Modulating stability and mechanical properties of silica–gelatin hybrid by incorporating epoxy-terminated polydimethylsiloxane oligomer

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ABSTRACT: Silica-gelatin hybrids, particularly GT-G hybrids prepared by crosslinking gelatin (G) with γ-glycidoxypropyltrimethoxysilane (GT), have attracted much attention in tissue engineering for diverse applications in hard or soft tissue regeneration; however, scaffolds with tunable properties are needed to meet specific requirements. In this work, a silica-gelatin hybrid (ES/GT-G) was synthesized by incorporating epoxy-terminated polydimethylsiloxane oligomer (ES) to modulate the properties of GT-G hybrid. The ES/GT-G hybrid sponge presented a 3D network structure with porosity 86.4% ± 0.9%, determined by the liquid displacement method, and average pore size 340 ± 36 μm, determined by SEM observation. Compared with GT-G hybrid material, the prepared ES/GT-G hybrid wet film showed a decrease of tensile strength from 2.79 ± 0.04 MPa to 1.87 ± 0.12 MPa, with an increase of elongation at break from 19.96 ± 0.66% to 29.86 ± 0.87%, and the ES/GT-G hybrid sponge exhibited a decline of compressive yield strength from 1.21 ± 0.04 MPa to 0.72 ± 0.06 MPa, based on the tensile and compression tests respectively. The introduction of ES enhanced the thermal denaturing temperature of GT-G by 5°C as determined by a DSC study, and increased *in vitro* biodegradation slightly, without significantly changing surface wettability and swelling behavior. These findings suggest that silica-gelatin hybrids with tunable properties are promising for applications from hard to soft tissue regeneration. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 43059.

INTRODUCTION

Organic-inorganic hybrid materials, which are usually prepared by combining organic polymer with an inorganic component at the molecular or nanometer level,[1,2] have been extensively evaluated as scaffolds in recent years since they can mimic the structure and properties of native extracellular matrix (ECM) for tissue formation.[3] Some natural biopolymers have been used to form the organic phase of hybrid materials such as chitosan, soybean, collagen, gelatin, etc.[4–6] Among them, gelatin is attractive since it is derived from collagen, a main constituent of the ECM of all tissues, and is recognizable and adhered to by many cell types. It may serve as a scaffold without immunogenicity concerns, and possesses major advantages with respect to biodegradability, biocompatibility, and high versatility.[7] Generally, the choice of artificial tissue scaffold requires consideration of several parameters, including mechanical properties relative to those of the host tissue, adequate porosity and pore size, controlled and matched biodegradability, and biocompatibility.[8] Some drawbacks, however, such as dissimilar mechanical

properties and the ready biodegradability of pure gelatin hinder its application in tissue engineering. Various physical and chemical methods have therefore been developed to crosslink/hybridize gelatin to improve its physicochemical properties.[6,9–11] Among them, formation of a silica-gelatin hybrid[12,13] is an effective strategy for the purpose of biomedical applications.

According to previous studies,[6,12,14] silica-gelatin hybrids are generally prepared using γ-glycidoxypropyltrimethoxysilane (GT) as a coupling agent, which covalently bonds amino groups on the gelatin chains via its epoxy groups, while forming a crosslinked network by hydrolysis of Si-OCH$_3$ to Si-OH with further condensation. Most recently, some researchers have intensively investigated modulating the micro/nano structure, mechanical behaviors, biological functions, and other properties of this silica-gelatin hybrid system to achieve effectiveness in tissue engineering applications. Reported methods included treating GT-gelatin hybrid in an ammonium hydroxide solution to obtain biomimetic anisotropic porous structure[15] and changing curing temperature and the ratio of GT to gelatin to control the
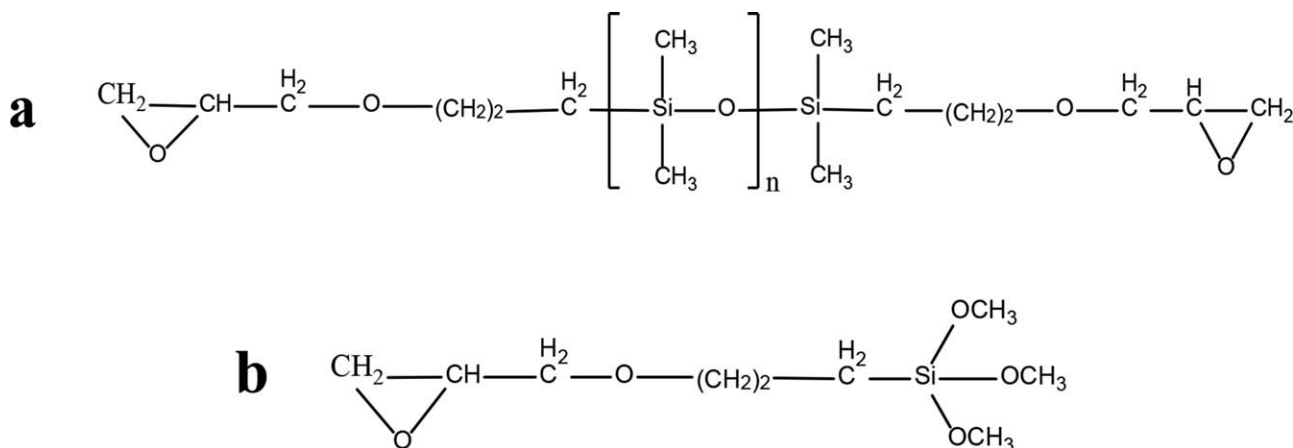
Scheme 1. The schematic molecular structure of (a) ES and (b) GT.

biomimetic structure and mechanical properties and biodegradability of the GT-gelatin hybrid.[16] In these hybrid systems, however, the inorganic (silica) contribution came from GT, and was not varied independently, thus limiting the range of tunable properties.[12] A silica-gelatin hybrid was therefore investigated to alter silica content independently and control the ratio of organic phase to inorganic phase through incorporating tetraethyl orthosilicate, and subsequently modulate degradability and mechanical properties for various tissue engineering applications.[12,13,17] Other reported methods incorporated an inorganic component such as bioactive glass[18] or magnetite nanoparticles[19] into a silica-gelatin system to achieve matched physicochemical and biological properties.

Most studies, however, focused on applications in hard tissue such as bone tissues, while few aimed to modulate the properties of silica-gelatin hybrid material for its application in soft tissue regeneration. The mechanical properties of hard and soft tissue differ considerably. For instance, the Young's modulus of cortical bone is in the range of 7–30 GPa, while that of skin is in the range of 4.6–20 MPa.[20,21] There is therefore still a need to explore new ways to fabricate silica-gelatin hybrids with tunable physicochemical properties to satisfy diverse tissue regeneration requirements.

