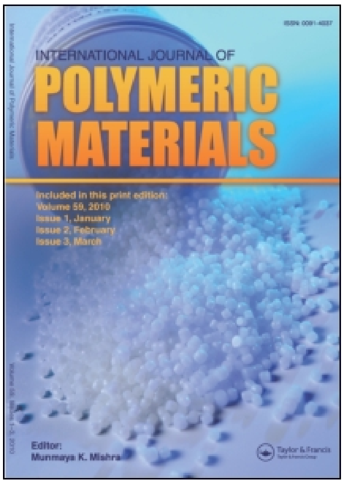

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Microporous Composites Prepared by Coagulation of a CS/PEG Solution in NaOH

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Novel biodegradable three-dimensional composites with a porous structure have been prepared by coagulation of a chitosan (CS)/poly ethylene glycol (PEG) solution in NaOH. It is confirmed that PEG acts not only as a modifier to improve the mechanical properties, but also as a porogen for the CS matrix to induce porous structure. The porous structure and mechanical properties of the composites can be tailored with the amount of PEG and glutaraldehyde (GA). The addition of GA can improve the interaction strength and compatibility between the PEG phase and CS matrix. The resulting composites have smaller pore size, lower porosity, and better mechanical properties.

Keywords chitosan, porous structure, strength

INTRODUCTION

Tissue engineering is a rapidly developing field and considered as a promising alternative to donor tissue procedures [1]. When the hard tissue is damaged or fails, a number of bioactive implant materials based on natural and synthetic polymers have been used to repair bone defects. Especially, chitosan (CS) has been extensively used in bone tissue engineering since after exploring its capacity to promote growth and mineral-rich matrix deposition by osteoblasts

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in culture [2]. Varieties of biomimetic materials based on CS with structural and mechanical equivalence to bone have been developed to repair bone defects. Ding prepared CS/calcium phosphate composites by a simple mixing-and-heating method [3,4]. The prepared composites have high initial strength (27 MPa) and might be an acceptable material candidate for bone tissue repair. Novel CS three-dimensional (3-D) rods via an in situ precipitation method by Hu et al. [5,6] showed much higher mechanical properties with bending strength and modulus of 92.4 MPa and 4.1 GPa, respectively. And they were successfully used as a fracture fixation device in animal experiments. All of these CS-based composites have a nonporous structure. It is well-known that a porous structure is necessary for most biomaterials because it can enhance cell adhesion, proliferation and differentiation [7–9]. However, the mechanical properties of CS-based composites are mainly dependent on the pore size. For example, the CS-based 3-D scaffolds prepared by freeze-drying often have big pore size (in tens and hundreds of micrometers) and have poor mechanical properties [10]. Therefore, a suitable balance between the mechanical properties and pore structure is needed. Using a compatible polymer as a porogen can be a good solution to obtain smaller-sized porous CS-based 3-D composites with good mechanical properties. Polyethylene glycol (PEG) is highly compatible with CS and soluble in water. It is expected that PEG can act not only as a modifier to improve the mechanical properties but also as a porogen for CS to induce porous structure.

In the present work, a micro/nanoporous CS-based 3-D composite with good mechanical properties was fabricated from the coagulation of a CS/PEG blend solution in NaOH. Meanwhile, glutaraldehyde (GA) was further added to improve the mechanical properties and chemical stability of the resulting composites. Properties including phase, porous structure, mechanism, and bending strength of the samples were investigated.

EXPERIMENTAL

Materials

Chitosan (pharmaceutical grade, 95% deacetylated, molecular weight evaluated from its intrinsic viscosity, 1.2×10^5) were a product of Zhejiang Aoxing Biotechnology Co., Ltd., China. PEG (average molecular weight: 20000) and glutaraldehyde (GA) were supplied by Chinese Pharmacy Group Shanghai Reagent Company (Shanghai, China). NaOH and acetic acid were analytical grade reagents.

