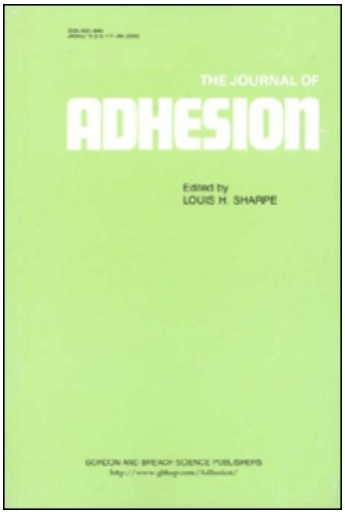

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Adhesion to Soft Tissues by Gelatin-Polyanion Hydrogels*

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(In final form January 31, 1996)

This study describes high potentiality of hydrogels composed of geleatin and poly(L-glutamic acid) (PGLA) as a biological glue for soft tissues compared with a conventional fibrin glue. The mixed aqueous solution of gelatin and PGLA set to a hydrogel by use of water-soluble carbodiimide as rapidly as the fibrin glue. The cured hydrogel exhibited firm adhesion to soft tissues with a higher bonding strength than the fibrin glue. In addition, the inflammatory response to the hydrogel subcutaneously implanted in mice was mild and the hydrogel was gradually absorbed with time in vivo.

KEY WORDS: Gelatin; poly(L-glutamic acid); water-soluble carbodiimide; biodegradable hydrogel; biological adhesive; soft tissue adhesion; bond strength of gelatin-polyanion hydrogel; inflammatory response.

INTRODUCTION

Biological adhesives have been used for tissue adhesion and hemostasis in surgery[1–4]. However, they have several problems which need to be overcome for their clinical use. For example, the stiffness of the solid formed after polymerization of cyanocrylate is much higher than that of normal soft tissues, although the cured material has an acceptable strength for use as a surgical adhesive. In addition, formaldehyde produced from degradation is toxic. Fibrin glue, the most widely used surgical adhesive, does not always have sufficient mechanical strength. The possibility of infection transfer cannot be completely ruled out because the origin of the fibrin glue is human blood. Thus, it is necessary to develop new, non-toxic biological adhesives applicable for human use. The present study was undertaken to assess the efficacy of hydrogels formed by chemical crosslinking of gelatin and poly(L-glutamic acid) as a bilogical glue. Its bonding strength was measured by using isolated mouse skins to compare it with that of the conventional fibrin glue.

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EXPERIMENTAL

Materials

The gelatin used was alkaline-processed ($Mw = 99{,}000$, isoelectric point (pI) 5.0, Nitta Gelatin Co., Ltd., Osaka, Japan). Poly(L-glutamic acid) (PGA, DP = 550) was kindly supplied by Ajinomoto Co., Ltd., Tokyo, Japan. As crosslinking agents, two types of water- soluble carbodiimide (WSC), 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (EDC) and 1-cyclo-hexyl-3-(2-morpholinoethyl) carbodiimide metho-p-toluene sulfonate (CMC), were obtained from Wako Pure Chemical Industries Ltd., Osaka, Japan. Phosphate-buffered saline solution (PBS(-), pH 7.4) was purchased from Nissui Pharmaceutical Co., Ltd., Tokyo, Japan. The fibrin glue (BOLHEAL®, The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) was used as a control adhesive.

Measurement of Gelation Time

The gelation of mixtures of gelatin and PGA aqueous solution by addition of WSC was measured at 37 °C. Briefly, 0.35 ml of PBS(-) containing different concentrations of WSC was added to 1 ml of gelatin-PGA aqueous solution under constant stirring. The duration time from the WSC addition until stirring stopped was measured and defined as the gelation time of hydrogels in this study. The gelation time of the fibrin glue was measured after mixing a fibrinogen solution with a thrombin solution of the same volume (0.675 ml).

The water content of gelatin-PGA hydrogels formed was calculated from their weight before and after swelling in PBS(-) for 24 hrs at 37 °C and expressed as the weight ratio of water in hydrogel to the whole wet hydrogel. The water content of gelatin-PGA hydrogel formed in the presence of 0, 1, 2, 5, and 10 wt% of PGA 91.1 ± 0.2, 92.1 ± 0.3, 92.8 ± 0.4, 93.3 ± 0.2, and 94.4 ± 0.1 %, respectively. The water content increased with the PGA concentration, because of the increased amount of carboxyl groups in the hydrogels.

