JOURNAL OF Applied Polymer SCIENCE

Reinforcement of pH-Responsive γ-Poly(glutamic acid)/Chitosan Hydrogel for Orally Administrable Colon-Targeted Drug Delivery

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ABSTRACT: Orally administrable hydrogel was prepared by crosslinking chitosan (CS) with γ-poly(glutamic acid) (γ-PGA) for an excellent pH-responsive colon-targeted drug delivery system. The stable crosslinked amide bond appeared in the shifted region of FTIR spectroscopy, and the tensile strength and elastic modulus were also reduced by crosslinking of CS and γ-PGA. The surfaces of crosslinked hydrogel have a homogeneous pore array with pore size corresponding to the varied blending ratio. The swelling ratio was dramatically changed by increasing the pH from 3 to 6, and the responsiveness of swelling ratio to the reversible pH changes between 3 and 10 was reliable for 72 h. The drug diffusion rate was mainly dependent on the pH, and a water-soluble tetrazolium (WST-1) assay indicated that cytocompatibility of the hydrogel was in an acceptable range. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: hydrogel; drug delivery; pH responsibility; chitosan; γ-poly(glutamic acid); PGA

Received 31 March 2010; accepted 24 February 2012; published online
DOI: 10.1002/app.37565

INTRODUCTION

Hydrogels, novel hydrophilic polymers, which consist of chemically or physically crosslinked three-dimensional networks, have been used in a wide range of biological studies and medical applications, including drug-delivery systems[1,2] and wound dressing.[3,4] Natural polymers such as chitosan (CS),[5–11] poly(L-glutamic acid),[6,8] alginate,[5] gelatin, hyaluronic acid, γ-poly(glutamic acid) (γ-PGA),[6,7,10,11] and collagen[12] have been intensively used in those various biological applications because of their inherent biocompatibility and excellent biodegradability.

Specifically, hydrogels with good pH responsiveness, biocompatibility, and large swelling capability[1,2,5,6,9] have been recognized as an excellent carrier in drug delivery systems. Additionally, there are two more requirements for the hydrogels in an orally administrable drug delivery system. One is the chemical stability in various digestive organs. For example, the CS hydrogel beads[13] for commercial drug delivery systems have been usually coated with Eudragit S100 for preventing deformation by the acidic environment in the stomach. The other consideration is a smart pH responsiveness of the swelling behaviors for an *in vivo* system.

Previously, we have reported that the polyelectrolyte complex (PEC) of γ-PGA/CS hydrogel had an excellent pH responsiveness; the swelling behaviors were successfully controlled by the pH conditions.[14] However, the stability was too weak in the strong acidic condition of the stomach (below pH 3) for an application of orally administrable drug delivery. In this study, we reinforced the chemical stability of the γ-PGA/CS hydrogel while maintaining the excellent pH responsibility by an appropriate chemical crosslinking for the practical applications in colon-targeted administrable drug delivery.

EXPERIMENTAL

Materials

CS (degree of deacetylation = 85%, weight-average molecular weight = 400,000 g/mol) was purchased from Jakwang, Korea. Phytocollagen, the main active component of which is γ-PGA obtained from fermented soybeans, was purchased from Ichimaru Pharcos (Shinsei, Japan). *N*-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide (EDC), *N*-hydroxysuccinimide (NHS), and water-soluble tetrazolium (WST-1) salts were purchased from Sigma. Neo-human dermal fibroblast (NHDF) cell lines were

Additional Supporting Information may be found in the online version of this article.

