PREPARATION OF RAPIDLY CURABLE HYDROGELS FROM GELATIN AND POLY(CARBOXYLIC ACID) AND THEIR ADHESION TO SKIN

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Abstract

Papidly curable hydrogels were prepared through chemical crosslinking of gelatin with poly(carboxylic acid)s including poly(L-glutamic acid) (PLGA), hyaluronic acid (HA), and poly(acrylic acid) (PAA) by use of water-soluble carbodiimide (WSC). The effects of the nature of added poly(carboxylic acid)s on gelation of mixed gelatin-poly(carboxylic acid) aqueous solutions and adhesion of the resulting hydrogels to the mouse skin were evaluated. The addition of poly(carboxylic acid)s reduced the gelation time of gelatin aqueous solutions except for HA. Mixed gelatin-PLGA solutions were cured more rapidly than other mixed solutions and the gelation time was shortened with the increasing PLGA molecular weight. The resulting gelatin-PLGA hydrogels exhibited stronger adhesion to the mouse skin than gelatin-HA and gelatin-PAA hydrogels. The bonding strength increased with the increase in PLGA molecular weight up to 83,000 and thereafter decreased. The longer gelation time and lower adhesion of the gelatin-PAA hydrogels than the gelatin-PLGA hydrogels seem to be due to poorer compatibility of gelatin with PAA than with PLGA. The mixed gelatin-PLGA solution underwent phase separation when the concentration and molecular weight of PLGA became higher than a threshold. The insignificant or suppressive effect of HA addition might be ascribed to the HA-WSC reaction which was the least effective in hydrogel formation.

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INTRODUCTION

Surgical adhesives have been explored aiming at tissue adhesion, hemostasis, and sealing of air and body fluid leaks (Refs. 1-15). However, adhesives currently available have several disadvantages to be improved for their clinical use. For example, the cured solid form of cyanoacrylate (CA) after polymerization is much stiffer than the normal soft tissues although the strength of cured materials is acceptably high for use as a surgical adhesive. Besides, the viscosity of CA is too low to restrict the application to the target site to be adhered. The most widely used surgical adhesive is currently fibrin glue (Refs. 5,6,8), but this is low in mechanical strength and is associated with a risk of viral infection transfer because of its human blood origin. Thus, there is a great demand for developing new biological adhesives applicable to patients without above mentioned problems.

A tissue adhesive is required to possess the following properties for its use in the body. It should be liquid before curing, solidify instantly on living tissues, and bond to them in the presence of water. In addition, appropriate flexibility and biodegradability are needed to the solidified glue. Biodegradable hydrogel is one candidate of adhesives, provided it meets the above requirements. We found that addition of water-soluble carbodiimide (WSC) to a gelatin aqueous solution yielded a hydrogel (Ref. 16). However, the gelation time was not as short as that of conventional fibrin glues. WSC is a coupling agent to effectively form amide bond between carboxyl and amino groups at room temperature even in the presence of water (Refs. 17,18).

Thus, in this study, poly(carboxylic acid)s were added to gelatin solution in an attempt to shorten the gelation time, because their addition might promote gelation of gelatin aqueous solution as a result of increased concentration of carboxyl groups. Poly(L-glutamic acid) (PLGA), hyaluronic acid (HA), and poly(acrylic acid) (PAA) were used as poly(carboxylic acid)s. The gelation time of gelatin aqueous solutions mixed with these poly(carboxylic acid)s was determined to assess the effect of the nature and molecular weight of these poly(carboxylic acid)s. We measured also bonding strength of the resulting hydrogels to the mouse skin.

RESULTS AND DISCUSSION

Gelation of Mixed Aqueous Solutions by WSC

Figure 1 shows the effect of poly(carboxylic acid) molecular weight on the gelation time of mixed gelatin aqueous solutions compared with that of the gelation aqueous solution alone. The gelatin aqueous solution without any poly(carboxylic acid) set to a hydrogel approximately 25 sec after WSC addition. Apparently, the gelation time of gelatin-PLGA solutions was much shorter than that of gelatin-HA and gelatin-PAA solutions over the molecular weight range studied. The PLGA addition to the gelatin solution reduced the gelation time to about 5 sec, which is comparable to the gelation time of the conventional fibrin glues (Ref. 16). The gelation time of gelatin-PLGA solutions tended to decrease with an increase in PLGA molecular weight, probably because their mutual entanglement increased with the increasing PLGA molecular weight. At least in this concentration range, the compatibility between PLGA and gelatin molecules was good.