Measurement of Bonding Strength of Gelatin-PGA Hydrogels to Soft Tissues

The tear strength between two dermal sides of mouse skin bonded with gelatin-PGA hydrogels was measured as follows. After applying the gelatin-PGA aqueous solution (100 μl) and WSC solution (35 μl) to the dermal side of one skin (1×2 cm^2), the other skin was lapped on it to form a bonding area of 1×1 cm^2. After 50 g/cm^2 bonding pressure for several times up to 200 min, the bonding strength of the gelatin-PGA hydrogel was measured by a tensile autograph machine (Shimadzu Ltd., Kyoto, Japan) at a separation rate of 10 mm/min. A similar measurement of bonding strength was performed for fibrin glue by applying 135 μl of it to the mouse skins.

The tensile strength of gelatin-PGA hydrogels (10×10 mm^2, 0.9 mm thickness) themselves were measured in a similar way.

Estimation of In-vivo Degradability of Gelatin-PGA Hydrogels

Figty μl of gelatin aqueous solution, with or without 10 wt% PGA and 17.5 μl of EDC solution, were at the same time injected subcutaneously into the back of female Balb/c mice (6 weeks old) to allow the formation of hydrogels therein. The hydrogels were taken out at 3 day, 1, 2, 4, and 12 weeks after implanation. The dry weight of the hydrogels was measured to estimate the hydrogel degradation. In addition, histological sections were prepared to observe the inflammatory response around the implantation site of the gelatin-PGA hydrogels.

RESULTS AND DISCUSSION

Gelation of Mixtures of Gelatin and PGA Solution by WSC

As is apparent in Figure 1, the gelatin aqueous solution was set to a gel within 2 min by the addition of WSC, forming hydrogels, irrespective of the presence of PGA. The gelatin time decreased with an increase in the concentration of WSC. The addition of PGA to the gelatin aqueous solution was effective in reducing not only its gelation time but also the WSC concentration necessary for the hydrogel formation. The shortest gelation time was obtained at a PGA concentration of 0.5 wt%. A similar effect of PGA addition was observed, irrespective of the type of WSC used, although the gelation time for CMC was longer than that for EDC. It is known that WSC is a coupling agent to form an amide bond between carboxyl and amino groups[5]. As the gelatin molecule has both carboxyl and amino groups, it is intermolecularly crosslinked by WSC, leading to gelation. It is conceivable that the PGA addition could enhance the rate of gelation of gelatin aqueous solution because of the increased carboxyl groups. The gelation time of the fibrin glue was about 6 sec (Fig. 1).

Bonding Strength of Gelatin-PGA Hydrogels

The effect of the loading time on the bonding strength of gelatin-PGA hydrogels and fibrin glues is shown in Figure 2. The bonding strength of gelatin-PGA hydrogels increases with the loading time up to 10 min and thereafter levels off. In the case of fibrin glue, loading for more than 1 min did not result in any increase in the bonding strength and the maximum level of the strength was much lower than that of gelatin-PGA hydrogels.

Figure 3 shows the bonding strength of gelatin-PGA hydrogels as a function of loading weight. The bonding strength of the gelatin-PGA hydrogel became higher with an increase in loading. This can be explained in terms of the anchoring effect of gelatin tissues. It is likely that load application enhanced the penetration of gelatin-PGA and WSC molecules into soft tissues. As a result, the hydrogels formed would be infiltrated more deeply into the tissue structure, and lead to an increased bonding strength. The loading time was fixed at 10 min for the following experiment unless otherwise mentioned.