Polydimethylsiloxane (PDMS) with functional groups is a particularly interesting elastometic modifier, which may be incorporated into the GT-gelatin hybrid system owing to its low glass transition temperature, high flexibility, good thermal stability and other unique properties such as low toxicity, good biocompatibility, and high oxygen permeability.[22,23] A few studies reported gelatin modified with functionalized PDMS. For example, gelatin modified with epoxy-terminated PDMS material was prepared to improve the flexibility and hydrophobicity of hybrids,[24,25] and gelatin with higher stability and hydrophobicity was achieved by modification of PDMS containing epoxy and fluoroalkyl groups.[26] Our previous study reported a cross-linked collagen fiber matrix prepared by modifying skin collagen fiber, from which gelatin is derived, with an epoxy-terminated PDMS oligomer.[27] In addition, PDMS-modified chitosan was prepared by the reaction between epoxidized PDMS and chitosan, exhibiting enhancement in flexibility and water

swellability.[23] Of particular interest, an organic-inorganic hybrid was obtained by cross-linking chitosan with both mono epoxy-terminated PDMS and GT, showing improved physical and mechanical properties due to the incorporation of soft siloxane segments into chitosan.[28] It is therefore conceivable to tailor physicochemical properties such as mechanical properties, hydrophobicity and biodegradation of GT-gelatin hybrids by incorporating epoxy-terminated PDMS, as an organic component, to obtain elastomeric silica-gelatin hybrid materials.

The main objective of this work was to investigate the influence of incorporating an epoxy-terminated polydimethylsiloxane oligomer (ES) on the physicochemical properties of GT-gelatin hybrid material. A silica-gelatin hybrid material was synthesized by simultaneously cross-linking and hybridizing gelatin with GT and ES. The microstructure, thermal stability, mechanical properties, surface wettability, and biodegradability of as-prepared hybrid material were evaluated for their potential application as a wound dressing, drug delivery material or scaffold in soft tissue regeneration.

EXPERIMENTAL

Materials

Gelatin (G, biochemical reagent) was obtained from Chengdu Kelong Chemical Reagent Company. Epoxy-terminated siloxane oligomer [ES, number-average molecular weight about 4000 g/mol, Scheme 1(a)] was supplied by Shanghai Qihua Textile Materials Company, Ltd. γ-glycidoxypropyltrimethoxysilane [GT, a silane coupling agent with relative molecular weight 236.34 g/mol, Scheme 1(b)] was purchased from Jingzhou Jianghan Fine Chemical Company, Ltd. 2,4,6-Trinitrobenzenesulfonic acid (TNBS) was purchased from Aladdin reagent company. All chemicals were used without further purification.

Preparation of Hybrid Materials

A novel silica-gelatin hybrid was prepared by simultaneous functionalization of gelatin with GT, a silane coupling agent, and ES, a functional polydimethylsiloxane oligomer having a highly flexible Si-O-Si backbone. A mole ratio of 1/1 between the amino groups in gelatin and the total epoxy groups in both GT and ES molecules was selected. Furthermore, the amount of GT and ES was calculated on the basis of moles of free amino

groups located in hydroxylysine, lysine and arginine residues in the gelatin molecule. The procedures used are outlined as follows. Gelatin (3 g) was dissolved in distilled water at 40°C with stirring to obtain a 10% (w/v) G solution. The pH value of this solution was adjusted to 10.0 using 1 M NaOH. Subsequently, 0.15 g GT and 1.35 g ES (a GT/ES mole ratio of 2) were dissolved in adequate isopropanol to obtain a mixed silicone solution which was then added into G solution dropwise at 40°C with stirring. After that, the mixture was stirred for 10 h at 40°C, and the pH value was maintained at 10.0 by adding 1 M NaOH as required. The resulting solution (30 mL) was poured into a silicon substrate (12 cm×12 cm), and air-dried at room temperature for 3 days to obtain an ES/GT-G hybrid film with a thickness of about 0.3 mm. For the preparation of an ES/GT-G hybrid sponge, another 30-mL portion was poured into a polyvinyl chloride pipe mold with a diameter of 14 mm and a height of 30 mm, and freeze-dried at −55°C for 48 h to form a porous matrix.

To investigate the effect of ES on the properties of the hybrid material, a GT-G hybrid film and sponge were prepared following the procedures described above with a mole ratio of 1/1 between the amino groups of G (3 g) and the epoxy groups of GT (0.3 g), but without incorporating ES. A pure G film and sponge were also prepared as controls following the above mentioned procedures.

Degree of Crosslinking
The degree of crosslinking of hybrid samples based on the amount of free –NH$_2$ in the gelatin before and after cross-linkage was defined according to eq. (1).

$$\text{Degree of crosslinking (\%)} = \left(\frac{\text{NH}_0 - \text{NH}_t}{\text{NH}_0}\right) \times 100 \qquad (1)$$

where NH$_0$ is the amount of free -NH$_2$ in the gelatin before crosslinking, and NH$_t$ is the amount of free -NH$_2$ after crosslinking. The amount of free -NH$_2$ was detected using the 2,4,6-trinitrobenzenesulfonic acid (TNBS) method.[29] The data reported are the mean of three determinations.

Fourier Transform Infrared-Attenuated Total Reflectance Spectroscopy (FTIR-ATR)
The micro-molecular structure analysis of pure G film, and GT-G and ES/GT-G hybrid films were conducted using FTIR-ATR spectroscopy (Thermo Fisher Scientific, USA) over the range 650–4000 cm^{-1}. The experiments rely on the OMNI-Sampler TM equipment. All spectra were obtained at room temperature by averaging 16 scans at a resolution of 4 cm^{-1}.

Differential Scanning Calorimetry (DSC)
Calorimetric measurements were carried out on an N33-DSC 200 PHOX (NETZSCH). Pure G, GT-G and ES/GT-G dried samples (3–5 mg) sealed in aluminum dishes were placed into the DSC instrument. The heating rate was maintained constant at 10°C/min over the temperature range of 20°C–150°C under a nitrogen atmosphere with a flow rate of 40 mL/min. Three independent measurements were performed for each sample.

Morphological Analysis
The morphology of vertical and horizontal cross-sections of sponges was obtained using a scanning electron microscope (JSM-5900 SEM, Philips). Sponge specimens with adequate sizes were placed in a vacuum environment and observed at an operating voltage of 5 kV. Pore dimension was measured by analyzing horizontal cross-section SEM micrographs using Image J (NIH) software. At least 20 pores were assessed from different areas of the same sample. The porosity of hybrid sponges was determined by the liquid displacement method as previously described[27]. Briefly, a pre-weighed sample (W_S) was immersed in a graduated tube containing 25 mL anhydrous alcohol whose weight was recorded as W_1. After 24 hours, liquid above the 25 mL scale line was removed, and the weight of the remains was recorded as W_2. Then, after carefully removing the sample filled with alcohol, the weight of the remains was recorded as W_3. The porosity of the sample was calculated according to eq. (2). The reported porosity was the average of three separate examinations.

$$\text{Porosity (\%)} = \left(\frac{W_2 - W_3 - W_S}{W_1 - W_3}\right) \times 100 \qquad (2)$$

Mechanical Properties
To examine the mechanical properties of hybrid materials, tensile and compression tests were conducted using an electronic universal testing machine (Shenzhen Suns Technology Co., Ltd. UTM6203). For the stretch test, the wet film, obtained by immersing dried film in distilled water to about 70% water uptake, was used. Membrane specimens were cut to dumbbell shape with length 50 mm and width 10 mm. The tensile speed was applied at 10 mm/min until rupture of the test specimen occurred. The Young's elastic modulus was defined as the slope of the initial linear region of the recorded stress-strain curve. Tensile strength was defined as the maximum load before the sample collapsed and was calculated by considering the sample's contact surface areas, while the elongation at break was recorded at the point of peak load.

