Thermally Crosslinked Anionic Hydrogels Composed of Poly(vinyl alcohol) and Poly(γ-glutamic acid): Preparation, Characterization, and Drug Permeation Behavior

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ABSTRACT: pH-sensitive anionic hydrogels composed of poly(vinyl alcohol) (PVA) and poly(γ-glutamic acid) (γ-PGA) were prepared by the freeze drying method and thermally crosslinked to suppress hydrogel deformation in water. The physical properties, swelling, and drug-diffusion behaviors were characterized for the hydrogels. In the equilibrium swelling study, PVA/γ-PGA hydrogels shrunk in pH regions below the pK_a (2.27) of γ-PGA, whereas they swelled above the pK_a. In the drug-diffusion study, the drug permeation rates of the PVA/γ-PGA hydrogels were directly proportional to their swelling behaviors. The cytocompatibility test showed no cytotoxicity of the PVA/γ-PGA hydrogels for the 3T3 fibroblast cell lines. The results of these studies suggest that hydrogels prepared from PVA and γ-PGA could be used as orally administrable drug-delivery systems. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 3768–3775, 2008

Key words: drug delivery systems; hydrogels; mechanical properties; polyamides; swelling

INTRODUCTION

Hydrogels are a crosslinked network of hydrophilic polymers that can absorb large amounts of water and swell, while maintaining their three-dimensional structure.[1] In past decades, hydrogels have received much attention for biological applications, such as drug-delivery systems, wound dressing, and tissue engineering scaffolds, because of their biocompatibility with blood, tissues, and cells in the human body.[2,3]

Hydrogels, which have ionic functional groups, are applicable for swelling-controlled drug-delivery systems.[4] Their pH-sensitive swelling behavior depends on the pK_a values of functional acidic groups (e.g., carboxylic and sulfonic acids) or basic groups (e.g., ammonium salts) that either accept or release protons in response to changes in the environmental pH. The ionization of hydrogels causes a difference in the osmotic pressure between the hydrogel phase and the buffer phase and results in a solution flux into the hydrogel.[5–8] Anionic hydrogels are composed of polymers that have pK_a values in the acidic range. Therefore, they can shrink in acidic pH's and swell in basic pH's. This property makes them a candidate for orally administrable drug-delivery systems, which have to pass the gastric pH in a shrunken state to suppress drug release.[9–14]

Poly(γ-glutamic acid) (γ-PGA), produced from *Bacillus subtilis*, is a natural homopolyamide, which is composed of D- and L-glutamic acid units connected by amide linkages between α-amino and γ-carboxylic acid groups (Fig. 1).[15] Because the pK_a value of the β-carboxyl pendent groups in γ-PGA is 2.27, it can shrink in the gastric pH range (pH 2–4). Therefore, γ-PGA is a suitable candidate polymer for orally administrable drug-release systems in association with its good biocompatibility and hydrophilicity. Previously, we tried to make a hydrogel with γ-PGA alone, but its great hydrophilicity, induced from the high charge density, led to low mechanical properties. To overcome this problem, we decided to use poly(vinyl alcohol) (PVA) as a scaffold material by considering its biocompatibility, pH stability, high elasticity, and high swelling behavior.[16,17]

In this study, anionic hydrogels were prepared by the freeze drying method with γ-PGA as an anionic polymer and PVA as a scaffold material, and the two polymers were crosslinked with each other to suppress hydrogel deformation in water by heating at different temperatures for ester bond formation between the carboxylic acid groups of γ-PGA and the hydroxyl groups of PVA. Also, to evaluate the potency of the hydrogels as orally administrable drug-release systems, their physical properties, swelling behaviors, drug-diffusion behaviors, and cytocompatibility were estimated.

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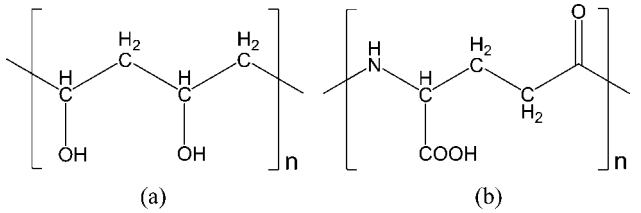

Figure 1 Molecular structures of (a) PVA and (b) γ-PGA.

