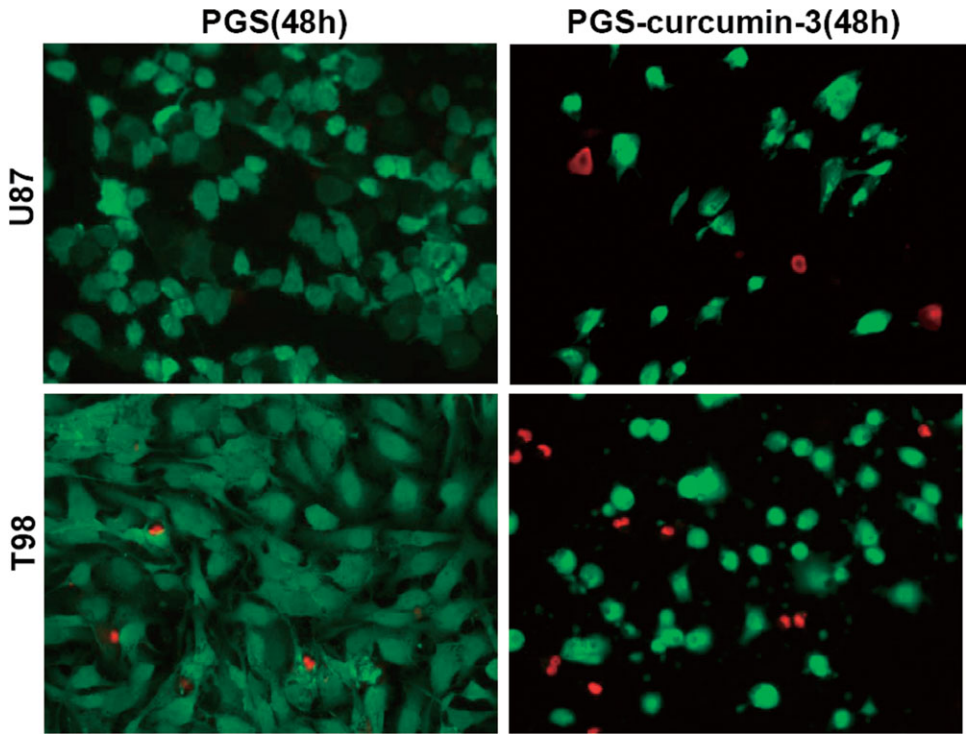

FIGURE 11. The photographs of U87 and T98 cells exposed to PGS–curcumin-3 for 48 h. PGS was used as a control. The live cells were stained in green and the dead cells were stained in red. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

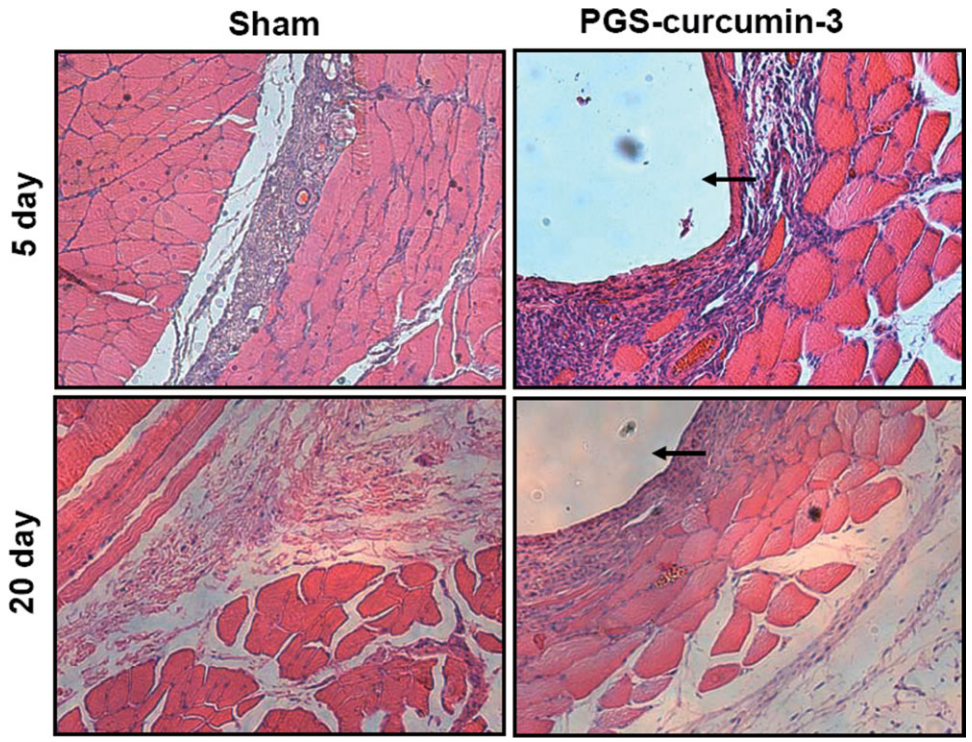

FIGURE 12. Photomicrographs (100×) of hematoxylin and eosin sections of the adjacent tissue after PGS and poly(glycerol–sebacate–curcumin) polymer implantation. Interface surfaces are denoted by arrows. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

DISCUSSION

Malignant glioma is the most common type of primary brain tumor in adults. Treatment for brain gliomas includes surgery, radiation therapy, and chemotherapy. In view of the characterization that brain gliomas is recurrent and does not tend to be metastatic, local drug delivery might be a better treatment way. Presently, carmustine-loaded polymers (Gliadel® Wafer) are commonly used locally for brain gliomas after surgery.[15] Enlightened by the Gliadel Wafer, we designed the poly(glycerol–sebacate–curcumin) polymers.

Curcumin is a polyphenolic natural compound and shows inhibitory effects on human glioblastoma cells. PGS polymer is biocompatible and biodegradable[16] and has been proven to be an ideal drug delivery carrier[11]; therefore, based on the fact that the phenolic hydroxyl group in curcumin structure could conjugate chemically with sebacate, we combined the unitary polymer, poly(glycerol–sebacate–curcumin) polymers. The *in vitro* degradation and drug release profile of poly(glycerol–sebacate–curcumin) polymer are in a linear pattern. The *in vitro* antitumor assay shows that poly(glycerol–sebacate–curcumin) polymer significantly inhibits human malignant glioma cells, U87 and T98 cells. The constant long-time drug release is more suitable for glioma treatment.

CONCLUSIONS

Based on the fact that the phenolic hydroxyl group in curcumin structure could conjugate chemically with sebacate, we combined the unitary polymer, poly(glycerol–sebacate–curcumin) polymers. Poly(glycerol–sebacate–curcumin) polymer inhibits human malignant glioma cells and showed constant, linear curcumin release pattern. We suggest that local use of this polymer would be a potential method for brain tumors.

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