For the compression test, cylinder shaped sponges with 14 mm diameters and approximately 30 mm heights were compressed from 0 kN to 3 kN at a rate of 1 mm/min. The initial linear portion of the obtained stress-strain curve at low strain correlates to the elastic behavior and its slope is the compressive modulus of the sponge, while compressive yield strength is defined as the load carried at the yield point divided by the original cross-sectional area of the sponge.[30] Three specimens were tested for each sample.

Surface Wettability
The contact angle, a measurement of surface wettability of hybrid membranes, was measured using a high-speed video contact angle measuring instrument (OCAH200, Dataphysics) by the sessile drop method. The film samples were placed on the object stage horizontally. Static contact angles were examined at room temperature with 4-μL double distilled water droplets. For each result, five measurements on different parts of the surface were carried out.

Swelling Behavior
The swelling behavior of hybrid materials was characterized based on the degree of swelling and the water uptake percentage. Briefly, dried samples (1 cm×1 cm) were immersed in
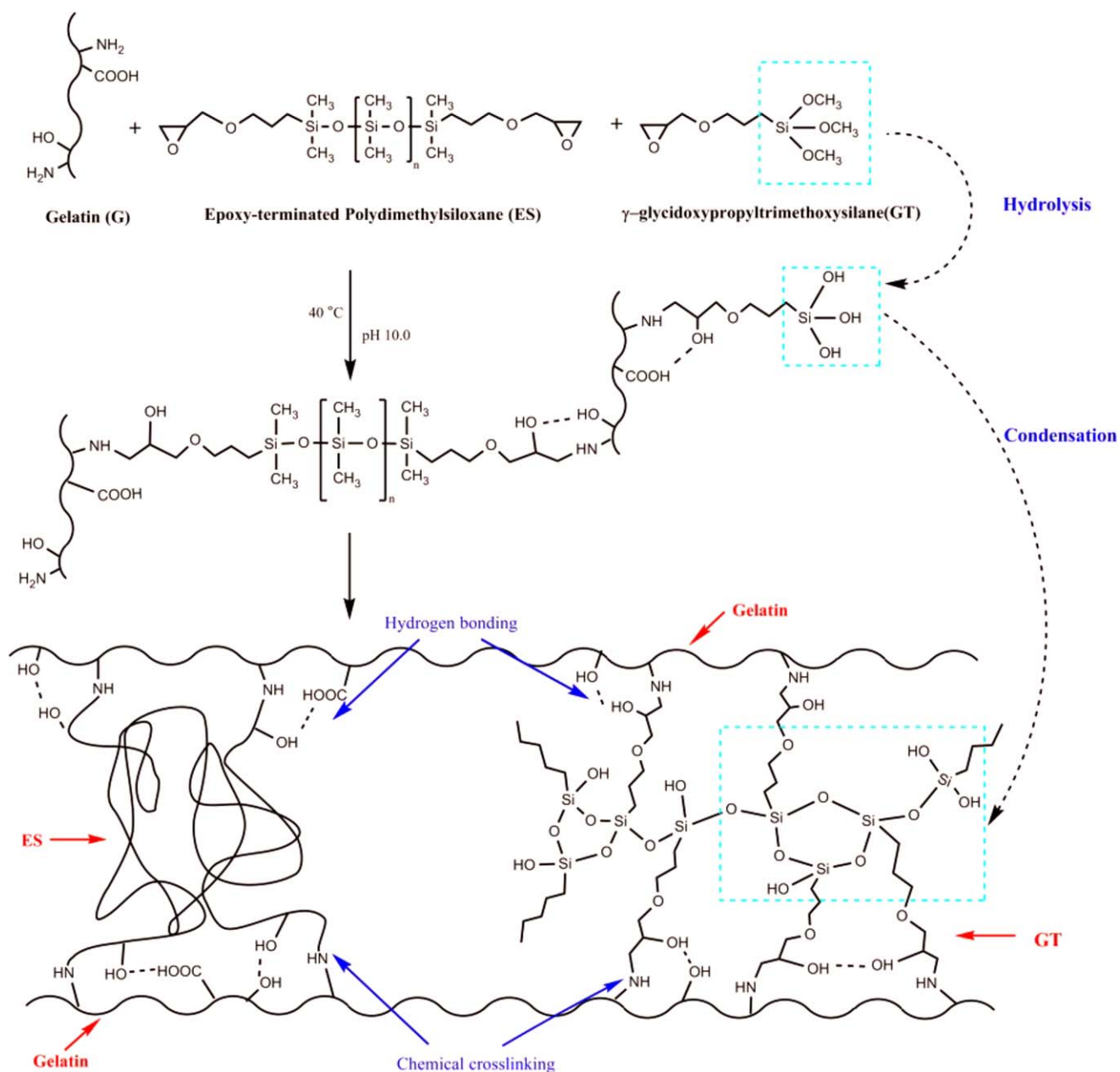
Scheme 2. Schematic representation of synthesis of silica-gelatin hybrid ES/GT-G. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

distilled water for different periods of time. At each immersion interval, i.e. 1, 3, 6, 9, 12 hours, the swollen samples were removed from water, excess water was removed with filter paper and the film was immediately weighed. Meanwhile, the side length of wet samples was precisely measured. To obtain the average value and standard deviation (SD), at least 3 species per sample were tested. The degree of swelling (Δa_s) was calculated using equation (3).

$$\Delta a_s(\%) = \left(\frac{a_s - a_0}{a_0} \right) \times 100 \qquad (3)$$

where a_0 and a_s are the side length of samples before and after swelling, respectively. The water uptake percentage (ΔW_s) was calculated according to equation (4).

$$\Delta W_S(\%) = \left(\frac{W_S - W_0}{W_0} \right) \times 100 \qquad (4)$$

where W_0 and W_s are the weight of samples before and after immersion, respectively.

In Vitro Biodegradation Test
The biodegradability of hybrid materials was evaluated by soaking them in phosphate buffer solution (PBS, pH=7.8) for 1, 2, 3, 4, 5, 6 and 7 days. At each interval, the remains were removed and oven-dried at 40°C to constant weight. The weight loss of samples (ΔW_d) in PBS was calculated using equation (5).
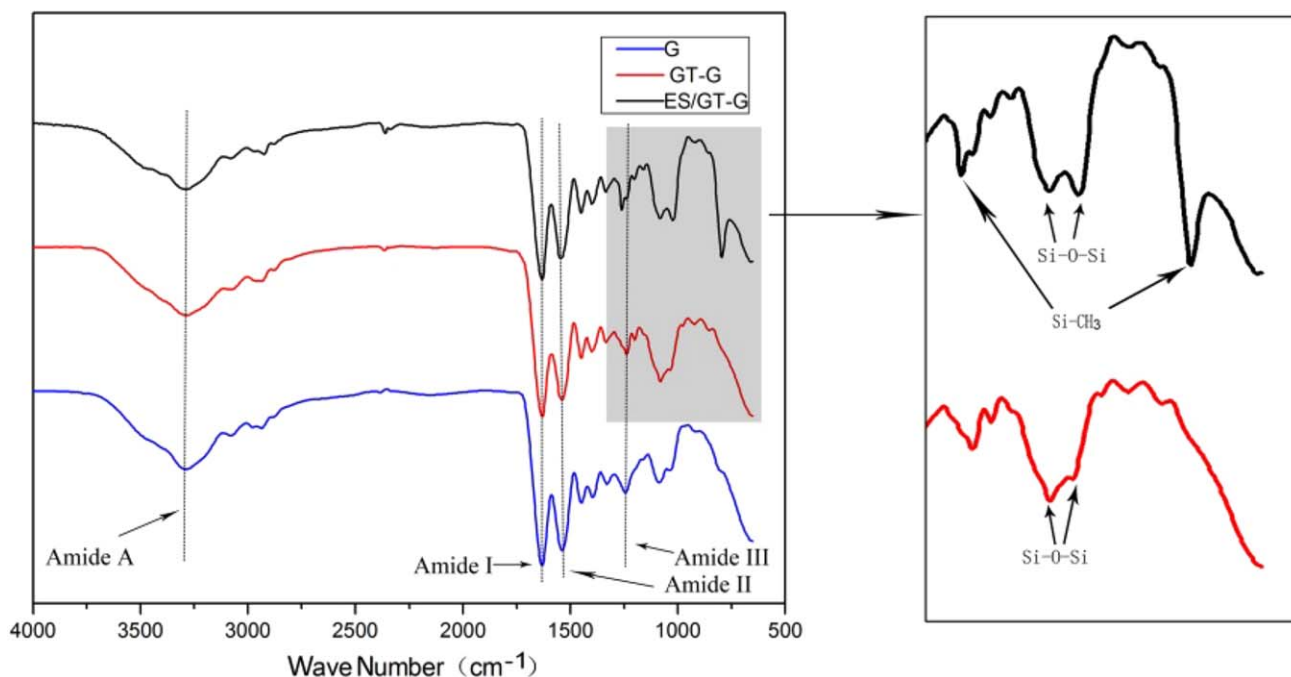
Figure 1. The FTIR-ATR spectra of G, GT-G, and ES/GT-G. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary. com.]