The gelation time of the gelatin-PLGA solution was influenced by the PLGA concentration; gelation time was shortened with an increase in the PLGA concentration up to 5 wt% but increased again at the PLGA concentration of 10 wt%. This prolonged gelation observed

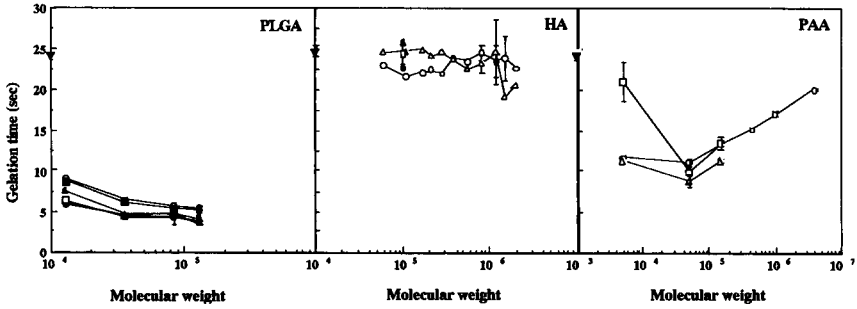

Figure 1 The effect of polyanion molecular weight on the WSC-mediated gelation time of mixtures of gelatin and a polyanion (PLGA, HA and PAA) at polyanion concentrations of (○)0.1, (△)0.5, (□)1, (●)2, (▲)5 and (■)10wt%. ▼ indicates the gelation time of gelatin in the absence of polyanions. (Gelatin;10wt%, WSC;50mM.)

at the PLGA concentration of 10 wt% may be explained in terms of compatibility between gelatin and PLGA. Although no apparent macroscopic phase separation was noticed for the mixed solution made from 10 wt% gelatin and 10 wt% PLGA, it seems that microscopic phase separation has occured in this mixed solution, because higher concentrations caused macroscopic phase separation for the mixed gelatin and PLGA solutions. On the contrary, addition of two other poly(carboxylic acid)s had different influence on gelation of gelatin solutions. Addition of PAA with a low molecular weight was effective in reducing the gelation time, but an increase in gelation time was observed when the PAA molecular weight became higher. This phenomenon also seems to be due to poor compatibility between gelatin and PAA molecules. In fact, when the PAA concentration was higher than 1 wt%, gelation was not observed for all PAA samples because of macroscopic phase separation. Even if the concentration was as low as 0.5 wt%, gelation experiment could not be performed for PAA with high molecular weights, as phase separation took place. For HA, its addition had no significant effect on gelation. Since the solution viscosity of HA with the molecular weight of 2,000,000 was too high to mix gelatin and HA solutions with a stirring bar prior to WSC addition, gelation time could not be estimated by the present experimental procedure. However, even if HAs with lower molecular weights were used at the concentrations up to 5 wt%, HA addition did not affect the gelation time of gelatin solutions.

Figure 2 shows the effect of WSC concentration on the gelation of mixed gelatin and poly(carboxylic acid) aqueous solutions. The gelation time of gelatin solutions was compared after mixing with PLGA, HA, or PAA having a similar molecular weight. Clearly, the gelation time of mixed gelatin aqueous solutions decreased with an increase in WSC concentration, irrespective of the nature of poly(carboxylic acid)s used. Among them, PLGA most remarkedly reduced gelation time of the gelatin solution. In other words, PLGA was the most effective in reducing the WSC concentration necessary for the hydrogel formation. This different effects of WSC concentration on the gelation of gelatin solutions mixed with different poly(carboxylic acid)s having a similar molecular weight and concentration may be explained in terms of different reactivities of WSC with different poly(carboxylic acid)s. By the reaction with WSC, the isotactic PLGA molecule will produce an intermediate which

is the most effective in amide formation with gelatin, while the polysaccharide HA will consume WSC, resulting in retardation of gelation, as seen in Figure 2.