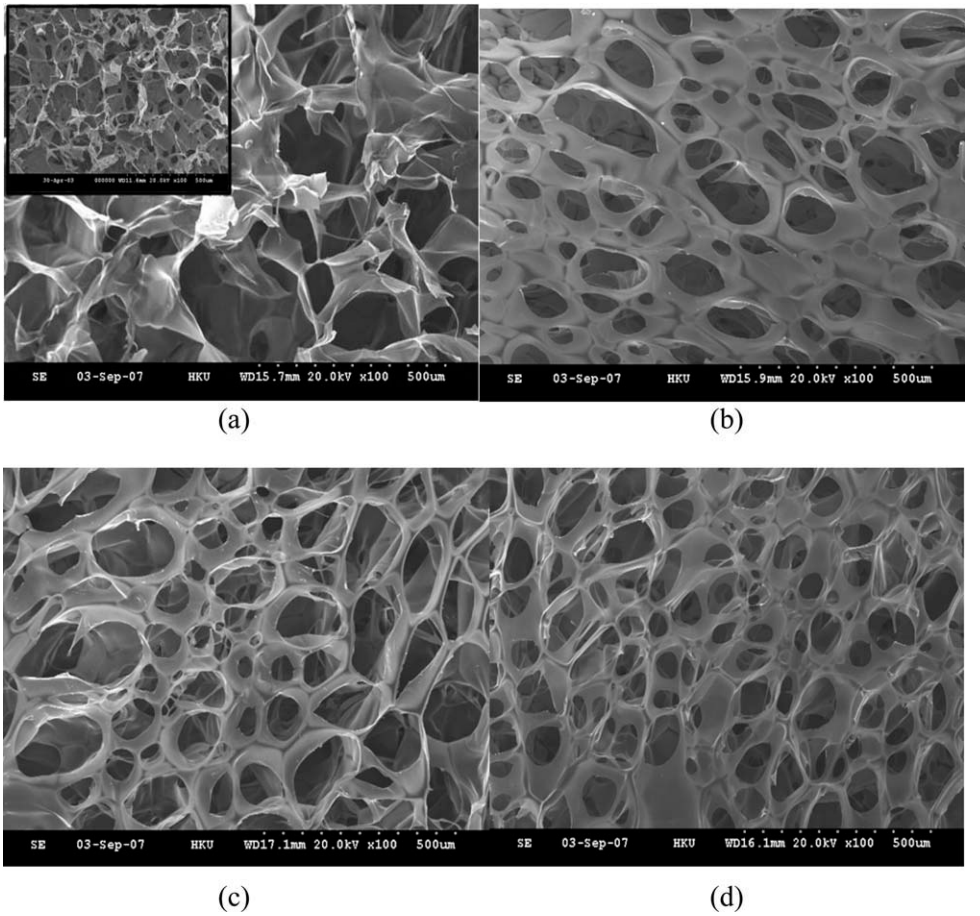

Figure 1. SEM images of the CS and crosslinked γ-PGA/CS hydrogels: (a) with the blending ratios (γ-PGA/CS) of 0.01*, (b) 0.2, (c) 0.6, and (d) 1.0. *The boxed image is the surface of CS hydrogel.

provided by the Korea Cancer Center Hospital. Dulbecco's modified Eagle's medium F12 growth media, fetal bovine serum, penicillin/streptomycin, and trypsin-EDTA were purchased from Gibco® (Okla).

Preparation of CS Hydrogel and Crosslinked γ-PGA/CS Hydrogel

CS solution was prepared in a 1% (v/v) acetic acid aqueous solution with 2% (w/v) CS. The solution was frozen at −20°C, lyophilized, and then the prepared CS hydrogel was washed with deionized water overnight and relyophilized. CS and γ-PGA solutions were mixed in a 1% (v/v) acetic acid aqueous solution with varied ratios of CS and γ-PGA (γ-PGA/CS = 0.01, 0.2, 0.6, and 1.0, w/w). These solutions were thoroughly agitated overnight, and thereafter lyophilized at −20°C. The completely dried hydrogels were thoroughly rinsed with deionized water and relyophilized. The lyophilized γ-PGA/CS hydrogels were rinsed with acetone and moved to an EDC- and NHS-containing acetone solution. The molar ratio of the EDC : NHS : γ-PGA-carboxylic acid groups was controlled as 10 : 10 : 1.[12] The crosslinking was then allowed to occur at 4°C overnight. The crosslinked CS/γ-PGA hydrogels were washed with a 4*M* Na_2HPO_4 solution for 2 h, with deionized water overnight, and lyophilized.