$$\Delta W_d(\%) = \left(\frac{W_d - W_0}{W_d} \right) \times 100 \qquad (5)$$

where W_0 and W_d are the weights of samples before and after soaking, respectively. The mean of three separate measurements was determined.

Trypsin was used to demonstrate the effect of proteolytic enzyme on the biodegradation of hybrid materials. Briefly, hybrid samples (about 40 mg) were immersed in PBS buffer solution (pH=7.8, 25 mL) including 0.1% (w/w) trypsin, at 40°C for 12 hours. Samples were retrieved each hour and dried at 40°C to constant weight. The weight loss (ΔW_D) was calculated according to eq. (6).

$$\Delta W_D(\%) = \left(\frac{W_0 - W_D}{W_0} \right) \times 100 \qquad (6)$$

where W_0 and W_D are the weights of samples before and after immersion, respectively. For each sample, at least three species were tested.

Statistical Analysis

The quantitative data were expressed as mean ± standard deviation, and statistical analysis was carried out using the one-way variance (ANOVA) test using Origin Pro 2015 software (Microcal Co, USA). The P values <0.05 (*) were considered significant.

RESULTS AND DISCUSSION

Synthesis of Silica-Gelatin Hybrid Material (ES/GT-G)

Silica-gelatin hybrid material ES/GT-G was prepared by simultaneous modification of gelatin with GT and ES, as shown in Scheme 2. It is essential that the formation of covalent bonds, i.e., establishment of a crosslinkage between gelatin and silicone

by nucleophilic attack of active ε-amino groups of lysine or hydroxylysine residues occurs in the gelatin on the electrophile oxirane ring within the GT and ES molecules. As reported previously in the literature,[31] under basic conditions (e.g. about pH 9-10), the formation of this crosslinkage takes place more readily. However, in a heterogeneous system, the reaction of amine groups of biopolymers (such as chitosan, gelatin) with epoxy rings linked to siloxane or silane moieties is quite difficult due to the very different solubility parameters of the mentioned reaction partners.[28] In addition, hydration of the trimethoxy groups on GT forms pendent silanol groups (Si-OH) through an alkali-catalyzed reaction. The condensation of Si-OH groups and the interaction of hydrogen bonds among amino groups, carboxyl groups and Si-OH groups would also contribute to the formation of hybrid.[16] As a consequence, the degree of cross-linking of hybrid ES/GT-G and GT-G was 38.4% ± 3.3% and 47.0% ± 1.7% respectively, according to the TNBS method. The incorporation of ES decreased the degree of cross-linking of GT-G slightly, likely due to the higher degree of hydrophobicity of ES and its lower miscibility with gelatin. Nevertheless, ES can be utilized to tailor the properties of silicagelatin hybrid to make the material more flexible and achieve other properties, which was confirmed in this work.

Fourier Transform Infrared-Attenuated Total Reflectance Spectroscopy (FTIR-ATR)

The FTIR-ATR spectra of G, GT-G and ES/GT-G are illustrated in Figure 1, demonstrating their surface chemical structure. As shown in Figure 1, pure G exhibited characteristic bands of amide A (3293 cm^{-1}), amide I (1631 cm^{-1}), amide II (1538 cm^{-1}) and amide III (1240 cm^{-1}) attributed to the O-H and N-H stretch, C=O stretch, N-H bending, and C-H stretch coupled with N-H in-planting bending vibrations, respectively.[9]
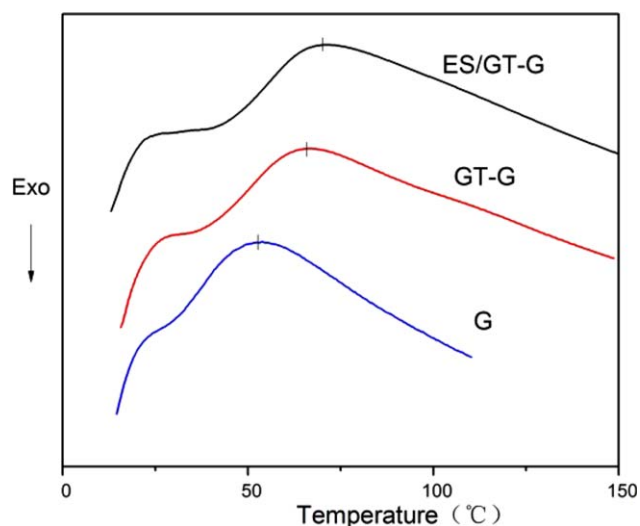
Figure 2. Representative DSC thermograms of G, GT-G and ES/GT-G samples. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

For GT-G and ES/GT-G, in addition to the amide bands of gelatin, a new absorption in the range of 1000-1100 cm^{-1} was assigned to the characteristic absorption peak of Si-O-Si bonds. As the hybrid incorporated ES, the characteristic bands of Si-O-Si increased in intensity. In addition, characteristic bands of 1260 cm^{-1} and 802 cm^{-1} in ES/GT-G spectrum, which did not appear in the GT-G spectrum, were assigned to Si-CH_3 bonds of the ES molecule.[26] Bands of Si-O-Si and Si-C bonds demonstrated successful crosslinking between functional polydimethylsiloxane oligomer and gelatin.[24]

Thermal Stability of Hybrid Materials

The thermal stability of hybrid materials was investigated based on their denaturation behavior compared with pure gelatin, as evaluated by differential scanning calorimetry analysis (DSC).

