Modification of hydroxyapatite/gelatin composite by polyvinylalcohol

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Over the past few years biomimetic synthesis [1–3] of artificial bone materials [4] such as hydroxyapatite [HAp], carbonated hydroxyapatite and fluoroapatite have gained increasing attention and some researchers have tried to prepare the apatite composite using collagen [5, 6], denatured collagen (i.e., gelatin [GEL]) [7–10], or polymer [11] as a template for the biomimetic reaction. For decades double diffusion process [12, 13] has been widely used to study the *in vitro* formation of biologic apatite phase in a stationary system. Its drawback is the constantly changing environment, i.e., the depletion of the solutions of the constituting ions (Ca^{2+}, PO_4^{3-}) due to precipitation, leading to a changing supersaturation and possibly changes in the crystallization mechanism [12]. For several years HAp/COL nanocomoposites have been developed through the coprecipitation reaction of HAp nanocrystals in soluble collagen [5, 6] and the characteristic feature of this process is the dynamic reaction using active $Ca(OH)_2$ precursor [5] as a free Ca^{2+} source instead of $CaCl_2$ or $Ca(NO_3)_2$. Recently we have focused on the development of HAp/GEL nanocomposites [9, 10] using the commercial GEL materials.

The development of the apatite phase in GEL matrices is very complicated because the commercial GEL contains a variety of protein species and different stages of degraded products. From our experiments we found that single morphology of apatite phase could be obtained through vigorous stirring or the introduction of a fluoride source during the coprecipitation. The evolution of the strength in the compact body of HAp/GEL composite is governed by the morphology development of HAp particles and the chemical coordination of HAp crystals with GEL matrices. We noticed that the strength of the HAP-GEL composite is limited by the gelatin phases. The purpose of this study is to use a hydrophilic polymer, polyvinylalcohol (PVA), to modify the gelatin phase without alteration of the apatite phase. PVA is known to give a good biocompatibility and has a potential to bind with gelatin molecules because of its hydrophilic property. The characteristics of the PVA modified HAp-GEL composites will be reported. Cross-linkage between PVA and GEL will be investigated using FTIR and DTA. GA as cross-linkage agent is known to have cyto-toxicity, but the limited amount of GA does not cause a significant toxic problem in animal tests [14].

The preparation details of HAp/GEL nanocomposite were previously described by Chang *et al.* [9]. The amount of $Ca(OH)_2$ and H_3PO_4 was calculated to make 10 g of HAp and the input amount of GEL precursor was 3 g. Before the coprecipitation the $Ca(OH)_2$ powders were stirred vigorously in deionized (DI) water at room temperature for 12 hrs and the weighed GEL powders were dissolved in the mixture solution of DI water and phosphoric acid at 37 °C for 12 hrs to help the homogenization of less denatured GEL coils such as worm-like chains [10]. During the entire coprecipitation process we applied vigorous stirring and the pH was controlled as 8.0 using a digital pH controller. After the reaction, the obtained slurry in the solution was aged at the same temperature for 24 hrs and the slurries were collected using a vacuum filter. As-received PVA flakes (low molecular weight PVA, Aldrich, USA) were dissolved in DI water at 60 °C for days and the HAp/GEL composite slurries collected by vacuum filter were vigorously mixed with the PVA solution for hours using a magnetic stirrer at 37 °C. After mixing 0.3 g of GA (85% Aldrich, USA) was added to the above mixed slurries under stirring and after 30 min the slurries were shaped using a glass vacuum filter (diameter 20 mm). The shaped body was dried in an incubator at 37 °C. The added amount of PVA was 1 and 2 g, and the sample names are coded as HG3-PVA1 and HG3-PVA2, respectively.