EXPERIMENTAL

Materials

PVA, which has a degree of polymerization of 1500 and a weight-average molecular weight of 65,000, was provided from Shinyo Pure Chemicals Co., Ltd. (Osaka, Japan). γ-PGA (molecular weight = 400,000–1,000,000) was provided from Ichimaru Pharcos Co., Ltd. (Shinsei, Japan). Swiss albino 3T3 fibroblast cell lines were provided from the Korea Cancer Center Hospital. Dulbecco's Modified Eagle's Medium-F12 growth media, fetal bovine serum, penicillin/streptomycin, and trypsin–ethylene diamine tetraacetic acid (2.5 mg/mL trypsin and 200 μg/mL ethylene diamine tetraacetic acid in Phosphate Buffered Saline [PBS]) were purchased from Gibco BRL Co. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; thiazoyl blue) was purchased from Sigma Chemical Co., USA. All other chemicals were purchased from Sigma Chemical Co.

Preparation of the PVA/γ-PGA hydrogels

γ-PGA and PVA powders were dissolved in deionized water (dH_2O) at 80°C to form homogeneous solutions. Subsequently, these solutions were mixed with various weight ratios of PVA to γ-PGA (γ-PGA/PVA = 0, 0.1, 0.15, 0.2, and 0.25). For hydrogel formation, the mixed solutions were frozen at −20°C for 24 h and then lyophilized according to the method of Madihally and Matthew.[18] The PVA/γ-PGA hydrogels were isothermally annealed in an oven at different temperatures from 120 to 180°C for 2 h. These prepared PVA/γ-PGA hydrogel samples are designated in Table I.

Fourier transform infrared (FTIR) analysis

FTIR spectra were recorded on an FTIR instrument (model 8400S, Shimadzu Co., Japan) to prove ester bond formation during the annealing process. The powdered samples were mixed with exhaustively dried KBr, and transparent discs were prepared by compression.

Morphologies of the PVA/γ-PGA hydrogels

The morphologies of PVA/γ-PGA hydrogel surfaces and cross sections were observed with a scanning electron microscope (model S-3500N, Hitachi, Japan) operated at an accelerating voltage of 20 kV. Before observation, the hydrogel samples were coated with ultrathin layer of Au/Pd in an ion sputterer (model E-1010, Hitachi).

Mechanical properties

The mechanical properties were characterized with a Tahdi texture-recorder (Tahdi). The specimens were rectangular disks ($4 \times 1 \times 0.5$ cm^3). The crosshead speed was 5 mm/min. The ultimate tensile strength (σ) and the fraction stress elongation at a break point were determined, and Young's modulus, or elastic modulus (E), was calculated by the following equation:

$$\sigma = E\varepsilon \quad (1)$$

where ε is the elongation of the PVA/γ-PGA hydrogels.

Swelling behavior

To determine the equilibrium swelling ratios, the γ-PGA/PVA hydrogels were weighed and then placed in PBS for 12 h. The hydrogel surface was blotted with filter paper to remove adsorbed water, and the hydrogel was weighed immediately. The equilibrium swelling ratios of the PVA/γ-PGA hydrogels were calculated by the following equation:

$$\text{Equilibrium swelling ratio (\%)} = [(W_s - W_d)/W_d] \times 100 \quad (2)$$

where W_d is the weight of the dry hydrogel and W_s is the weight of the swollen hydrogel.

Drug-diffusion study

The drug-diffusion experiments were performed with a diaphragm cell according to the method of Falk et al.[19] (Fig. 2). The cell consisted of two chambers separated by the hydrogel (1.5 mm thick). The hydrogel samples were fixed on the center of the partition wall by silicon glue to prevent leakage between each chamber. The left chamber (donor cell) contained a known concentration (5 mg/mL) of *para*-acetaminophen solution dissolved in PBS,

TABLE I
Sample Preparation and Designation of the PVA/γ-PGA Hydrogels

Designation	γ-PGA/PVA weight ratio
PVA	0/100
PE 0.1	10/100
PE 0.15	15/100
PE 0.2	20/100
PE 0.25	25/100

PE is polyelectrolyte.

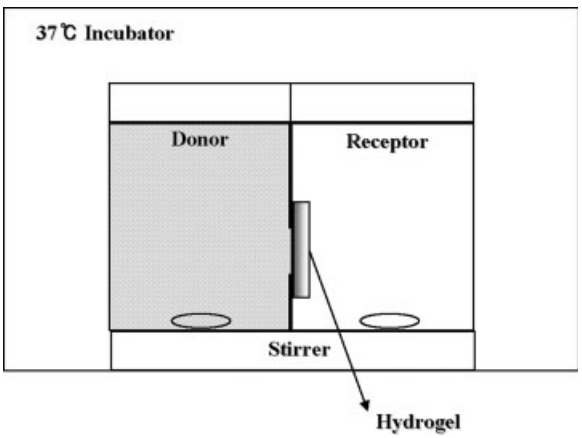

Figure 2 Schematic of the diaphragm cell for the drug-diffusion experiment.

whereas the right chamber (receptor cell) contained PBS solution only. The diaphragm cell was placed in a constant-temperature incubator (37°C) with oscillation at a frequency of 50 rpm. A sample (1 mL) was taken from the receptor cell periodically, and *p*-acetaminophen concentrations were determined with an UV–vis spectrophotometer (model OPTIZEN 2120UV, Mecasys Co., Ltd., Korea) at a wavelength of 265 nm to evaluate the drug-diffusion rates.