DSC curves (Figure 2) showed that the denaturation temperature (T_d) of gelatin was 51.8°C ± 0.3°C, while the T_d of GT-G and ES/GT-G was 66.7°C ± 0.3°C and 71.5°C ± 0.5°C, respectively. For hybrid GT-G, the T_d increased by 15°C as compared to pure gelatin, which was similar to the previous reported value of 17°C.[9] The increase is probably due to the increase of
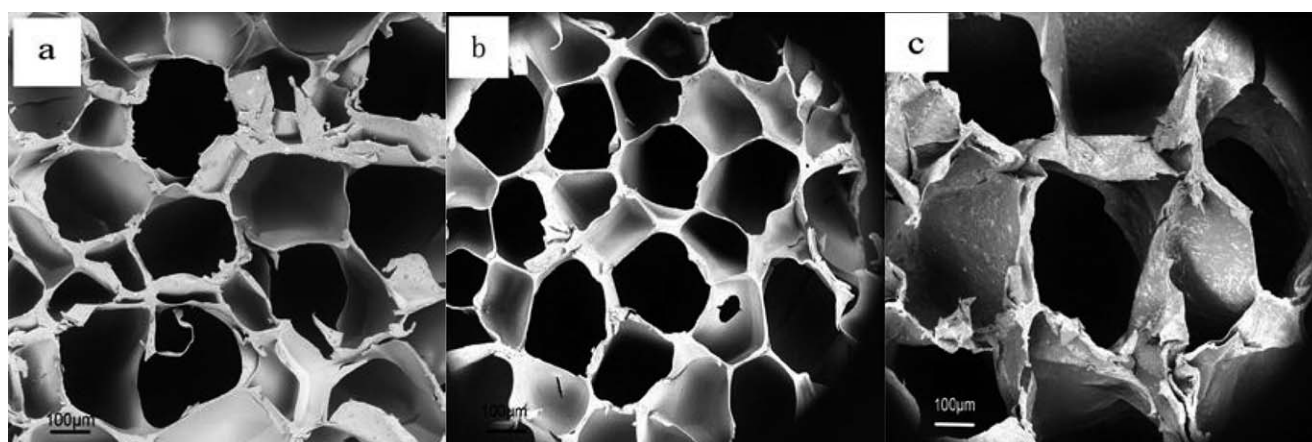

Figure 3. Horizontal cross-section SEM images of (a) G, (b) GT-G, and (c) ES/GT-G sponges. The diameters of pore sizes were about 250 ± 29 μm for G, 220 ± 27 μm for GT-G, and 340 ± 36 μm for ES/GT-G.
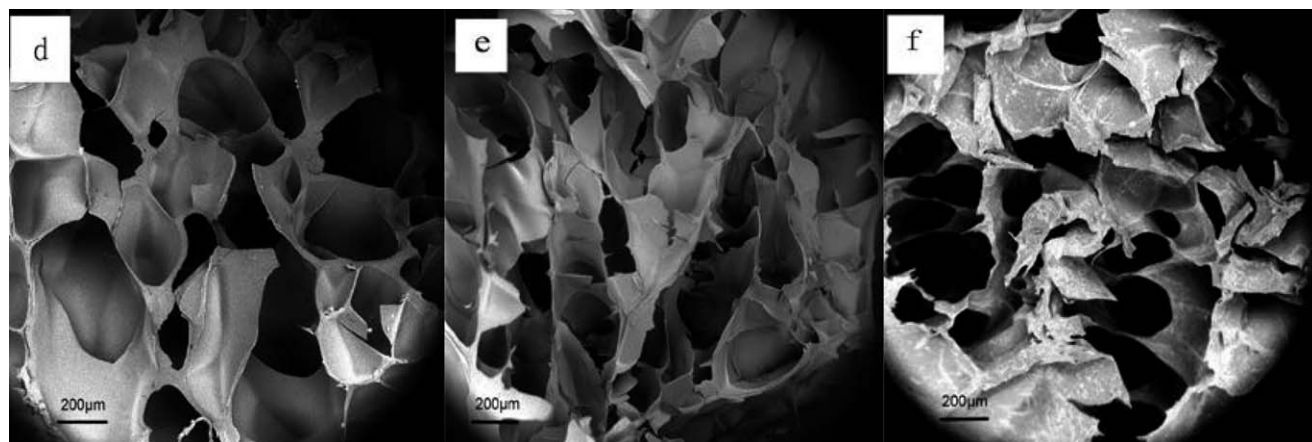

Figure 4. Vertical cross-section SEM images of (d) G, (e) GT-G and (f) ES/GT-G sponges.
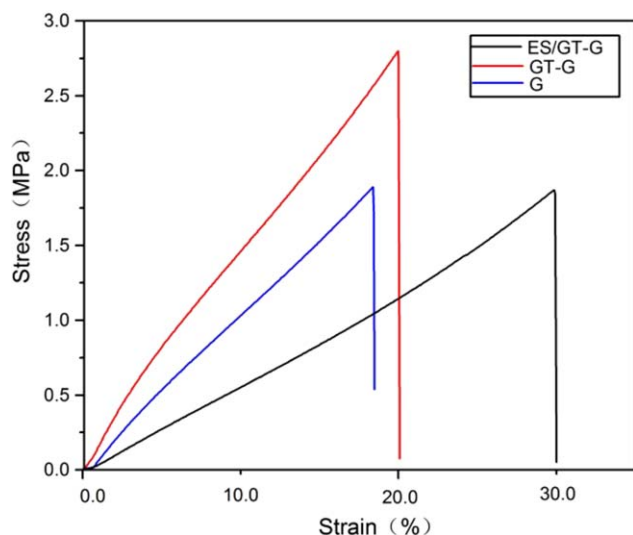
Figure 5. Typical stress-strain curves of G, GT-G, and ES/GT-G films. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

covalent bonds in gelatin chains as a result of modification by GT, indicating the success of cross-linkage and improved thermal stability. With respect to ES/GT-G hybrid, the incorporation of ES further enhanced the thermal stability of GT-G, as indicated by the additional T_d increment of 5°C. In general, a higher T_d value implies a higher degree of cross-linking. This observation, however, is somewhat contradictory to the amino group determination by the TNBS method, i.e. ES/GT-G having a lower cross-linking degree. This is thought to be due to the high thermal stability of the polydimethylsiloxane soft segment introduced by the siloxane oligomer ES in the ES/GT-G hybrid matrix. Additionally, the peak of GT-G and ES/GT-G hybrid samples appeared to be more flat than that of the G sample. This phenomenon could also indicate the improved thermal stability of hybrid films, as their degradation is more progressive.[32]

Morphological Analysis

The porosity and pore size of biomaterial scaffolds play a critical role in tissue regeneration. For different organisms scaffolds with different porosity and pore size are needed to favor the adhesion and growth of cells. The horizontal cross-section SEM image of pure G [Figure 3(a)] displayed a uniform porous network with mean pore size around 250 ± 29 μm, and that of GT-G [Figure 3(b)] resembled G in its structure with a mean pore size of 220 ± 27 μm, while ES/GT-G [Figure 3(c)] showed an ellipsoid shaped pore with an average size of 340 ± 36 μm. This observation suggested that the incorporation of ES increased the pore size of GT-G hybrid. This may mean that

since GT has a lower molecular weight and shorter molecular chain than ES, it is possible for GT to form a denser cross-linking with gelatin than ES.[33] Moreover, the incorporation of ES decreased the formation of hydrogen bonds between the remaining Si-OH and free amino or carboxyl groups in gelatin, and also of Si-O-Si bonds generated from the condensation of Si–OH, which resulted in a relatively loose network structure. In addition, the vertical cross-section images (Figure 4) of three samples suggested that they displayed an interconnected porous structure which could favor cell migration and proliferation. Furthermore, data from the liquid displacement method revealed that the three sponges exhibited high porosity (86.9% $\pm$ 0.5% for G, 85.4% $\pm$ 0.7% for GT-G and 86.4% $\pm$ 0.9% for ES/GT-G), which could also favor the growth and migration of cells.