Preparation of the Composites

The preparation of the composites was referred to the method reported by Hu et al. [5]. Selected amounts of CS and PEG with different mass ratios

(CS/PEG: 2/1 and 1/1) were dissolved in 2% acetic acid with continual stirring for about 3 h at room temperature to form a 5% solution. GA (crosslinking agent) solution (0.25%) was added at different concentrations (GA/amino-group of CS: mol/mol) to a given CS/PEG mixture under stirring. The concentrations were 0, 0.1%, respectively. The resulting solution was held for 24 h to remove the air bubbles trapped in the viscous liquid. A small amount of CS solution (5%) was spread on the internal surface of a cylindrical glass mold (about 185 mm in length and 28 mm in diameter). The mold was soaked in the NaOH solution (10%) for 30 min and its internal surface would be covered with a thin CS membrane. The molds were then filled up with the resulting mixture solution and immersed into NaOH solution (10%) for 12 h to get CS/PEG/GA cylindrical gel rods. The resulting rod-like gel could be easily taken out from the mold. They were washed with water until the pH of washed water became about 7. Then the CS/PEG/PEG rod-like gel was air-dried in an oven at 60°C for about 10 h to get CS/PEG/GA dried composites (about 60 mm in length and 8 mm in diameter).

Measurements of the Mechanical Properties of the Composites

Mechanical Property Tests for CS/PEG/GA Composites

Bending strength and modulus of CS/PEG/GA composites were determined by three-point bending tests, which were performed with a universal materials testing machine (SANS Testing Machine Co. Ltd., Shenzheng, China) with a crosshead speed of 2 mm/min. The span length was 38 mm. The ultimate bending strength (σ_b) and bending modulus (E_b) of the CS/PEG/GA composites were calculated according to Eqs. 1 and 2.

$$\sigma_b = 8F_{\max}L/\pi d^3 \tag{1}$$

$$E_b = 4L^3\Delta F/3\pi d^4\Delta l \tag{2}$$

where, $F_{\max}$ is the maximum load at failure (N), L is the supported span (mm), d is the diameter of sample (mm), and $\triangle F/\triangle l$ is the gradient of the linear portion of the load-displacement curve (N/mm).

Measurement of the Porosity of the Composite

The porosity of the composites was measured by the liquid displacement method. Composite samples with certain mass were first placed in a cylinder containing a certain volume (V_1) of ethanol, and treated by cycled vacumm pumping until no bubbles escaped. The total volume of samples and ethanol was marked as V_2. Thus, the solid volume of the samples could be expressed as $V_2 - V_1$. After the samples were removed, the volume of remaining ethanol was recorded as V_3. Then the volume of the ethanol $(V_1 - V_3)$ contained in the samples could be viewed as the volume occupied by the pores of samples and the total volume of the samples was $V=(V_2 - V_1)+(V_1 - V_3)=V_2 - V_3$. The porosity of the composites could be: $P\ (\%)=(V_1 - V_3)/(V_2 - V_3)\times 100\%$.

Scanning Electron Microscope (SEM) Observation

The morphology of the composites (fracture surface after bending tests) was examined in a JEM-6360 scanning electron microscope (SEM). All the samples were coated with gold to improve SEM imaging.

Fourier Transform Infrared (FTIR) Characterization

The FTIR spectra of the samples were measured with a FTIR-Nicolet 740 spectrometer in the wavenumber range of 500–4000 cm^{-1}. Samples for FTIR spectroscopic characterization were prepared by grinding the dry composite samples with KBr and compressing the mixtures to form a sheet.

Differential Scanning Calorimeter (DSC) Analysis

A Perkin-Elmer Pyris 7 DSC was used to measure the thermodynamic properties of the composite samples. The mass of each sample for testing in DSC was controlled at about 7 mg. All experiments were done with dry N_2 flowing through the calorimeter and with a heating rate of 10°C/min.