Cytocompatibility

The cytotoxicity of PVA/γ-PGA hydrogels was investigated with 3T3 fibroblast cell lines. The cell proliferation test was carried out by direct contact with the Swiss albino 3T3 fibroblast cell lines. The γ-PGA/PVA hydrogel samples were immersed in 70% v/v aqueous ethanol for 24 h for sterilization and then washed with PBS. Cells (1×10^6) were seeded into the γ-PGA/PVA hydrogels and cultivated for 14 days in a 37°C CO_2 incubator with 5% CO_2 and 100% humidity. The cell viability was evaluated by the MTT assay.

RESULTS AND DISCUSSION

Thermal crosslinking

γ-PGA and PVA are highly water-soluble polymers, and therefore, the hydrogels prepared from the two polymers could undergo irreversible deformation in water. To prevent this phenomenon, we performed the thermal crosslinking of PVA/γ-PGA hydrogels at different temperatures (Fig. 3). The hydrogels crosslinked at 150°C and above did not show deformation, but they still maintained a high absorbing capacity for water. Previous work by Arndt et al.[20] proved the ester bond formation during the annealing process of a hydrogel composed of PVA and poly(acrylic acid) by FTIR analysis. The chemical structure of γ-PGA is similar to the synthetic polymer poly(acrylic acid); thus, the main mechanism of the thermal crosslinking of the PVA/γ-PGA hydrogels was considered to be the ester bond formation between the carboxyl groups of γ-PGA and the hydroxyl groups of PVA. The FTIR spectrum of the thermally crosslinked hydrogel at 160°C, in comparison with those of pure PVA and γ-PGA, showed two new absorption bands at 1716 and 1106 cm^{-1}, which represented the C=O and C—O—C stretching vibrations (Fig. 4). The new C=O band at 1716 cm^{-1} proved ester bond formation during the thermal crosslinking process, but the absorption band at 1106 cm^{-1} indicated that unexpected ether bond formation also occurred between the hydroxyl groups of PVA.

Morphologies

The formation of a homogeneous structure during the hydrogel preparation process is important for

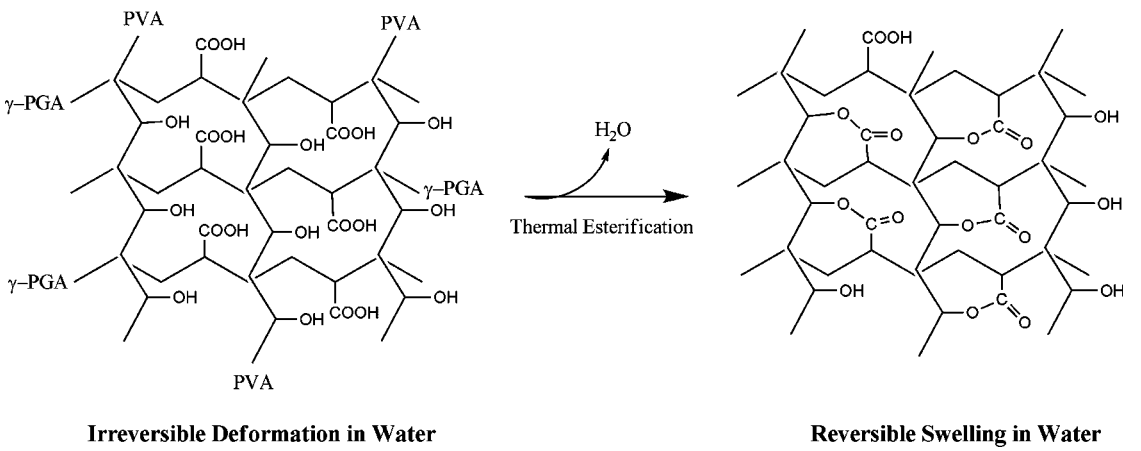

Figure 3 Scheme of thermal crosslinking: ester bond formation between the hydroxyl groups in PVA and carboxyl groups in γ-PGA.