Before vacuum filtering a part of the slurry was sampled for TEM observation and microstructures were characterized by transmission electron microscopy TEM (JEM-1210, Jeol, Japan). The filtered cake was dried at 37 °C in the incubator and apatite phase was confirmed using X-ray Diffraction (XRD D-5005, Siemens, Germany) via the crushed powders. For the dried material the microstructures were characterized using scanning electron microscopy (SEM, JSM-6700F, Jeol, Japan). The chemical interactions among HAp crystals, GEL macromolecules and PVA polymers were analyzed using the diffuse reflectance FT-IR (Magna 750-R. Nicolet, USA). Following collection of the raw spectra, the spectral band positions were identified by using GRAMS AI 7.0 software (Thermo Galactic, Salem, USA). Thermal analysis (TG-DTA, MacScience, Japan) was also carried out on the dried samples to evaluate the bond formation among HAp crystals, GEL molecules, and PVA polymer. The measurements were carried out between

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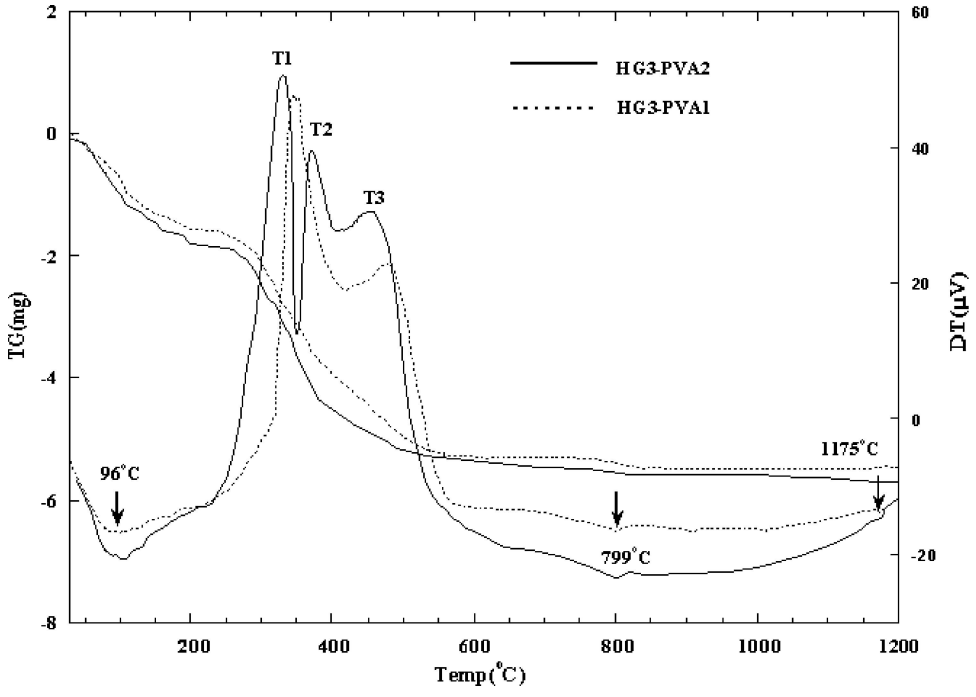


Figure 1 DT/TG analysis for HG3-PVA1 and HG3-PVA2 samples. There are typically three exothermic peaks (T1, T2, T3) and especially T2 peak is profoundly developed in HG3-PVA2. The higher amount of modification by PVA induced the strong T2 peak, corresponding to the thermal decomposition of polymer.

25 and 1200 °C at a heating rate of 10 °C/min. All experiments were carried out in platinum pans in air atmosphere, and Al_2O_3 powders (10 mg) were used as the reference.

The XRD showed that the crystal phase in the composite was HAp (ASTM 9-432). The peaks were broad and weak, which might be attributed to the dispersed tiny crystallites of the heterogeneous nucleation of HAp on the GEL matrices during the precipitation [9]. From TG analysis in Fig. 1 we could confirm that a large amount of organic components (about 7 wt%) was included in HG3-PVA2 compared to that in HG3-PVA1 and the cross-linkage reaction by GA was effectively working to form the chemical bonding between GEL macromolecules in HAp/GEL composite slurries and the added PVA molecules. From DT data in Fig. 1 we can observe three exothermal peaks (T1, T2, T3) for the HG3-PVA2 sample, which typically appeared in HAp/GEL nanocomposite [9]. The HG3-PVA1 sample also appeared to show three exothermic peaks, but the peak separation between T1 and T2 was small and not very clearly defined. It is certain that the increasing amount of chemical bonding between GEL and PVA molecules greatly contributed to the enhancement of the peak separation. The strong development of T2 peak in HG3-PVA2 is very notable and we can definitely observe several small peaks in the range 400–490 °C, indicating the multistep reaction probably influenced by excessive PVA. T1 and T2 correspond to the thermal degradation and pyrolyzation of organic molecules, respectively, and T3 is associated with the final decomposition of the residual organics. It can be said that the specific enhancement of the T2 peak in the HG3-PVA2 sample resulted from the increase of the structural energy of the organic part in the HAp/GEL composite. In gelatin macromolecules the T1 peak corresponds to the disorganization of the helical structure in denatured collagen fibrils and renatured collagen fibrils, and the T2 peak reflects the disintegration of each chain. The disorganization of the helical structure is the unique property of collagen fibrils and is hardly affected by the PVA modification. On the other hand, thermal disintegration of chains in GEL molecules can be easily affected by the modification of GEL molecules, so we can acertain that there was a huge amount of chemical bridge formation between PVA and GEL molecules. In HG3-PVA1 (Fig. 2A) the T3 peak indicating the combustion of organics is located at ∼510 °C and most of the residual organics disappeared at ∼575 °C. In Fig. 2B the T3 peak and the final burn-out temperature for HG3-PVA2 are lower that that of HG3-PVA1. PVA modification contributed to the decrease of T1 (thermal degradation) and T3 (combustion temperature), and the increase of T2. Higher amount of PVA modification for GEL molecules induced the strong development and shift of the T2 peak through the pyrolyzation process mainly governed by the PVA molecules.