Figure 4 shows the effect of gelatin concentration on the bonding strength of gelatin-PGA hydrogels. The bonding strength increased with an increase of gelatin

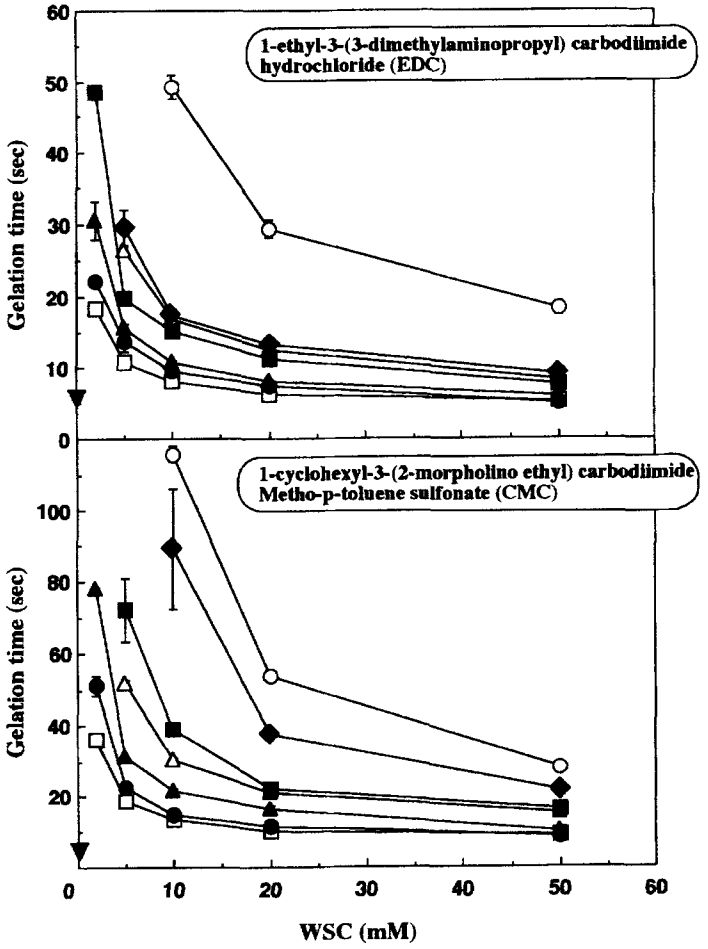

FIGURE 1 Galation of 10 wt% gelatin aqueous solution with WSC in the absence (○) and the presence of 0.1(Δ) 0.5(□), 1(●), 2(▲), 5(■), and 10 wt% (◆) of poly(L-glutamic acid) and fibrin glue (▼). (WSC; 50mM).

concentration up to 10 wt% and thereafter leveled off. The PGA addition was effective in increasing the bonding strength of gelatin hydrogels. Fig. 5 illustrates the relationship between the bonding strength of gelatin hydrogels and the concentration of PGA used for hydrogel preparation. The bonding strength of the gelatin hydrogel became higher as the PGA concentration increased. However, PGA did not dissolve in the gelatin aqueous solution at concentrations higher than 10 wt%. Thus, each concentration of gelatin and PGA was fixed at 10 wt% because that gave the highest bonding strength.

The effect of the WSC concentration on the bonding strength of gelatin-PGA hydrogels is shown in Figure 6. The bonding strength of hydrogels increased with increasing WSC concentration up to a concentration of about 50 mM. Higher WSC concentrations did not further enhance the bonding strength of the hydrogels. These

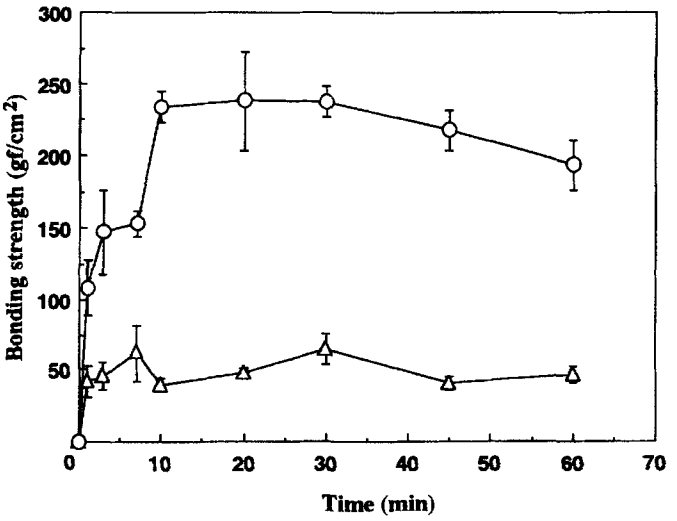

FIGURE 2 Comparison of the bonding strength between (○) gelatin-poly(L-glutamic acid) hydrogel and (Δ) fibrin glue. Gelatin-poly(L-glutamic acid) hydrogel was composed of 10 wt% gelatin, 10 wt% poly(L-glutamic acid), and 50 mM WSC.