RESULTS AND DISCUSSION

Morphology of the Composites

The morphologies of the CS/SP/GA composites have been characterized and shown in Figure 1. In comparing, it is found that the crack surfaces of the crosslinked CS rods are smoother (meaning a more brittle fracture) than pure CS rods (Figures 1a, b). This is attributed to the crosslinking of the matrix, resulting in stronger interaction of the CS molecules. Both of the pure CS and the crosslinked CS composites have a nonporous structure. With the addition of PEG, the bending fracture surface of the composites showed porous structure. Much bigger and more open pore structure occurred in the case of simply blending of CS/PEG (1–3 μm, in Figure 1c), comparing with the case that containing GA (0.2–0.4 μm Figure 1d). As shown in Figure 2, the degree of porosity is determined by the content of the PEG and GA. The formation of the pores must be related to the fact that PEG is a high water-soluble polymer. During the

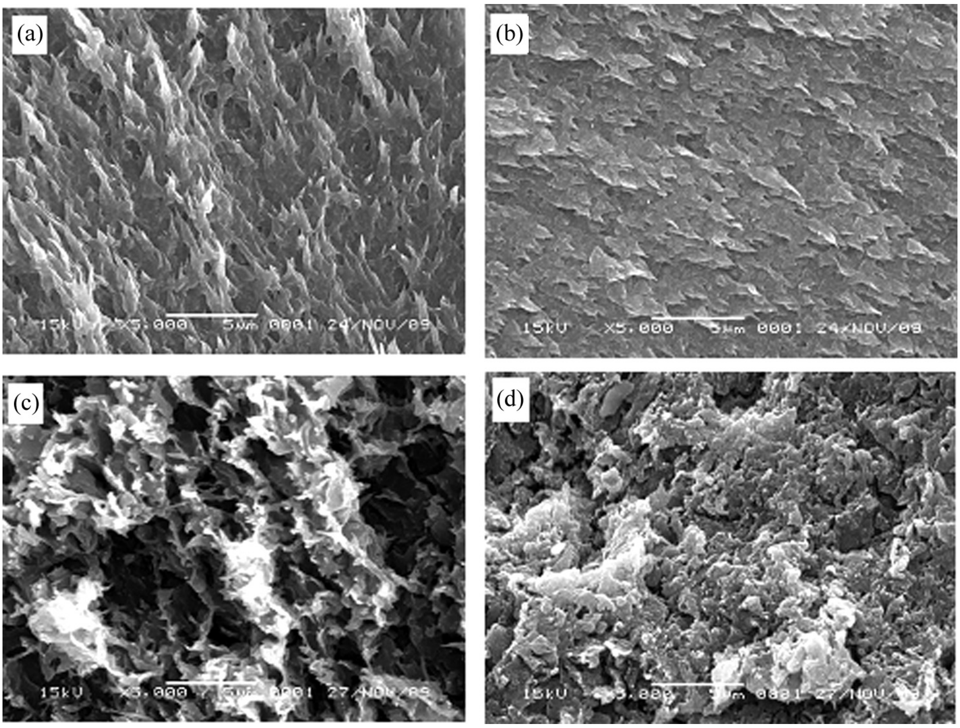

Figure 1: SEM observation of the bending fracture surface of the composites: (a) pure CS; (b) CS/GA (GA mol percentage: 0.1%); (c) CS/PEG (1/1); (d) CS/PEG (1/1)/GA (GA mol percentage: 0.1%).

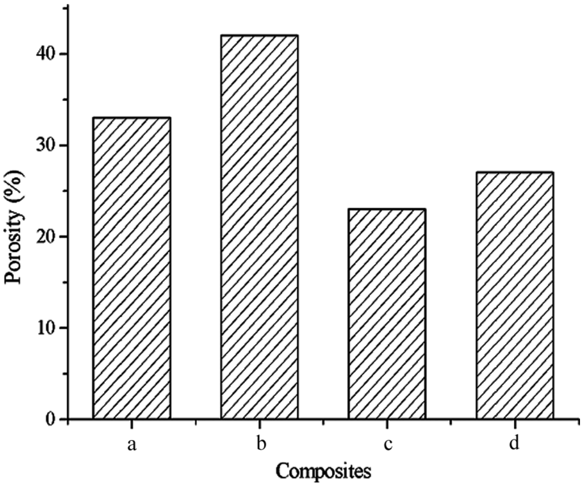

Figure 2: Porosity of the composites: (a) CS/PEG (2/1); (b) CS/PEG (1/1); (c) CS/PEG (2/1)/GA (GA mol percentage: 0.1%); (d) CS/PEG (1/1)/GA (GA mol percentage: 0.1%).

preparation of the composites, they were treated in an aqueous environment for a long time (in precipitation and washing steps). Thus, it is possible that some of the blended PEG component dissolved during the preparation.