Endothermic peaks by the hydrated water appeared at ∼96 °C. The water molecules are partially associated with the organic components combined with the individual apatite crystals. Other endothermic peaks were located just below 800 °C, corresponding to the release of lattice OH^- along the *c*-axis. At 1175 °C there is another endothermic peak, showing the CO_2 releasing from the carbonated apatite.

Fig. 2 HG3-PVA1 shows the typical FT-IR spectra indicating the chemical bond formation between the HAp phase and GEL, and the GA cross-linkage of PVA with the HAp/GEL composite severely modified the spectra as shown in HG3-PVA2. The organic-inorganic bond formation could be well identified from amide I, II, III bands at 1700–1200 cm^{-1} and PO_4 high band

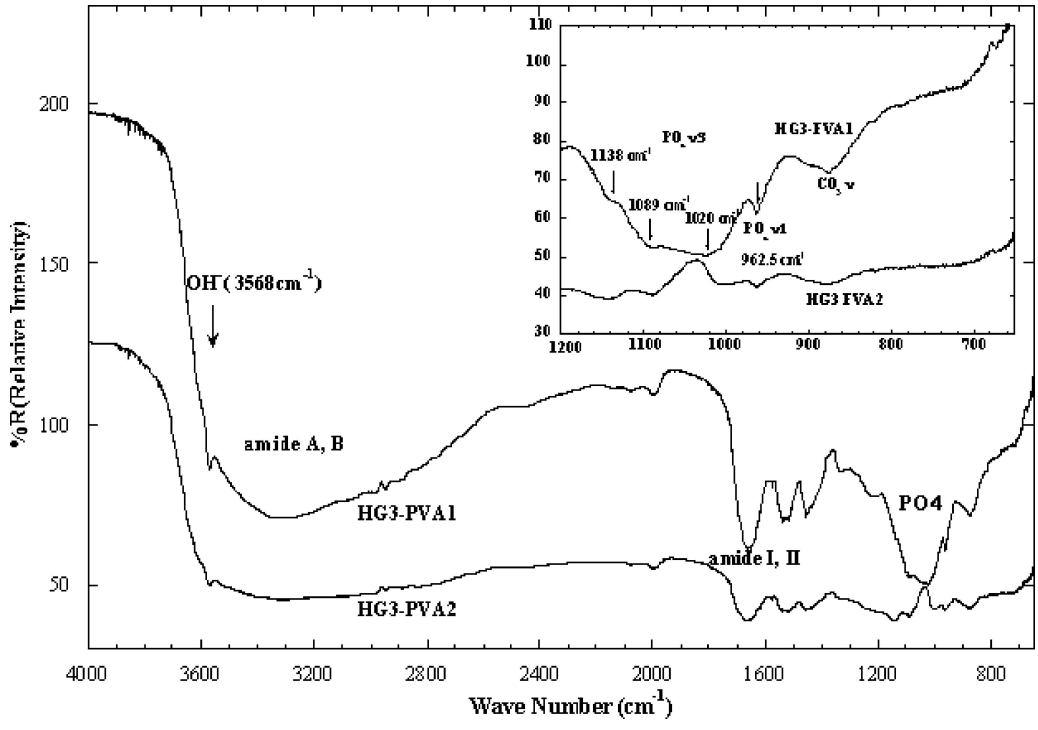


Figure 2 FT-IR spectra for HG3-PVA1 and HG3-PVA2. The organic coordination of HAp with GEL matrix is confirmed from amide I, II, III bands and PO_4 bands with the reaction temperature. We can observe the typical bands such as C═O stretching at 1700–1600 cm^{-1} for amide I, N-H bending at 1550–1500 cm^{-1} for amide II, and N-H deformation at 1300–1200 cm^{-1} for amide III band. As HAp related bands, there are OH^- stretching (3569 cm^{-1}), and phosphate contours: ν_1 962 cm^{-1} and ν_3 1089 cm^{-1}. The inset shows a big difference in the PO_4 band feature.

(ν_1, ν_3) at 1200–850 cm^{-1}. The OH^- band at 3568 cm^{-1} indicates the surface-free water on HAp crystallites and the intensity decreased with the increase of PVA content. Normally the spectral feature of PO_4 ν_3 domain (1200–980 cm^{-1}) well reflects the crystal development and the organic coordination of apatite phases. With the increase of PVA content the spectral feature of PO_4 ν_3 domain was highly modified as shown in the inset of Fig. 2. Normally in HAp/GEL nanocomposite