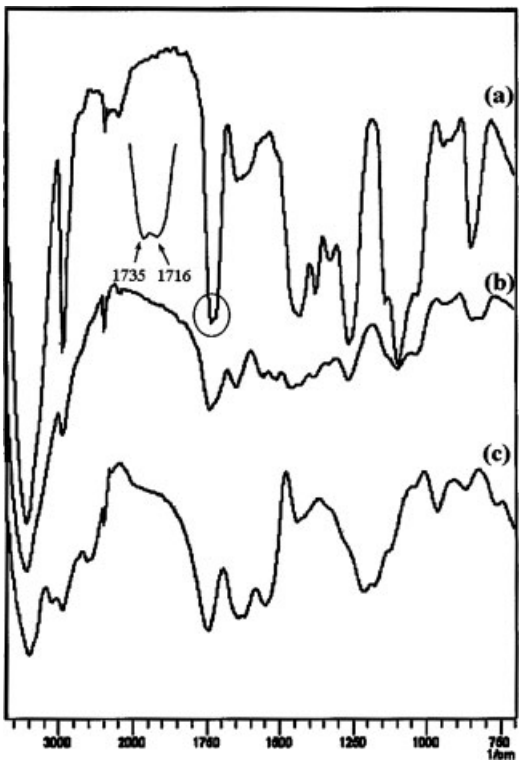

Figure 4 FTIR spectra of (a) the crosslinked hydrogel at 160°C, (b) PVA, and (c) γ-PGA.

the acquisition of reproducible results of swelling and drug-diffusion experiments. To evaluate the homogeneity of the PVA/γ-PGA hydrogels, their microstructures were observed with scanning electron microscopy (SEM). The surface morphologies of the PVA/γ-PGA hydrogels before and after thermal crosslinking are shown in Figure 5. The interconnected three-dimensional porous structures of the hydrogels were retained after thermal crosslinking. However, some other changes occurred with respect to pore size and morphology. The pore sizes of the crosslinked hydrogels were smaller than those of the noncrosslinked hydrogel, but a significant difference was not shown. Accompanying the reduction of the fibers between each pore, more sheet-like structures appeared together with the condensed walls. These results suggest that the morphological difference was mainly caused by thermal crosslinking.

Mechanical properties

The mechanical properties of the PVA/γ-PGA hydrogels were estimated by the measurement of their tensile strengths and elongations at break points with a texture analyzer (Fig. 6). As we expected, the ultimate tensile strength of the PVA/γ-PGA hydrogels increased, whereas the elongations at break points decreased after thermal crosslinking. From the results of tensile strength and elongation, Young's modulus was calculated by eq. (2). The Young's modulus of the PVA/γ-PGA hydrogels increased with increasing crosslinking temperature. This fact indicated that after thermal crosslinking, the PVA/γ-PGA hydrogels became more rigid. The results of the increased tensile strength might have been the combined effect of crosslinking and micromorphological changes after thermal esterification. The main factor that increased the mechanical properties of the PVA/γ-PGA hydrogels was the crosslinking effect established by thermal esterification; however, the sheetlike structure that appeared after thermal treatment may have also contributed to the increased mechanical properties.

Swelling behavior

The swelling behavior of ionic hydrogels in a buffer solution can be explained in terms of volume elasticity and charge quantity of the hydrogels. The equilibrium swelling ratio of ionic hydrogels is formed by the balance between volume elasticity and charge quantity. A degree of charge quantity determines the difference in osmotic pressure between a solid hydrogel phase and a liquid buffer phase, and an osmotic difference leads to water influx into a hydrogel. However, a large swelling degree resulting from a high charge quantity is suppressed by volume elasticity. The charge quantity and the volume elasticity of the PVA/γ-PGA hydrogels varied by thermal crosslinking temperature. In the crosslinking process, some ester bonds were formed between the carboxyl groups of γ-PGA and the hydroxyl groups of PVA. The ester bonds formed by thermal crosslinking decreased the amount of carboxyl groups, which determined the charge quantity, and increased the crosslinking points, which determined the volume elasticity. The results of the swelling experiments show the decreased swelling degree with increasing crosslinking temperature (data not shown).

Another factor that influences the swelling behavior is the environmental pH. The ionization degree of the carboxyl groups in γ-PGA was dependent on the buffer pH. In pH regions below the pK_a, the hydrogel charge quantity decreased by the deionization of the carboxyl groups, whereas above the pK_a, the charge quantity increased by ionization. This was the basic theory of the pH-sensitive hydrogel swelling of the anionic PVA/γ-PGA hydrogels.