Characterization of Crosslinked γ-PGA/CS Hydrogels

The FTIR spectra of CS, γ-PGA hydrogel, and γ-PGA/CS hydrogel were measured with an 8400S spectroscope (Shimazu, Japan) to assign the changes in chemical structure of the prepared CS and γ-PGA/CS hydrogel. Specifically, the amide bond that was formed by crosslinking the amine group of the CS and the carboxylic acid group of γ-PGA was measured. The morphological images of the CS hydrogel and crosslinked γ-PGA/CS hydrogels were obtained with a S-3500N SEM (Hitachi, Japan). The mechanical stabilities of the hydrogels were estimated by a TAHD plus (SMS, UK). The specimens were cut into rectangular disks (4 cm × 1 cm × 0.5 cm). The crosshead speed was 5 mm/min. The fractional stress elongation and the ultimate tensile strength were determined. The elastic modulus (E) was calculated by the following equation:

$$\sigma = E\varepsilon, \tag{1}$$

where σ is the ultimate tensile strength, E is the elastic modulus, and ε is the elongation of the hydrogel.

Swelling Behaviors

The swelling ratio of the hydrogel was estimated as follows: the γ-PGA/CS hydrogel was weighed and then soaked in phosphate

buffered saline (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 ratio of the γ-PGA/CS 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 dried hydrogel and W_s is the weight of the swollen hydrogel.

Drug Diffusion Behaviors

The applicability of the crosslinked hydrogel to a drug delivery system was estimated by drug diffusion study. The study was performed with a diaphragm cell according to the method proposed by Falk et al.[9] The cell consisted of two chambers separated by the hydrogel (diameter = 30 mm; thickness = 1.5 mm). The *para*-acetaminophen, which was extracted from commercial Tylenol by the Soxhlet method, was used as a model drug. The hydrogels were fixed on the center of the diaphragm by silicon glue. The donor cell contained a *para*-acetaminophen solution, which was dissolved in PBS at a known concentration (5 mg/mL), and the receptor cell contained only PBS. The diaphragm cell was incubated at 37°C. Supporting Information Data S4 denote the schematic of the diaphragm cell for the drug diffusion experiment. Time-dependent concentration exchange of *para*-acetaminophen was determined by periodical sampling with an OPTIZEN 2120 UV spectrometer (Mecasys, Korea) at a wavelength of 265 nm.[15]

Cytocompatibility

The cytotoxicity of the crosslinked hydrogel was investigated with NHDF cell lines. The control specimen was CS hydrogel. All of the hydrogel samples were immersed in 70% ethanol for 48 h for sterilization and then thoroughly washed with deionized water. Then NHDF cell lines (1×10^6) were seeded into the hydrogels and were cultivated for 24 h at 37°C with 5% CO_2 and 100% humidity. Cell viability was evaluated by WST-1 assay. Because WST-1 is cleaved to formazan, which has an absorbance maximum at 450 nm, the absorbance of WST-1 was measured at a wavelength of 450 nm.

RESULTS AND DISCUSSION

Characterization of Crosslinked γ-PGA/CS Hydrogels

The shifted peak of the newly formed amide bond appeared at 1647 cm^{-1} in the FTIR spectrum of the crosslinked hydrogel, whereas only the typical carbonyl peak of carboxylic acid at 1747 cm^{-1} was observed for γ-PGA (Supporting Information Data S1). This result indicates that the carboxylic acid moieties in the γ-PGA have been changed to crosslinking amide groups through the chemical reaction with amine functionalities in CS to form crosslink junctions in the hydrogel.

There are several well-known factors that affect drug release from polymer matrix, namely, surface area, diffusion coefficient, and concentration gradient of a drug. Among them, the surface area is closely correlated with the morphology. Thus, the homogeneity of the pore size at the surface of a hydrogel is an important structural requirement to obtain a reproducible steady result in swelling and drug diffusion.[16,17] Figure 1 shows the