Mechanical Properties

Appropriate mechanical properties of biomaterials are of utmost importance for tissue engineering applications. In this study, both tensile and compression behaviors of silica-gelatin hybrid materials were tested, to reveal their feasibility for hard or soft tissue regeneration applications, as a result of incorporation of a flexible and elastic polydimethylsiloxane oligomer.

Typical stress-strain curves recorded from three films are shown in Figure 5, and the corresponding mechanical parameters are reported in Table I. It can be seen that the elongation at break of ES/GT-G (29.86% $\pm$ 0.87%) is considerably higher than that of pure G (18.29% $\pm$ 0.78%) and hybrid GT-G (19.96% $\pm$ 0.66%), demonstrating that the incorporation of ES enhanced the tenacity of GT-G hybrid. In addition, incorporating ES decreased the tensile strength of GT-G from 2.79 ± 0.04 MPa to 1.87 ± 0.12 MPa, quite close to that of G film. This may be attributed to the soft and linear Si-O-Si segment of ES, which decreased the intermolecular and intra-molecular interaction of silica-gelatin hybrid matrix, thereby making the molecular chains more movable under external force.

Figure 6 shows typical stress versus strain curves of pure G, GT-G and ES/GT-G hybrid sponges as measured by the compression test, and the compression properties of three samples are presented in Table II. Pure G and ES/GT-G hybrid showed a typical stress versus strain curve of highly porous polymer scaffolds, i.e. an initial elastic response, followed by a plateau and rapid increase in stress. The GT-G sample showed a similar stress versus strain curve but a slight decrease in stress after reaching an elastic region.[15] In other words, the GT-G sample showed more brittle behavior while ES/GT-G showed more elastic compression behavior when compared to pure G sample. As can also be seen from Table II, the compressive yield strength and modulus of GT-G sponge were significantly improved

Table I. The Tensile Strength, Elastic Modulus, and Elongation at Break of Films

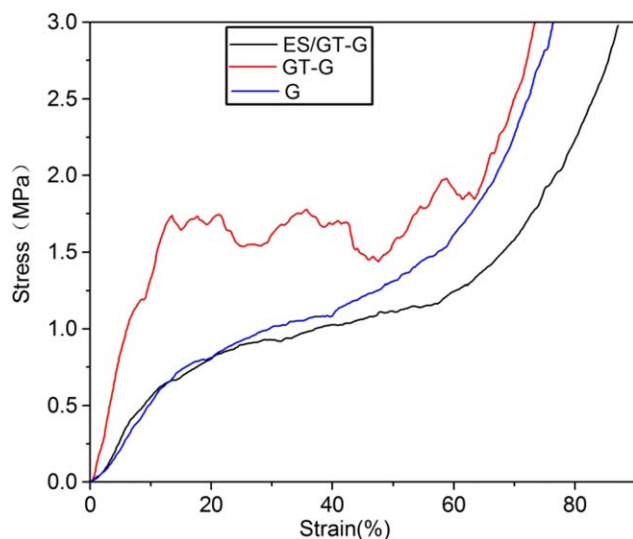|	G	GT-G	ES/GT-G
Tensile strength (MPa)	1.87 ± 0.22	2.79 ± 0.04	1.87 ± 0.12
Elastic modulus (MPa)	0.79 ± 0.13	1.49 ± 0.04	0.45 ± 0.05
Elongation at break (%)	18.29 ± 0.78	19.96 ± 0.66	29.86 ± 0.87

Figure 6. Typical stress versus strain curves of G, GT-G, and ES/GT-G sponges. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 7. Static water contact angles of G, GT-G, ES/GT-G. Columns are average values, bars indicate standard deviation. $**P < 0.01$ ($n = 6$) when GT-G and ES/GT-G compared with G.

compared to pure G sample, while that of ES/GT-G sponge decreased to a great extent as a result of incorporation of ES, which corresponds with a previous study.[23]

The ability to vary the stiffness of materials could meet the demands of diverse tissues in medical applications.[12] Therefore, it is expected that ES/GT-G hybrid materials with tunable mechanical properties via incorporating different amount of ES could find applications in wound dressing and soft tissue regeneration.

Surface Wettability and Swelling Behavior

Surface wettability and swelling behavior are the main factors affecting biocompatibility of biomaterials. Static water contact angles indicating surface hydrophilicity of film samples are presented in Figure 7. No significant difference was found between the contact angles of GT-G ($72.0° \pm 3.8°$) and ES/GT-G ($73.6° \pm 3.1°$), both of which were greater than that of pure G ($55.2 \pm 1.5°$). This may be attributed to hydrophobic groups (e.g. Si-O-Si) on the surface of hybrid membranes.[4] In spite of their lower surface wettability, silica-gelatin hybrids would still support cell adhesion and proliferation.[9]

From Figure 8(a), it is clear that the swelling rate of GT-G and ES/GT-G films initially increased rapidly but after 12 hours the rate of increase became slower. The degree of swelling degree of ES/GT-G and GT-G after soaking in water for 24 hours was $19.2\% \pm 1.0\%$ and $19.8\% \pm 0.8\%$ respectively. Both showed better anti-deformation in aqueous medium when compared with
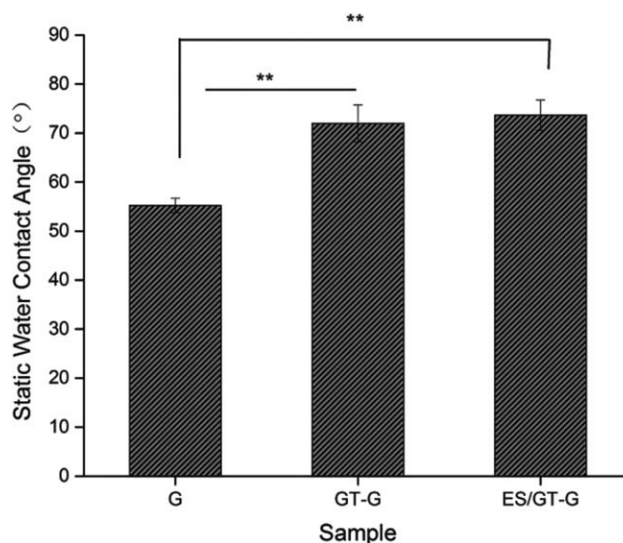
pure gelatin, whose degree of swelling was $76.0\% \pm 1.94\%$. In addition, the degree of swelling of ES/GT-G was slightly higher than GT-G only during the first hour, whereas there was no significant difference during the rest of the soaking time. Figure 8(b) showed that the water uptake of GT-G and ES/GT-G after 12 h was $181.5\% \pm 6.8\%$ and $201.5\% \pm 10.2\%$ respectively, significantly lower than that of pure G ($1000–3000\%$), demonstrating that the incorporation of silane/polydimethylsiloxane improved the hydrophobic character of gelatin significantly. Moreover, it should be noted that the water uptake of ES/GT-G was higher than that of GT-G, probably owing to the relatively loose network structure of ES/GT-G hybrid compared with that of GT-G, as demonstrated by SEM observation, which allowed water to penetrate into it more easily. This further implies that silica-gelatin hybrid incorporated with polydimethylsiloxane oligomer is promising as a biomimetic support in tissue engineering. In addition, it should be noted that the swelling behavior ($180\%–230\%$) of ES/GT-G is in the range of the requirement of wound dressings.[20]