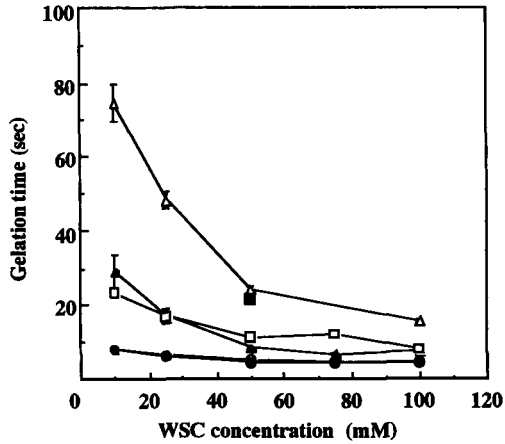

Figure 2 The effect of WSC concentration on the WSC-mediated gelation time of mixtures from gelatin and a polyanion [PLGA(Mw=83,000;○, Mw=130,000;●), HA(Mw=100,000;△) and PAA(Mw=50,000;▲, Mw=1500,000;□)]. ■denotes the gelation time observed in the absence of polyanions. (Gelatin;10wt%, polyanion;0.5wt%.)

Bonding Strength of Hydrogels to Skin

Figure 3 shows the bonding strength of WSC-mediated gelatin-poly(carboxylic acid) hydrogels to mouse skins as a function of poly(carboxylic acid) concentration. As is seen, the bonding strength depeneded on the nature and molecular weight of poly(carboxylic acid)s. The bonding strength of gelatin-PLGA hydrogels tended to increase with an increase in PLGA concentration in contrast to that of gelatin-HA and gelatin-PAA hydrogels. In addition to the significant effect on reduction of the gelation time of gelatin aqueous solutions, PLGA was also much effective in increasing the bonding strength of hydrogels compared with HA and PAA. The bonding strength of gelatin-PLGA hydrogels was higher than that of the conventional fibrin glue (50 gf/cm^2), depending on the PLGA concentration and molecular weight. When PLGA with a molecular

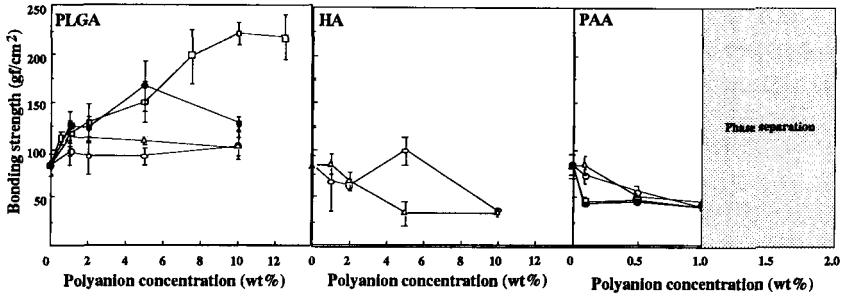

Figure 3 The effect of polyanion concentration on the bonding strength of WSC-mediated hydrogels prepared from mixtures of gelatin and a polyanion (PLGA, HA and PAA). PLGA molecular weight: 12,800(O), 35,600(Δ), 83,000(□) and 130,000(●); HA molecular weight: 100,000(O) and 800,000(Δ); PAA molecular weight: 5,000(O), 50,000(Δ), 150,000(□) and 450,000(●). (Gelatin;10wt%, WSC;50mM.)

weight of 83,000 was used, the bonding strength of gelatin-PLGA hydrogels increased as the PLGA concentration became higher up to 10 wt%. The solution mixtures from a gelatin aqueous solution of 10 wt% and a PLGA aqueous solution of concentrations less than 10 wt% were always clear, indicating that the compatibility between gelatin and PLGA molecules in this concentration range was good, leading to homogeneous mixing. Phase separation occurring at PLGA concentrations higher than 10 wt% may be closely related to the levelling off of bonding strength. Contrary to PLGA, HA and PAA addition had no effect on or decreased the bonding strength. The decreased bonding strength was observed with an increase in the concentration and molecular weight of these poly(carboxylic acid)s. This may be ascribed to poor compatibility of gelatin with PAA and HA molecules, as mentioned above.