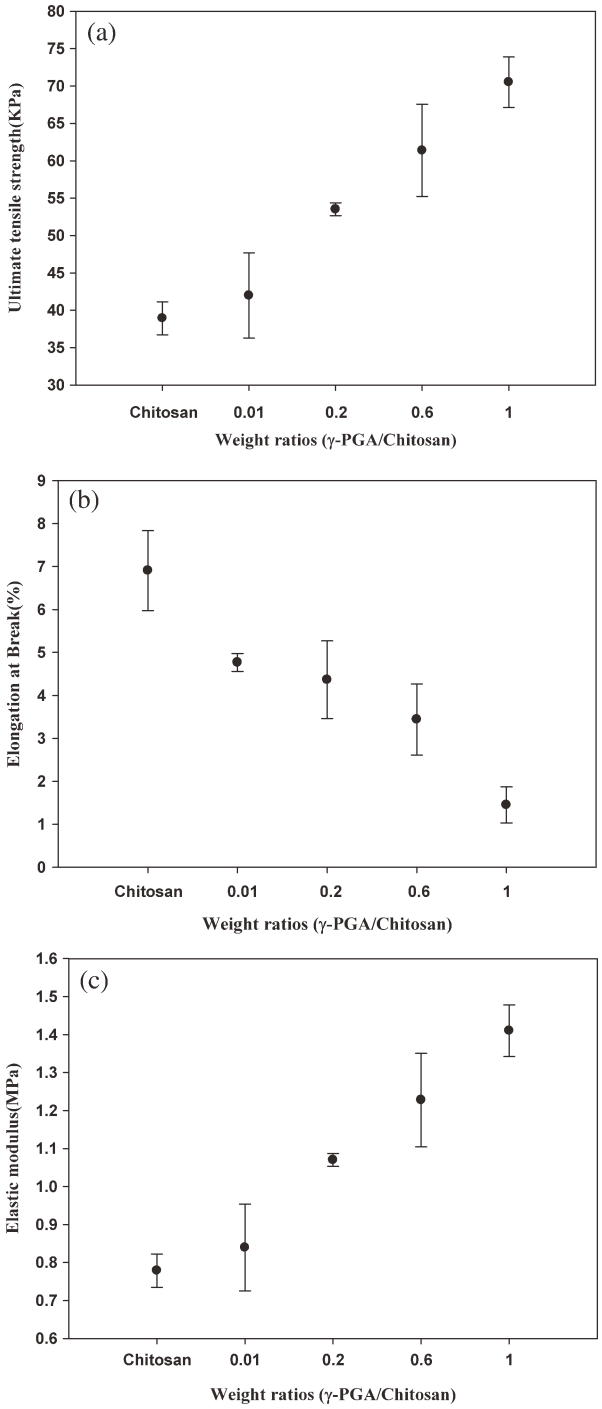

Figure 2. Mechanical properties of the crosslinked γ-PGA/CS hydrogels: (a) ultimate tensile strength, (b) elongation, and (c) elastic modulus.

structural changes at the surface of crosslinked hydrogels by the various blending ratios. CS hydrogel [the boxed image in Figure 1(a)] has a very bulky and uneven morphology, but the γ-PGA/CS crosslinked hydrogel has a homogeneous porous surface, and the size of the pores was dramatically decreased with the increased blending portion of γ-PGA (Supporting Information Data S2). Because CS has strong intermolecular hydrogen bonds, it aggregates during the process of CS hydrogel

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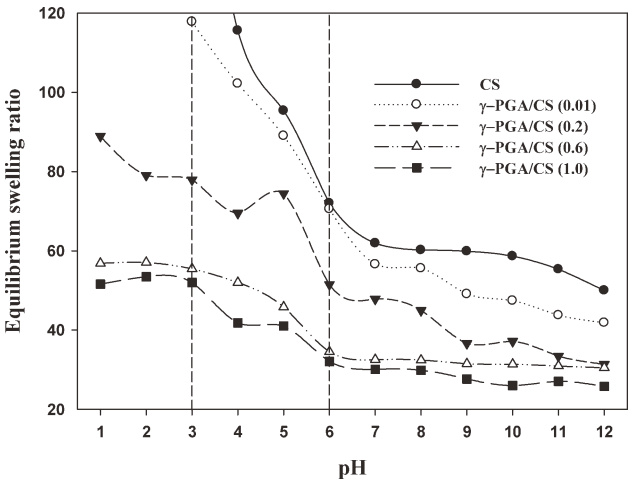

Figure 3. The swelling behaviors of the crosslinked γ-PGA/CS hydrogels with various blending ratios.