Above the pK_a of the carboxyl groups in γ-PGA, swelling occurred:

$$[\mathrm{RCOOH}]_{\mathrm{hydrogel}} + [\mathrm{OH}^-]_{\mathrm{buffer}} \rightarrow [\mathrm{RCOO}^-]_{\mathrm{hydrogel}} + \mathrm{H_2O}$$

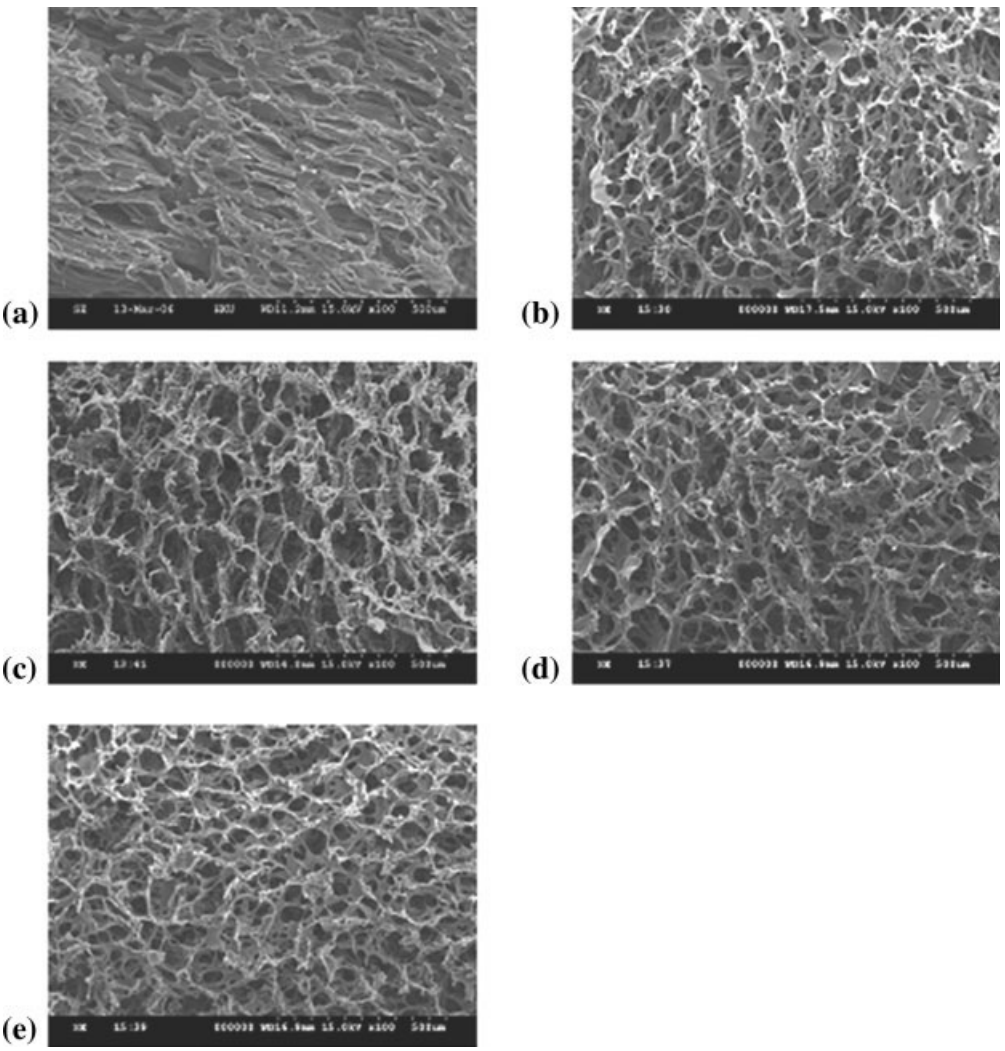

Figure 5 SEM micrographs for the thermally crosslinked PVA/γ-PGA hydrogels: (a) control (PVA hydrogel), (b) noncrosslinked PE 0.25, (c) crosslinked PE 0.25 at 140°C, (d) crosslinked PE 0.25 at 160°C, and (e) crosslinked PE 0.25 at 180°C.