Mechanism of the Formation of the Porous Structure

To confirm the dissolution of the PEG during the preparation, FTIR is used to study the microstructure of the CS/PEG/GA composites (as shown in Figure 3). The pure CS showed absorption bands at 1073, 1595 and 1649 cm^{-1}, attributed to C-O stretching, N-H bending, and residual acetylated amide I (C=O stretching), respectively. The absorption band at 3401 cm^{-1} is assigned to the overlapping of N-H and O-H stretching of CS. The main difference between the pure CS and crosslinked CS is that the peak at 1595 cm^{-1} becomes weaker with the addition of GA. This means that the amine groups of CS have reacted with GA to form a Schiff base. However, the Schiff base absorption (C=N) is not observed obviously. The main reason is that the C = N characteristic absorption can not distinguish with acetylated amide I in the FTIR spectrum. The characteristic absorption peaks of PEG is located at 845, 960, 1110, 1240, 1280, 1342, 1472 cm^{-1}. All of

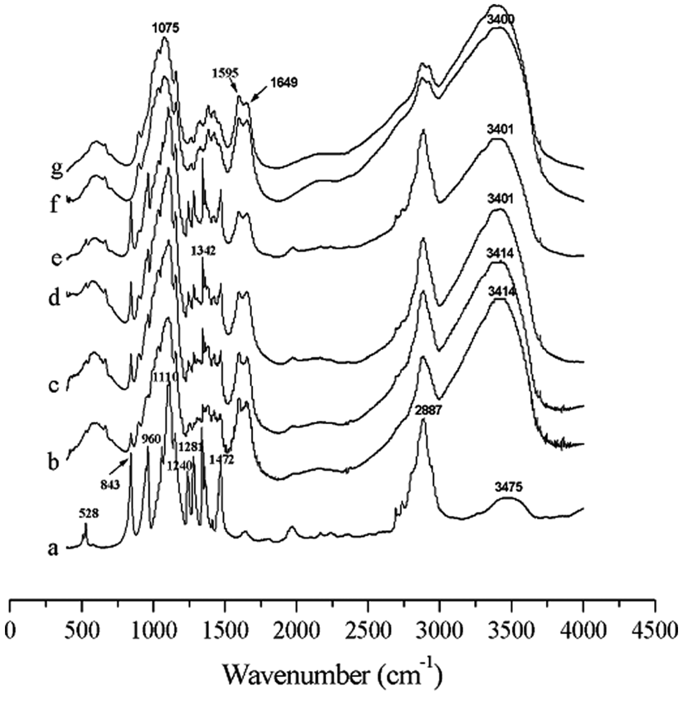

Figure 3: FTIR analysis of the composites: (a) PEG; (b) CS/PEG (2/1); (c) CS/PEG (1/1); (d) CS/PEG (2/1)/GA (GA mol percentage: 0.1%); (e) CS/PEG (1/1)/GA (GA mol percentage: 0.1%); (f) CS/GA (GA mol percentage: 0.1%); (g) CS.

these absorption peaks are observed in the CS/PEG/GA composites. This means that the blended PEG component did not dissolve completely during the preparation. The intensities of these peaks are determined by the amounts of the PEG and GA. The stronger peaks of PEG observed in the cases of CS/PEG (1/1) series than CS/PEG (2/1) series is attributed to the higher PEG content in blending. For a series of CS/PEG with the same mass ratio, the intensity of PEG characteristic peaks in the cases of CS/PEG containing GA is obviously stronger than that of CS/PEG with no addition of GA. This means that the addition of GA will reduce the dissolution of PEG significantly. It is attributed to the facts that GA also has high reactivity with the polar groups (hydroxyl groups) of PEG. Besides being a crosslinking agent for CS, GA acts as a compatibilizer for CS and PEG. Therefore, fewer PEG molecules dissolved in the cases of CS/PEG containing GA.