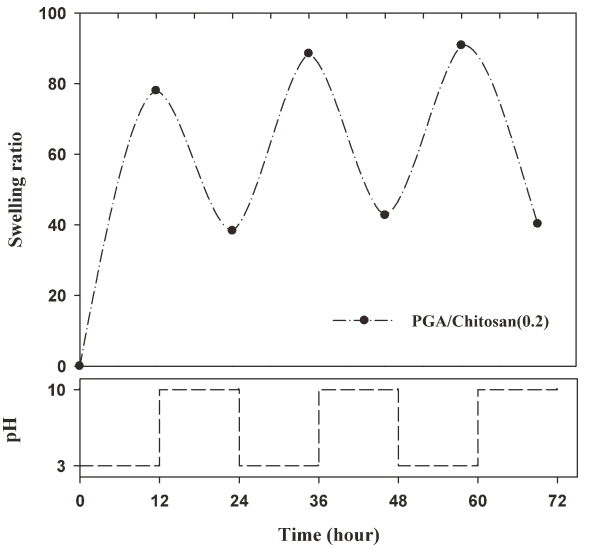

Figure 4. Reversible swelling behavior of the crosslinked γ-PGA/CS hydrogel (blending ratio of 0.2).

preparation, and a bulky surface forms between each pore in the hydrogel. However, because strong intermolecular hydrogen bonds are disturbed by the ionic interaction with γ-PGA, γ-PGA/CS hydrogel has no bulky surfaces.[14] The mechanical properties, which are closely correlated with corresponding stability, were estimated by measurements of the tensile strengths, elongations, and elastic modulus at the break points (Figure 2). The tensile strength and the elastic modulus were increased, and the elongation at the break point was decreased with the increase of the γ-PGA blending ratio. The ultimate tensile strength and the elastic modulus of the γ-PGA/CS crosslinked hydrogel were significantly lower than the PEC γ-PGA/CS hydrogel (no crosslinking) in our previous study.[14] This means that the tensile strength and the elastic modulus were also reduced by crosslinking of CS and γ-PGA. These results were well matched with the FTIR results. The corresponding stability was increased by the formation of the crosslinking amide junction between CS and γ-PGA.

Swelling Behaviors

The swelling equilibrium is mainly determined by a balance of the elastic retractile responses of the polymer network and the net osmotic pressure. For application in colon-targeted drug delivery, the hydrogel should not be dissolved in the stomach (below pH 3). In our previous study, the simply blended (no chemical crosslinking) γ-PGA/CS and CS hydrogels were totally dissolved in an acidic (below pH 3) solution. However, the chemically crosslinked γ-PGA/CS hydrogel in this study remained intact without dissolving in the series of acidic solutions from pH 1 to 3.

Figure 3 shows the results of swelling behaviors of the γ-PGA/CS hydrogels with various blending ratios in PBS solution (from pH 1 to 12). The equilibrium swelling ratios of the crosslinked γ-PGA/CS hydrogels gently decreased with pH in the strong acidic condition (from pH 1 to 3). The most dramatic change was observed between pH 3 and 6. This behavior is adequate for colon-targeted drug delivery, because the hydrogels can deliver a drug safely to release in colon, while passing through the strong acidic environment of the stomach. Specifically, the hydrogel with a blending ratio of 0.2 shows an ideal behavior for colon-targeted drug delivery. In the strong acidic range (below pH 3), the equilibrium swelling ratio was saturated (more than 80), that is, there will be no meaningful release in a strong acidic condition. Drug release can begin above pH 3 and sustained until pH 9. The responsiveness of the hydrogel to a reversible condition was also excellent; the swelling ratio was reliable for 72 h and reversible with pH changes between 3 and 10 (Figure 4).

Drug Diffusion Behaviors

The drug diffusion study was performed with a model drug, *para*-acetaminophen (Figures 5 and 6). The released concentration of the model drug at pH 7.4 was time dependently increased (Figure 5). Release rate was dependent on the pH

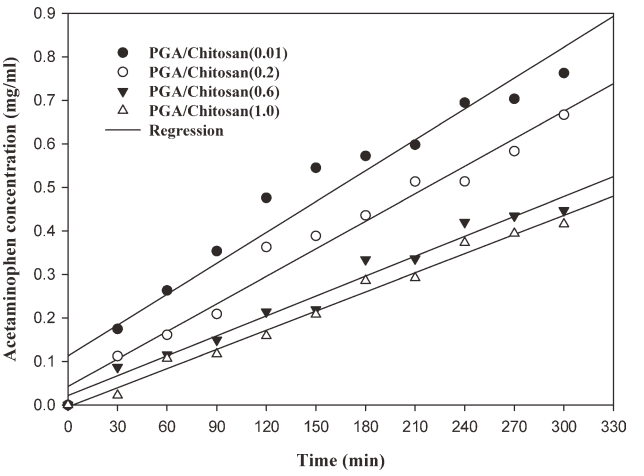

Figure 5. Drug diffusion behaviors of the crosslinked γ-PGA/CS hydrogels with various blending ratios at pH 7.4.