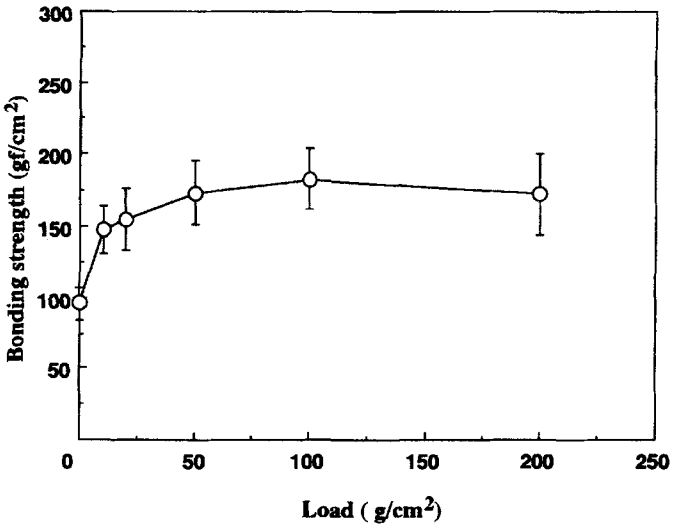

FIGURE 3 Effect of the load weight on the bonding strength of gelatin-poly(L-glutamic acid) hydrogel formed with WSC. (gelatin; 10 wt%, poly(L-glutamic acid); 10 wt%, EDC; 50 mM).

bonding strengths were essentially the same irrespective of the type of WSC used of crosslinking.

The tensile strength of gelatin-PGA hydrogels themselves increased with increasing concentration of PGA and WSC (Figs. 7 and 8). A similar trend was observed for the bonding strength of hydrogels on the mouse skin, as is shown in Figures 5 and 6. The tensile strengths of gelatin-PGA hydrogels was comparable with their bonding

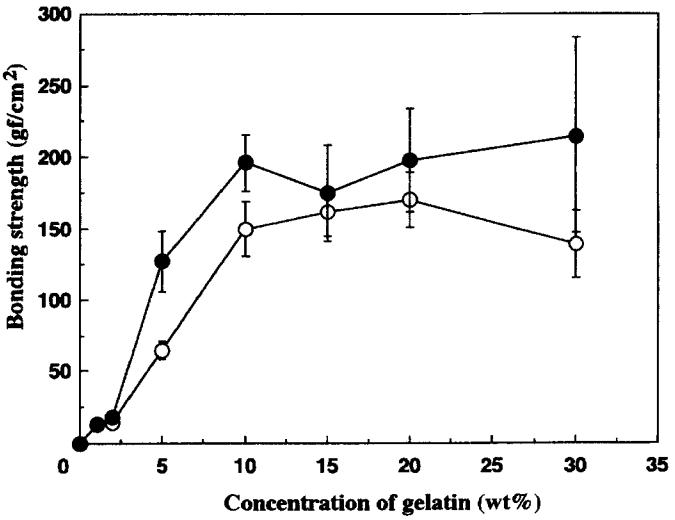

FIGURE 4 Effect of the gelatin concentration on the bonding strength of gelatin hydrogel formed with WSC in the absence (○) and the presence (●) of poly(L-glutamic acid). (poly(L-glutamic acid); 10 wt%, EDC; 50 mM).

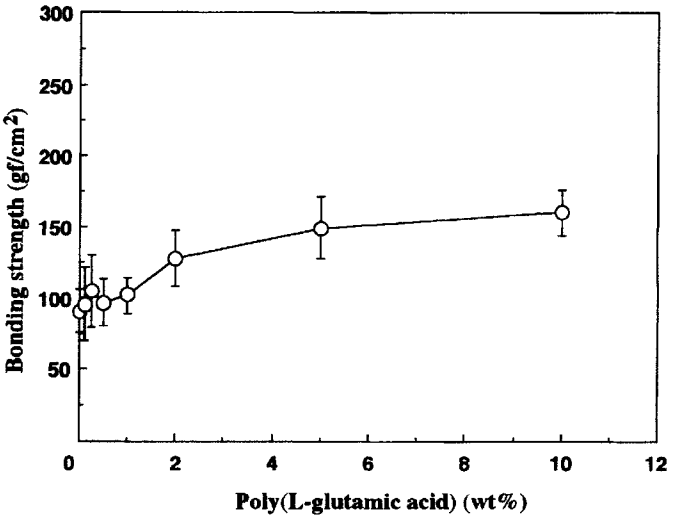

FIGURE 5 Effect of poly(L-glutamic acid) concentration on the bonding strength of gelatin-poly(L-glutamic acid) hydrogel. (gelatin; 10 wt%, EDC; 50 mM).