DSC was carried out to further determine the dissolution behavior of PEG (as shown in Figure 4). PEG is a polymer with high crystallizability and its melting temperature (T_m) is 68°C. Although CS has crystalline regions, the crystalline melting temperature is not found because of its rigid-rod polymer backbone having strong inter- and intramolecular bonding. This behavior is frequently detected in many polysaccharides such as cellulose and chitin derivatives. Lower T_m of PEG within the composites is observed. It is because of the formation of the interaction between CS and PEG molecules leading to a decrease in the crystallizability of PEG. For a series of CS/PEG with the same mass ratio, the intensity of the PEG melting peaks in the cases of CS/PEG

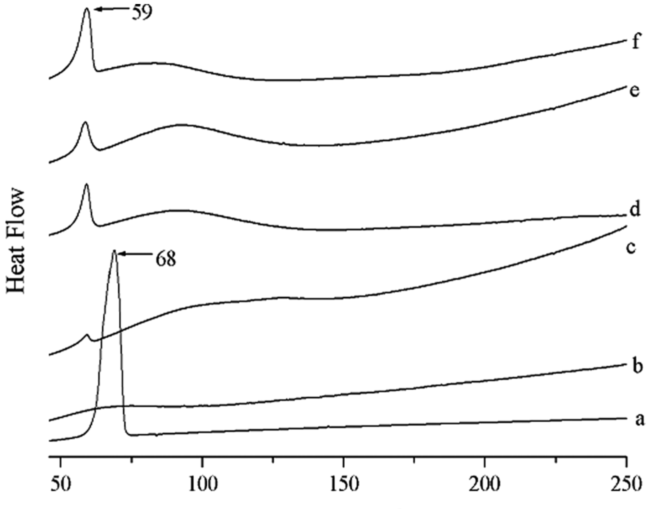

Figure 4: DSC curves of the composites: (a) PEG; (b) CS; (c) CS/PEG (2/1); (d) CS/PEG (1/1); (e) CS/PEG (2/1)/GA (GA mol percentage: 0.1%); (f) CS/PEG (1/1)/GA (GA mol percentage: 0.1%).

containing GA is obviously stronger than that of CS/PEG with no addition of GA. Such results are consistent with the results from FTIR.

In sum, the FTIR and DSC results have confirmed that a part of the blended PEG component dissolved during the preparing process. There is a competition between the interactions of the CS matrix-PEG phase and PEG phase-H_2O molecules during the preparation process. The addition of GA can improve the interaction strength and compatibility between the PEG phase and CS matrix. This will lead to an increase in the difficulty of dissolution of PEG. As a result, the pore size and porosity decrease as GA added. In other words, the phase separation and pore structure can be tailored with the addition of the GA.

Mechanical Properties of the Composites

A desirable material for biodegradable implants should provide enough initial mechanical strength to support the strength of the healing tissue during an early stage. The bending properties of the composites are shown in Figures 5 and 6. The bending strength and modulus of the pure CS rods were 24.5 MPa and 0.25 GPa, respectively. It can be seen in Figures 5 and 6, both the bending strength and modulus of CS/PEG composites are lower than pure CS. Lower mechanical properties of the CS/PEG composites occurred as higher content of PEG blended. It is due to the formation of a much higher porous structure of the matrix that is caused by the dissolution of the PEG component.