The resulting gelatin-PLGA hydrogels were found to undergo their cohesive failure at the adhesion breaking (data not shown). On the contrary, the skin bonded with fibrin glue exhibited failure at the interface between the glue and the soft tissue. This adhesion failure difference may be explained in terms of the direct bonding of gelatin-PLGA hydrogels to the soft tissue. Probably, some of PLGA molecules activated with WSC have directly reacted with the proteins on the tissue surface, resulting in curing of gelatin-PLGA mixed solutions on the uneven soft tissue. As a result, firm anchoring between the cured hydrogel and the tissue took place. On the contrary, such firm anchoring may not be formed for the fibrin glue, since this

adhesive system does not contain any mediator that promotes the direct bonding between the soft tissue and the cured hydrogel. As is ssen in Figure 3, the bonding strength of hydrogels prepared from PLGA with the highest molecular weight was lower than that of hydrogels from PLGA with lower molecular weights when the PLGA concentration was 10 wt%. This finding can be also explained in terms of PLGA compatibility with gelatin.

EXPERIMENTAL PART

The gelatin used was extracted form bovine bone by the alkaline-process (Mw=99,000, pI 5.0, Nitta Gelatine Co., Ltd., Osaka, Japan). Sodium poly(L-glutamic acid) (PLGA, Mw=83,000) and other PLGAs were kindly supplied by Ajinomoto Co., Ltd., Tokyo, Japan and Professor T. Hayashi, Osaka prefectural University, respectively. Hyaluronic acid (HA, Mw=2,000,000) was supplied by Denki Kagaku Kogyo, Co. Ltd., Tokyo, Japan and HAs of varying molecular weights were prepared through acid hydrolysis of this HA. A water-soluble carbodiimide (WSC), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and poly(acrylic acid) (PAA) with different molecular weights were obtained from Wako Pure Chemical Industries Ltd., Osaka, Japan. The fibrin glue used was BOLHEAL® (Lot No. SDH2026) of The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan.

Phosphate-buffered saline solution (0.35 ml, pH 7.4) containing different amounts of WSC was added to 1 ml of mixtures from gelatin and poly(carboxylic acid) aqueous solutions at different concentrations. The time period required for a magnetic stirring bar to stop stirring from the time of WSC addition was measured at 37 ^{o}C and defined as the gelation time of the mixed solutions. Bonding study was performed using 100 μl of mixtures from gelatin and poly(carboxylic acid) aqueous solutions and 35 μl of WSC aqueous solution of different concentrations. The mixtures were applied to the dermal side of one mouse skin (1x2 cm^2), and the other mouse skin was lapped on it to have the bonding area of 1x1 cm^2. After loading to 50 g/cm^2 for 10 min at 25 °C, the bonding strength of hydrogels was measured by a tensile machine Autograph (Shimadzu Ltd., Kyoto, Japan) at 25 °C. Both the gelation and adhesion experiments were done 6 times independently.

CONCLUSIONS

A gelatin aqueous solution was cured by WSC addition in the presence of poly(carboxylic acid)s. The most effective poly(carboxylic acid) to reduce the gelation time was PLGA. The mixed gelatin-PLGA solutions set to biodegradable hydrogels by WSC addition as rapidly as the conventional fibrin glue. The hydrogels could adhere more strongly to the mouse skin than the WSC-mediated gelatin-HA and gelatin-PAA hydrogels. The bonding strength of gelatin-PLGA hydrogels became maximum at the PLGA molecular weight of 83,000.The different addition effects of poly(carboxylic acid)s were explained in terms of their compatibility with gelatin and their reactivity with WSC. It was concluded that the mixtures of gelatin with PLGA containing WSC was promising as a biological adhesive.

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