strengths. Cohesive failure was often observed for the bonding with gelatin-PGA hydrogels. These findings give evidence that the adhesion of gelatin-PGA hydrogels to soft tissues is excellent compared with the fibrin glue which was broken in the interfacial region between the glue and the soft tissue.

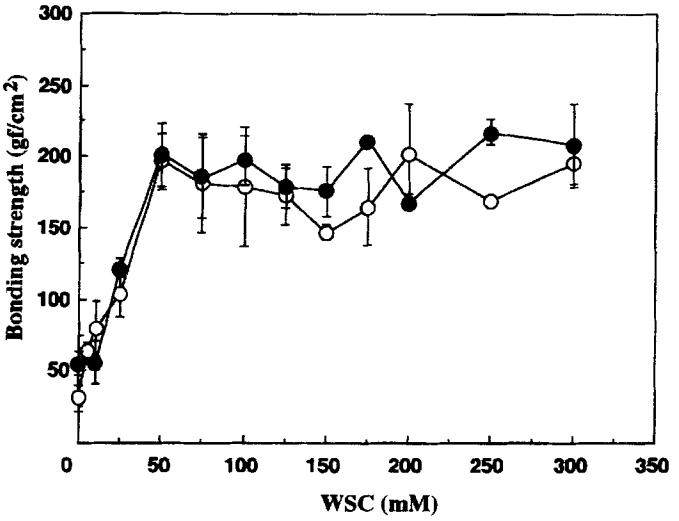

FIGURE 6 Bonding strength of gelatin-poly(L-glutamic acid) hydrogel formed with (○)EDC and (●)CMC. (gelatin; 10 wt%, poly(L-glutamic acid); 10 wt%).

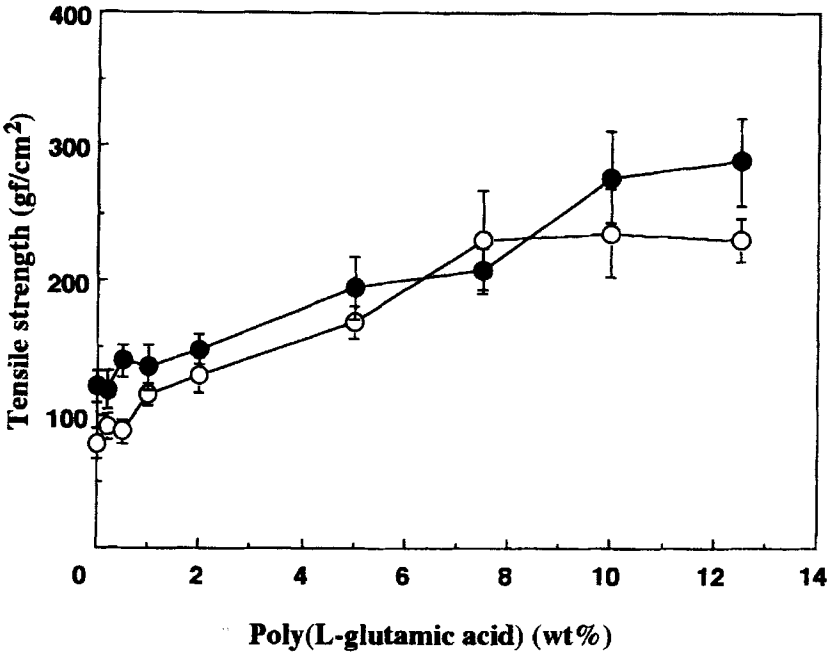

FIGURE 7 Effect of poly(L-glutamic acid) concentration on the tensile strength of gelatin-poly(L-glutamic acid) hydrogel formed with (○)EDC and (●)CMC. (gelatin; 10 wt%, WSC; 50 mM).