Below the pK_a of the carboxyl groups in γ-PGA, deswelling occurred:

$$[\mathrm{RCOO^-}]_{\mathrm{hydrogel}} + [\mathrm{H^+}]_{\mathrm{buffer}} \rightarrow [\mathrm{RCOOH}]_{\mathrm{hydrogel}}$$

Figure 7 shows the pH-sensitive swelling behaviors of the thermally crosslinked PVA/γ-PGA hydrogels. The hydrogels crosslinked at 160°C showed high pH sensitivity, which might have resulted from the maximum balance between the charge quantity and the volume elasticity. In the reversible swelling experiments, the γ-PGA/PVA hydrogels showed a reversible swelling in response to the change in buffer pH, but after 2 days, the equilibrium swelling degree at pH 1 and 6 increased gradually by partial deformation (Fig. 8).

Drug-diffusion study

To evaluate the applicability of PVA/γ-PGA hydrogels as orally administrable drug-delivery systems, a drug-diffusion study was performed with a model drug, *p*-acetaminophen. The drug-diffusion behavior of the hydrogels was directly related to the swelling behavior. Figure 9 shows the time-dependent drug-diffusion behavior of the PVA/γ-PGA hydrogels. The drug-diffusion rate, the slope in Figure 9, decreased as the crosslinking temperatures increased. The drug-diffusion behaviors at pH 1.0 (below the pK_a) and pH 7.4 (above the pK_a) were also measured with the PVA/γ-PGA hydrogels crosslinked at 160°C, which showed a high pH sensitivity in the swelling experiment. The results show that the drug-diffusion rate decreased remarkably at pH 1.0 compared with that at pH 7.4. In sum, the drug-diffusion rates were proportional to the equilibrium swelling degrees of the PVA/γ-PGA hydrogels. Therefore, we could control the drug-release rate by changing the crosslinking temperature and buffer pH. Also, the PVA/γ-PGA hydrogels could suppress the drug release in the gastric pH range, and there-

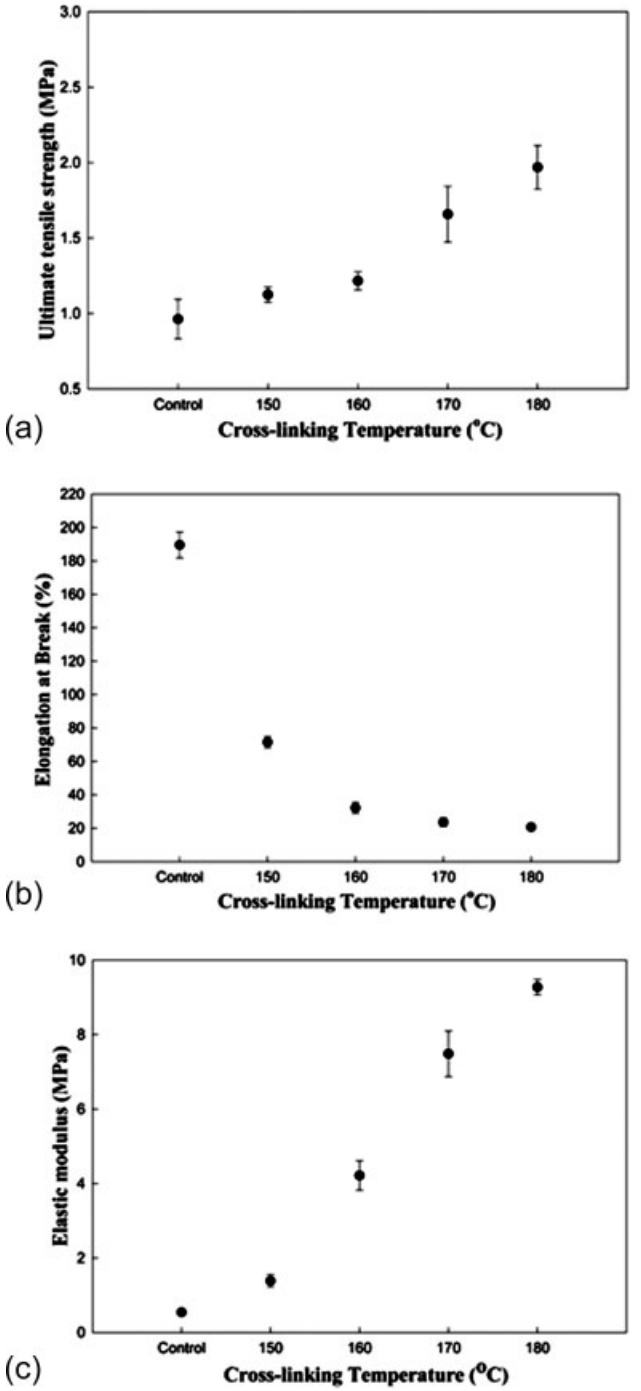

Figure 6 Mechanical properties of the thermally crosslinked PVA/γ-PGA hydrogels: (a) ultimate tensile strength, (b) elongation at break points, and (c) elastic modulus.

fore, they are suitable material for orally administrable drug-release systems.