In Vitro **Biodegradation Ability**

In addition to hydrophilicity, the biodegradability of biomaterials is another crucial factor determining their suitability for application in tissue regeneration. To investigate the biodegradation of hybrid materials, the weight loss of samples in PBS in the absence [Figure 9(a)] and presence [Figure 9(b)] of trypsin was measured. Figure 9(a) showed that the weight loss of two

Table II. The Compression Properties of G, GT-G, and ES/GT-G Sponges

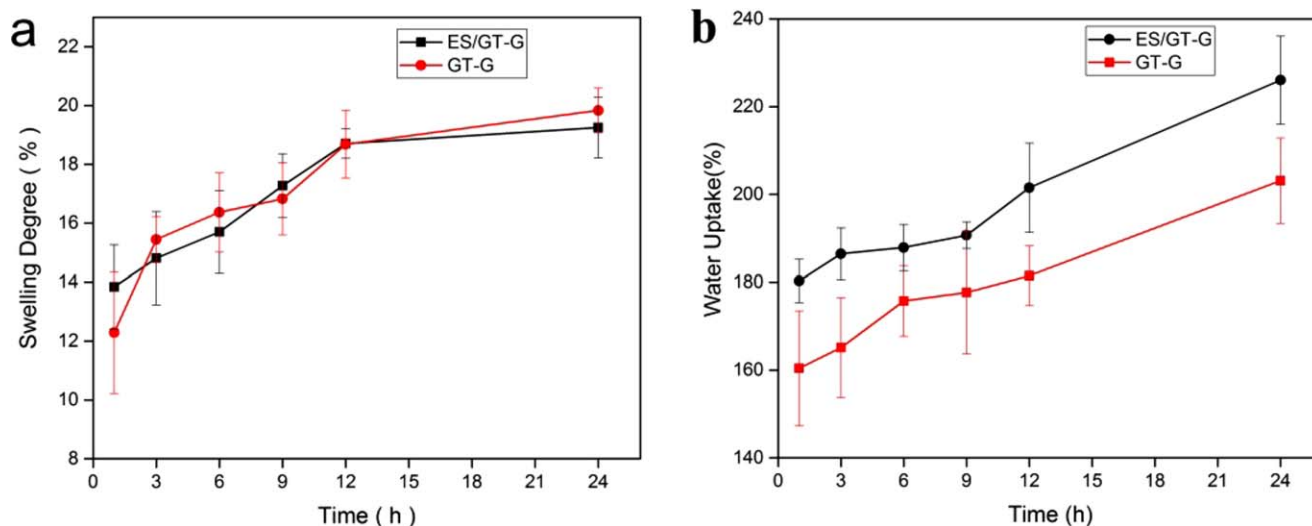|	G	GT-G	ES/GT-G
Compressive yield strength (MPa)	0.79 ± 0.07	1.21 ± 0.04	0.72 ± 0.06
Compressive modulus (MPa)	5.45 ± 0.26	15.17 ± 0.22	5.39 ± 0.30
Strain at 3 MPa stress (%)	75.52 ± 1.93	73.52 ± 2.38	87.50 ± 0.67

Figure 8. (a) The swelling degree and (b) water uptake of GT-G and ES/GT-G. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

hybrid samples showed no obvious difference in the first four days, while a higher degradation rate was observed for ES/GT-G from the fifth day. Within one week the weight loss of ES/GT-G reached $33.0 \pm 2.0\%$, whereas that of GT-G was $15.6 \pm 1.3\%$. On the whole, silica-gelatin hybrid films showed lower biodegradation as compared to pure G, which dissolved completely in one day.

Figure 9(b) showed that the weight loss of samples in one hour was $43.0 \pm 1.4\%$ for ES/GT-G, and $20.8 \pm 2.0\%$ for GT-G, while pure G degraded completely in one hour. Additionally, the weight loss of GT-G was $69.6\% \pm 2.0\%$ in 12 hours, whereas the ES/GT-G hybrid sample dissolved almost completely in 7 hours. As a consequence, the biodegradation stability of GT-G hybrid was significantly improved owing to the cross-linking of silane GT with gelatin, consistent with a previous report.[16]

However, ES/GT-G hybrid exhibited lower biodegradation stability than GT-G sample owing to the incorporation of polydimethylsiloxane oligomer ES. Nevertheless, it is clear that ES/GT-G hybrid showed much better biodegradation stability than pure gelatin. This may be due primarily to two reasons: (1) the crosslinking degree of ES/GT-G was lower than that of GT-G as determined by the TNBS method. More remaining hydrophilic groups (e.g. free amino groups) would allow easier enzyme attack, increasing the degradation rate and (2) the relatively loose network structure of ES/GT-G hybrid compared with GT-G increased the accessibility of trypsin to the cleavage sites, which could accelerate the degradation of samples. Therefore, it can be considered that the biodegradation behavior of silica-gelatin hybrids could be tuned by introducing epoxy-terminated polydimethylsiloxane oligomer.
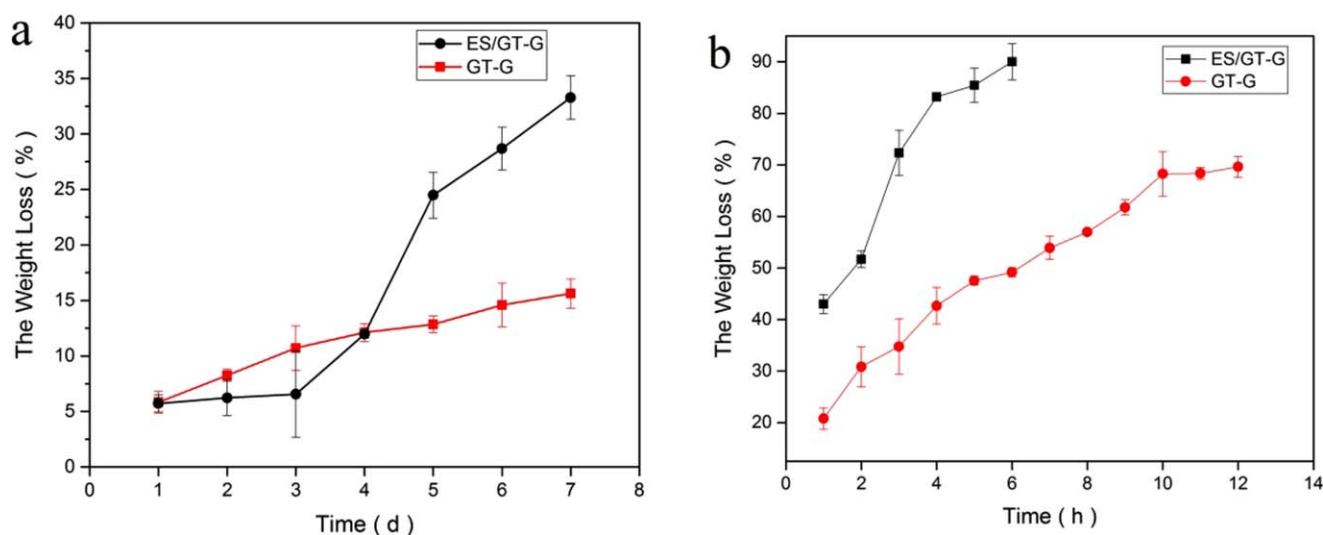