In-vivo Degradation of Gelatin-PGA Hydrogels

Figure 9 shows the time course of the *in-vivo* hydrogel degradation. The hydrogel prepared from only gelatin was completely digested within 4 weeks. On the contrary, the PGA addition prolonged the period of hydrogel degradation and about 50% of gelatin-PGA hydrogels still remained at 4 weeks. In addition, no severe

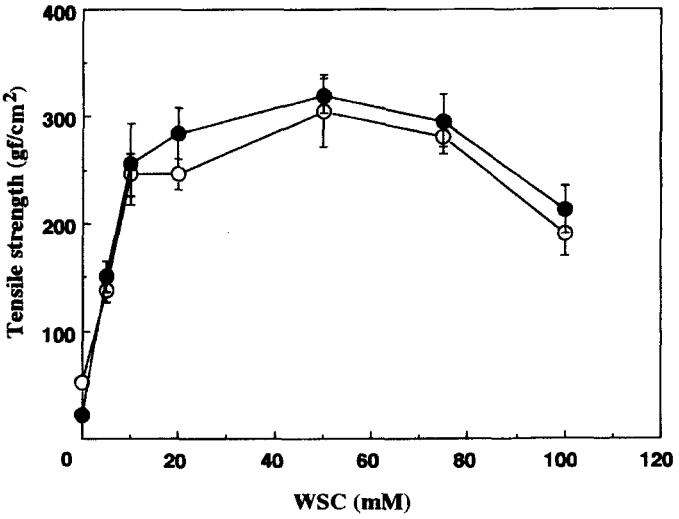

FIGURE 8 Tensile strength of gelatin poly(L-glutamic acid) hydrogel formed with (○)EDC and (●)CMC. (gelatin; 10 wt%, poly(L-glutamic acid); 10 wt%).

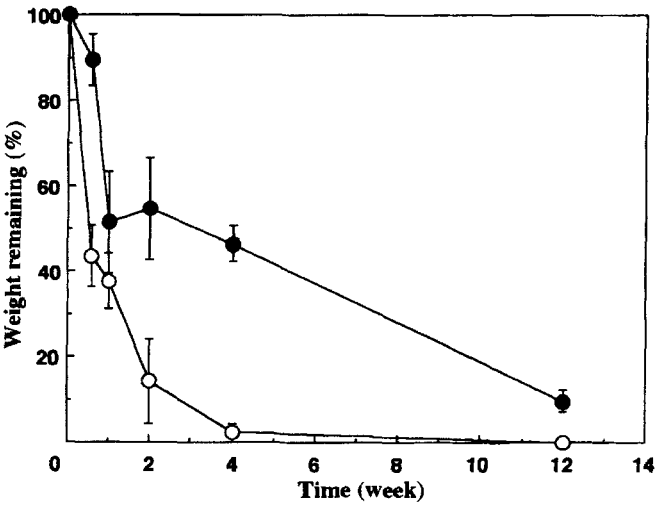

FIGURE 9 In vivo weight loss of gelatin hydrogel formed with WSC in the absence (○) and the presence of poly(L-glutamic acid) (●). (gelatin; 10 wt%, poly(L-glutamic acid); 10 wt%, WSC; 50 mM).

inflammatory reaction was observed around the implantation site over a period of 12 weeks.

CONCLUSIONS

The present study verifies that the gelatin-PGA hydrogel formed by WSC is a promising biological adhesive. The bonding strength of the gelatin-PGA hydrogel to soft tissues was much higher than that of the conventional fibrin glue. The gelatin-PGA glue could be solidified within a short time when compared with the fibrin glue. In addition, the inflammatory reaction induced by the implanated hydrogel was weak and the hydrogel was degraded with time in the body. The gelain-PGA glue before gelation was more viscous than the fibrinogen/thrombin solution of fibrin glue, which is another advantage in its use as a surgical adhesive.

WSC allowed chemical reactions to proceed between the reactive groups of gelatin and PGA molecules without involvement of any foreign materials and the resulting hydrogels did not contain WSC fragments in the crosslinked structure[5]. Although the remaining WSC will be converted into water-soluble urea derivatives which are less reactive and not toxic (unpublished data), we have to check the non-toxicity of gelatin-PGA hydrogels prior to their clinical use. It would seem that the amount of WSC required for crosslinking is so low that it would not cause any problems.

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