Cytocompatibility

A cytocompatibility test is very important for biological applications of hydrogels. To investigate the

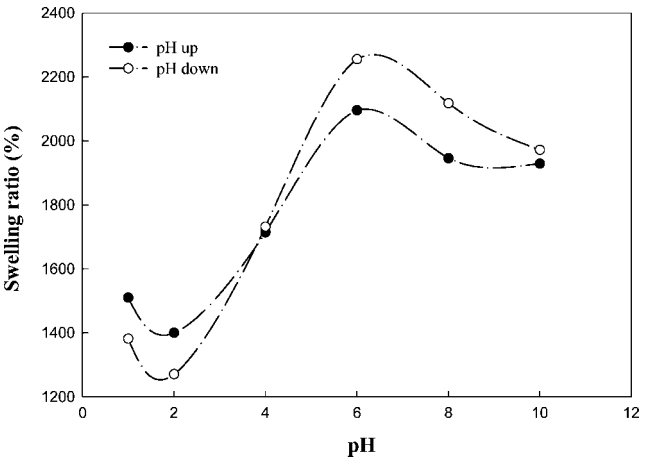

Figure 7 Equilibrium swelling ratios of the PVA/γ-PGA hydrogels as a function of buffer pH.

cytocompatibility of the PVA/γ-PGA hydrogels, the cell attachment and proliferation degrees were evaluated with Swiss albino 3T3 fibroblast cell lines by MTT assay (Fig. 10). The result showed no cytotoxicity of the PVA/γ-PGA hydrogels. Also, a significant difference was found for the thermally crosslinked PVA/γ-PGA hydrogels compared with noncrosslinked PVA. In cell attachment and subsequent proliferation, the surface structure of a hydrogel is a critical factor. In the SEM observation study, the microstructures of the thermally crosslinked hydrogels were different from those of the noncrosslinked hydrogels. The annealing process contributed to form the more sheetlike structures of the hydrogels, and the sheetlike structures provided a good environment for the cells to attach. Therefore, the

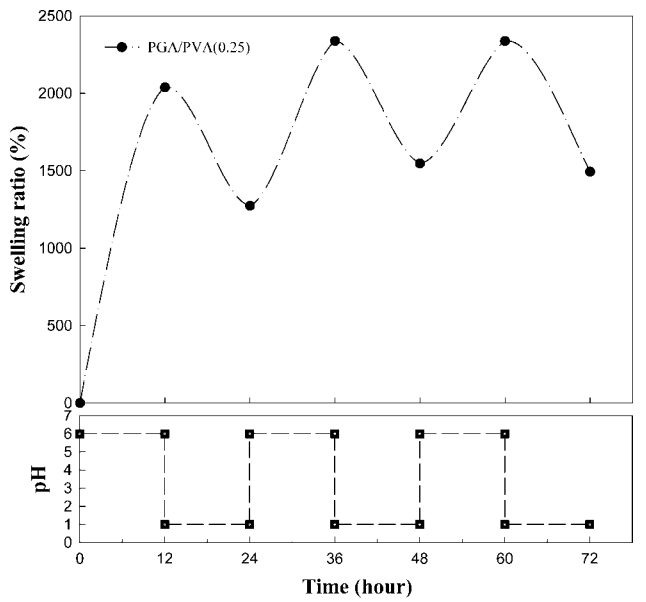

Figure 8 Reversible swelling behavior of the PVA/γ-PGA hydrogels.

increased cell proliferation may have originated from the good attachment rate of the cells.

CONCLUSIONS

pH-sensitive anionic hydrogels composed of PVA and γ-PGA were introduced as orally administrable drug-delivery systems. To suppress hydrogel deformation in water, the hydrogels were crosslinked by a thermal crosslinking method at different temperatures. The thermal crosslinking mechanism was not clear, but the suggested main mechanism was ester bond formation between the carboxyl groups of γ-PGA and the hydroxyl groups of PVA. The physical properties test revealed a more rigid structure of the thermally crosslinked hydrogel compared with the noncrosslinked hydrogel. In the swelling experiments, the anionic hydrogels displayed pH-sensitive swelling characteristics. The anionic hydrogels shrunk in the acid pH region, below the pK_a (2.27) of the carboxyl pendent groups, whereas they swelled in the neutral and basic pH regions above the pK_a. The drug-diffusion rates of the anionic hydrogels were directly proportional to the equilibrium swelling degrees. In the cytocompatibility test, the thermally crosslinked anionic hydrogels did not show any cytotoxicity for the 3T3 fibroblast cell lines. From these results, we expect that the anionic hydrogels examined in this study will be potent drug-delivery systems for oral administration.