Figure 9. *In vitro* biodegradation of hybrid materials in PBS solution in the absence (a) and presence (b) of trypsin. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CONCLUSION

A novel silica-gelatin hybrid material (ES/GT-G) was prepared by simultaneously crosslinking gelatin with both γ-glycidoxypropylthrimethoxysilane and polydimethylsiloxane oligomer with double-terminated epoxy groups (ES). As compared with GT-G hybrid (gelatin crosslinked only with γ-glyci-doxypropylthrimethoxysilane), ES/GT-G hybrid possessed enhanced thermal stability, flexibility, and biodegradability, which were verified by the thermal denaturation test, tensile and compression test, and enzyme degradation test *in vitro*, respectively. There was, however, no significant difference in surface wettability and swelling behavior between ES/GT-G and GT-G hybrids. Overall, it may be concluded that the physico-chemcal properties of silica-gelatin hybrids could be modulated via incorporation of functional polydimethylsiloxane oligomer, making them promising for applications particularly in wound dressing and soft tissue regeneration. Further work is in progress to systematically investigate the effect of the proportion of γ-glycidoxypropyltrimethoxysilane to epoxy-terminated polydimethylsiloxane on the properties, in particular biodegradation and biocompatibility, of this novel silica-gelatin hybrid, in order to meet the demands of regeneration of diverse tissues.

REFERENCES

1. Gross, S.; Bauer, M. *Adv. Funct. Mater* **2010**, *20*, 4026.

2. Sanchez, C.; Julián, B.; Belleville, P.; Popall, M. *J. Mater. Chem* **2005**, *3536*, 3559.

3. Olszta, M. J.; Cheng, X.; Sang, S. J.; Kumar, R.; Kim, Y. Y.; Kaufman, M. J.; Douglas, E. P.; Gower, L. B. *Sci. Eng. R* **2007**, *58*, 77.

4. Silva, S. S.; Oliveira, J. M.; Benesch, J.; Caridade, S. G.; Mano, J. F.; Reis, R. L. *J. Bioact. Compat. Polym.* **2013**, *28*, 385.

5. Raghavan, R. N.; Muthukumar, T.; Somanathan, N.; Sastry, T. *Mater. Sci. Eng. C* **2013**, *33*, 1983.

6. Ren, L.; Tsuru, K.; Hayakawa, S.; Osaka, A. *J. Sol-Gel Sci. Technol.* **2001**, *21*, 115.

7. Young, S.; Wong, M.; Tabata, Y.; Mikos, A. G. *J. Control. Release* **2005**, *109*, 256.

8. Hutmacher, D. W. *Biomaterials* **2000**, *21*, 2529.

9. Tonda-Turo, C.; Gentile, P.; Saracino, S.; Chiono, V.; Nandagiri, V. K.; Muzio, G.; Canuto, R. A.; Ciardelli, G. *Int. J. Biol. Macromol.* **2011**, *49*, 700.

10. Bigi, A.; Cojazzi, G.; Panzavolta, S.; Rubini, K.; Roveri, N. *Biomaterials* **2001**, *22*, 763.

11. Khan, M. A.; Khan, R. A.; Noor, F. G.; Rahman, M.; Noor-A-Alam, M. *Polym. Plast. Technol. Eng.* **2009**, *48*, 808.

12. Mahony, O.; Tsigkou, O.; Ionescu, C.; Minelli, C.; Ling, L.; Hanly, R.; Smith, M. E.; Stevens, M. M.; Jones, J. R. *Adv. Funct. Mater.* **2010**, *20*, 3835.

13. Mahony, O.; Sheng, Y.; Turdean-Ionescu, C.; Hanna, J. V.; Smith, M. E.; Lee, P. D.; Jones, J. R. *J. Sol-Gel Sci. Technol.* **2014**, *69*, 288.

14. Lei, R.; Tsuru, K.; Hayakawa, S.; Osaka, A. *Biomaterials* **2002**, *23*, 4765.

15. Lei, B.; Shin, K. H.; Koh, Y. H.; Kim, H. E. *J. Biomed. Mater. Res. Part B* **2014**, *102B*, 1528.

16. Xue, Y.; Wang, L.; Shao, Y.; Yan, J.; Chen, X.; Lei, B. *Chem. Eng. J.* **2014**, *251*, 158.

17. Lei, B.; Shin, K. H.; Jo, I. H.; Koh, Y. H.; Kim, H. E. *Mater. Chem. Phys.* **2014**, *145*, 397.

18. Lei, B.; Wang, L.; Chen, X.; Chae, S. K. *J. Mater. Chem. B* **2013**, *1*, 5153.

19. Dashnyam, K.; Perez, R. A.; Singh, R. K.; Lee, E. J.; Kim, H. W. *RSC Adv.* **2014**, *4*, 40841.

20. Morgado, P. I.; Aguiar-Ricardo, A.; Correia, I. J. *J. Membr. Sci.* **2015**, *490*, 139.

21. Brian, S.; Yanzhe, Y.; Arunesh, M.; Brent, S.; Truong, N. K. *J. Biomed. Mater. Res. Part B* **2008**, *85b*, 573.

22. Nahrup, J. S.; Gao, Z. M.; Mark, J. E.; Sakr, A. *Int. J. Pharm.* **2004**, *270*, 199.

23. Rutnakornpituk, M.; Ngamdee, P.; Phinyocheep, P. *Carbohydr. Polym.* **2006**, *63*, 229.

24. Zhang, M.; Wu, Y.; Zhang, Q.; Xia, Y.; Li, T. *J. Appl. Polym. Sci.* **2011**, *120*, 2130.

25. Jing, X.; Li, T. D.; Jiang, Q. W.; Qiao, C. D.; Cheng, J. Y. *Colloids Surf. B* **2013**, *103*, 375.

26. Wojciechowska, P.; Pietras, P.; Maciejewski, H. *Adv. Polym. Tech.* **2014**, *33*, S1.

27. Du, W.; Han, X.; Li, Z.; Li, Y.; Li, L.; Wang, K. *J. Appl. Polym. Sci.* **2015**, DOI: 10.1002/app.42727.

28. Enescu, D.; Hamciuc, V.; Ardeleanu, R.; Cristea, M.; Ioanid, A.; Harabagiu, V.; Simionescu, B. C. *Carbohydr. Polym.* **2009**, *76*, 268.

29. Kuijpers, A. J.; Engbers, G. H.; Krijgsveld, J.; Zaat, S. A.; Dankert, J.; Feijen, J. *J. Biomater. Sci., Polym. Ed.* **2000**, *11*, 225.

30. Williams, J. M.; Adewunmi, A.; Schek, R. M.; Flanagan, C. L.; Krebsbach, P. H.; Feinberg, S. E.; Hollister, S. J.; Das, S. *Biomaterials* **2005**, *26*, 4817.

31. Di, Y.; Heath, R. J. *Polym. Degrad. Stab.* **2009**, *94*, 1684.

32. Torres-Giner, S.; Gimeno-Alcaniz, J. V.; Ocio, M. J.; Lagaron, J. M. *ACS Appl. Mater. Interfaces* **2009**, *1*, 218.

33. Zaman, H. U.; Khan, M. A.; Khan, R. A. *J. Adhes. Sci. Technol.* **2013**, *27*, 413.