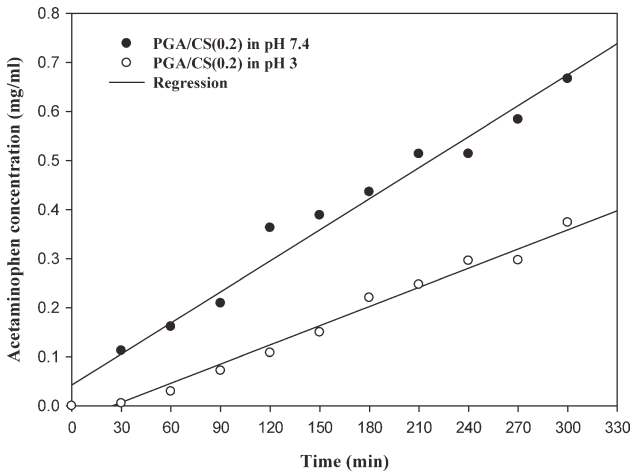

Figure 6. Drug diffusion behaviors of the crosslinked γ-PGA/CS hydrogels (blending ratio of 0.2) at pH 3 and 7.4.

(Figure 6 and Supporting Information Data S3) rather than the blending ratio (Figure 5 and Supporting Information Data S3). The drug release in pH 7.4 occurred immediately, and the concentration increased more rapidly (almost twice as fast) than in pH 3 (Figure 6). The release of *para*-acetaminophen at pH 3 was delayed for 30 min. This observation means that the γ-PGA/CS hydrogels could suppress the drug release in the gastric pH range, for example, we could control the drug release rate by the blending ratio and the pH condition.

Cytocompatibility

Cytocompatibility is also one of the important factors to be considered for evaluating the potential of a hydrogel in application of an orally administrable drug carrier. The cell viability on the crosslinked hydrogels was estimated with NHDF cell lines by WST-1 assay (Figure 7). WST-1 was cleaved to formazan by interaction with glycolytic NAD(P)H enzyme of viable cells, and the amount of formazan dye formed directly correlates to the number of metabolically active cells. The cell viability within the γ-PGA/CS hydrogels was higher than the control CS hydrogel, which is nontoxic to cells. This means that the crosslinked γ-PGA/CS hydrogels make a comfortable environment for cell attachment and offers good cytocompatibility for the NHDF cell lines.

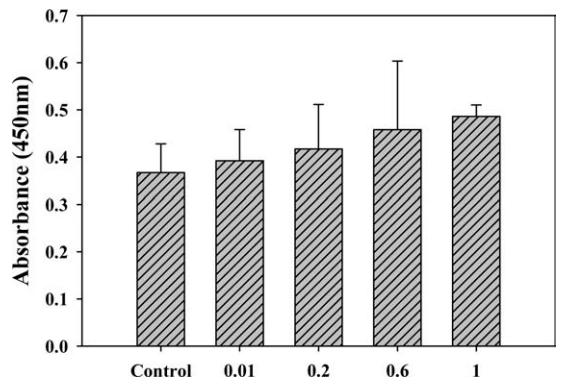

Figure 7. Cytocompatibility test of the crosslinked γ-PGA/CS hydrogels.

CONCLUSION

The pH-sensitive γ-PGA/CS hydrogels were introduced as orally administrable drug delivery systems. The γ-PGA/CS hydrogels was successfully reinforced by a chemical crosslinking method using EDC/NHS. Mechanical property testing revealed a more reinforced structure of the chemically crosslinked hydrogel, and the carbonyl peaks were shifted after the chemical crosslinking. The FTIR results indicate that the amide bonds were successfully formed with the amine group of CS and the carboxylic group of γ-PGA. In the swelling and drug diffusion behaviors, we could control both by the blending ratio of CS and γ-PGA and the pH condition. The γ-PGA/CS hydrogels showed cytocompatibility. From the adequate mechanical stability, excellent swelling and drug releasing behavior, and good cytocompatibility, we expect that the crosslinked hydrogels have excellent potential in medicinal applications, especially as orally administrable, pH-sensitive drug carriers, for colon-targeted drug delivery.

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