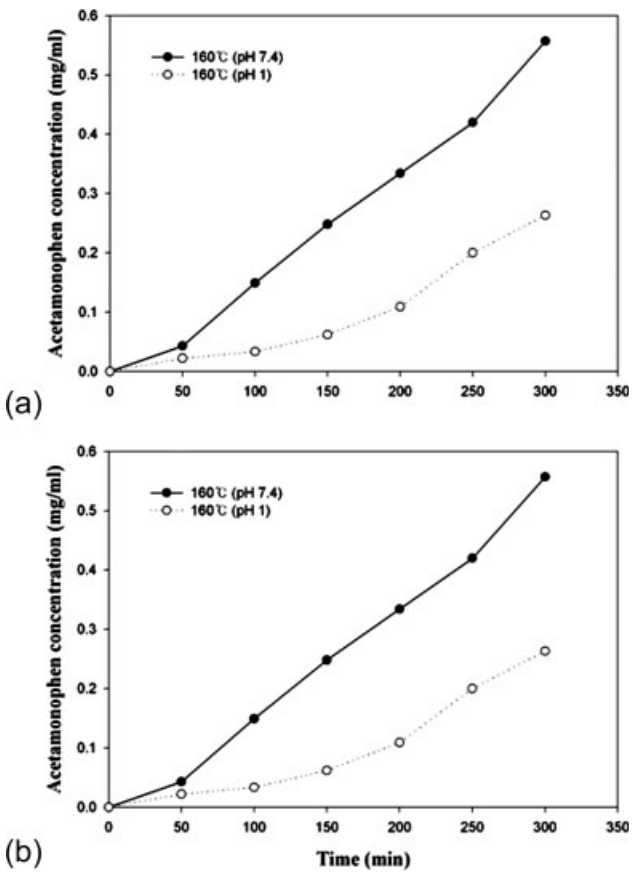

Figure 9 Drug-diffusion behaviors of the thermally crosslinked PVA/γ-PGA hydrogels: (a) the effect of crosslinking temperature and (b) the effect of buffer pH (pH's 7.4 and 1).

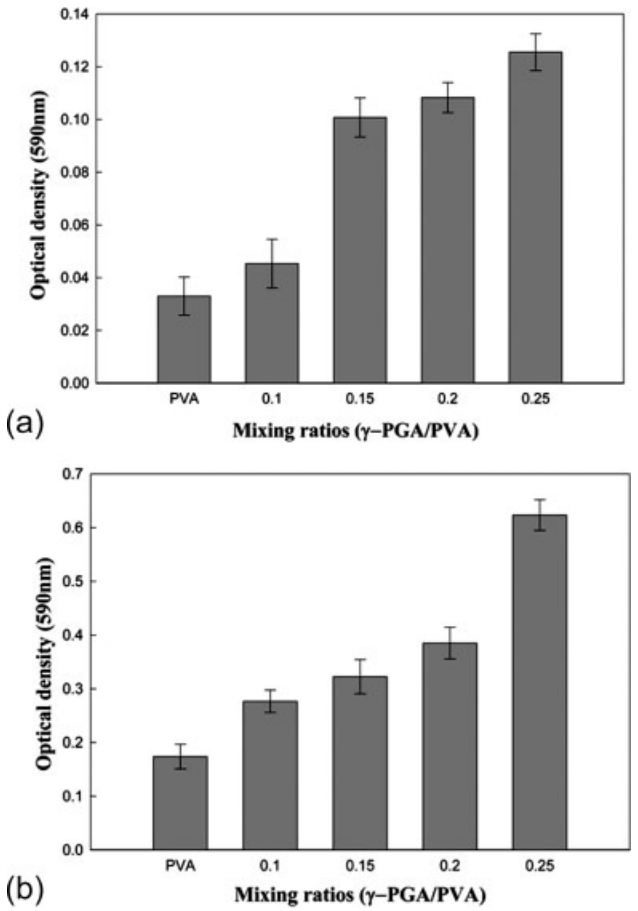

Figure 10 *In vitro* cytocompatibility of the thermally crosslinked PVA/γ-PGA hydrogels determined by the 3T3 fibroblast cell line: (a) cell attachment and (b) cell proliferation.

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