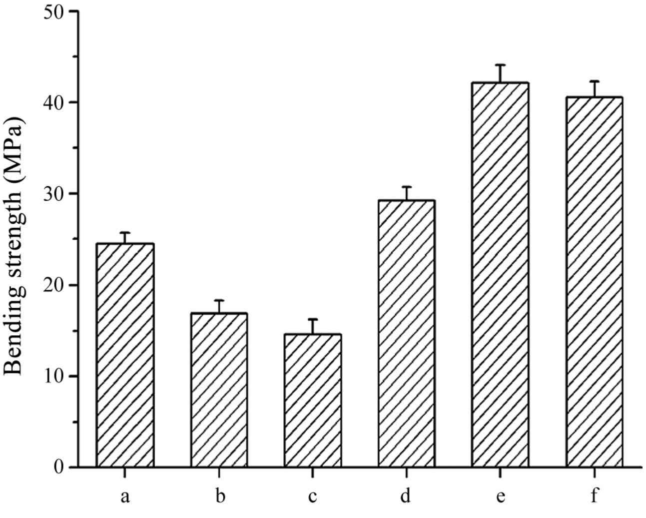

Figure 5: Bending strength of the composites: (a) CS; (b) CS/PEG (2/1); (c) CS/PEG (1/1); (d) CS/GA (GA mol percentage: 0.1%); (e) CS/PEG (2/1)/GA (GA mol percentage: 0.1%); (f) CS/PEG (1/1)/GA (GA mol percentage: 0.1%).

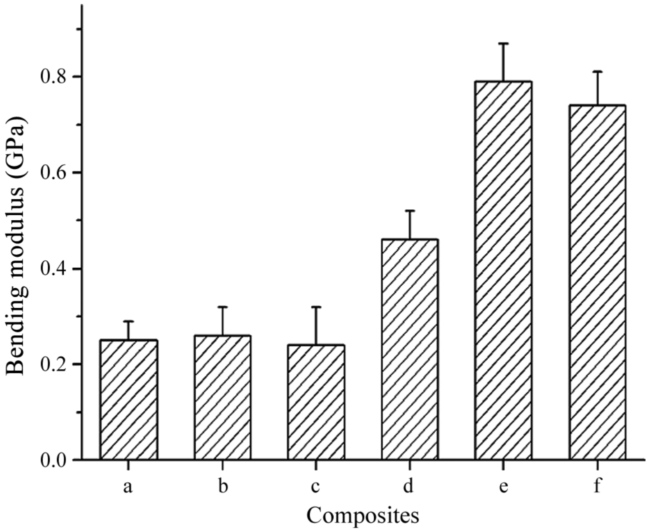

Figure 6: Bending modulus of the composites: (a) CS; (b) CS/PEG (2/1); (c) CS/PEG (1/1); (d) CS/GA (GA mol percentage: 0.1%); (e) CS/PEG (2/1)/GA (GA mol percentage: 0.1%); (f) CS/PEG (1/1)/GA (GA mol percentage: 0.1%).

The mechanical properties of the composites are improved remarkably with the addition of GA. It is mainly because of the crosslinking of the matrix. In comparing, interestingly, the porous crosslinked CS/PEG composites even have higher mechanical properties than nonporous crosslinked CS. On the one hand, much less of the PEG component dissolved during the preparing process, resulting in lower porous structure. On the other hand, for the crosslinked CS/PEG composites, the interfacial interaction between the PEG phase and CS matrix has been improved remarkably with the addition of GA. This good interfacial property is very helpful to stress transitions between the matrix and PEG phase while the composites are performed with outside force. The high crystalline PEG phase acts as physical crosslinking points of the composite. For example, in the case of the crosslinked CS/PEG (1/1) composite, the strength and modulus achieve 40.5 MPa and 0.74 GPa, increasing 65 and 196% compared with CS, respectively. The mechanical properties of which are near and even stronger than other bone replacement materials, such as PMMA [11], bone cement [11], and CS/calcium phosphate composites [4]. It might be acceptable for use in low-load-bearing bone replacement materials.

CONCLUSIONS

Novel biodegradable 3-D composites with porous structure have been prepared by coagulation of a CS/PEG solution in NaOH. It is confirmed that PEG acts not only as a modifier to improve the mechanical properties but also as a porogen for the CS matrix to induce porous structure. The porous structure and mechanical properties of the composites can be tailored with the amount of PEG and GA. This type of porous CS/PEG/GA composites with good initial mechanical properties might be acceptable for use in low-load-bearing bone replacement materials.

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