samples the phosphate ν_1 band is centered at 962 cm^{-1} with a narrow band spectra. That is, P-O stretching of the PO_4 ν_1 mode is very stable and hardly affected by the parameters of organic-inorganic reactions and crystal growth. On the other hand the phosphate ν_3 domain is highly influenced by the quality of the apatitic phase and the ratio of apatite to organics. The ν_3 band at 1020 cm^{-1} is assigned to the P-O stretching in the symmetric PO_4 mode of the HAp crystal and there are several other modes such as an asymmetric PO_4 mode at 1089 cm^{-1} and a labile nonapatitic PO_4 mode at 1138 cm^{-1}.

Fig. 3 shows the assembly of needle-shaped particles, which is the typical TEM morphology for the HAp/GEL composite. The needle-shaped particles are self-assembled because of the self-assembling ability of renatured gelatin and the assembled bundles can make a rod-shaped aggregate because of the self-similarity between the bundles, as shown in the enlarged view of highly magnified SEM microstructure (Fig. 4B). From Fig. 4A and B we can observe the grain-type microstructures, and the grain-like regime is actually an aggregation of rod-shaped composite bundles. When the GEL solution is dropped into the reactor through a peristaltic pump the liquid GEL droplet spreads to form a film in the batch solution and apatite crystals are deposited on the GEL film. The apatite slurries embedded in GEL matrices are self-assembled and rod-shaped and the assembled slurries are distributed over the GEL film. The composite HAp/GEL films, which are bent and warped, are stacked to make a bulk body during the shaping process using the vacuum filter. From the comparison between Fig. 4A and B SEM microstructure was becoming smoother with the increase of PVA content. The addition of an excess amount of PVA often

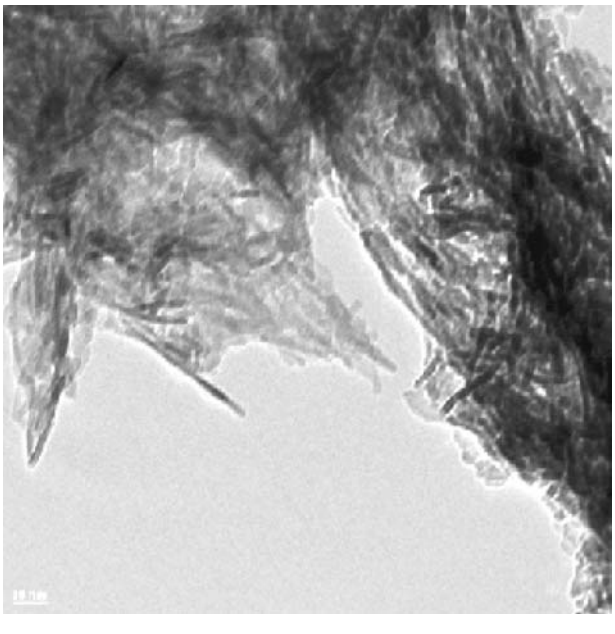
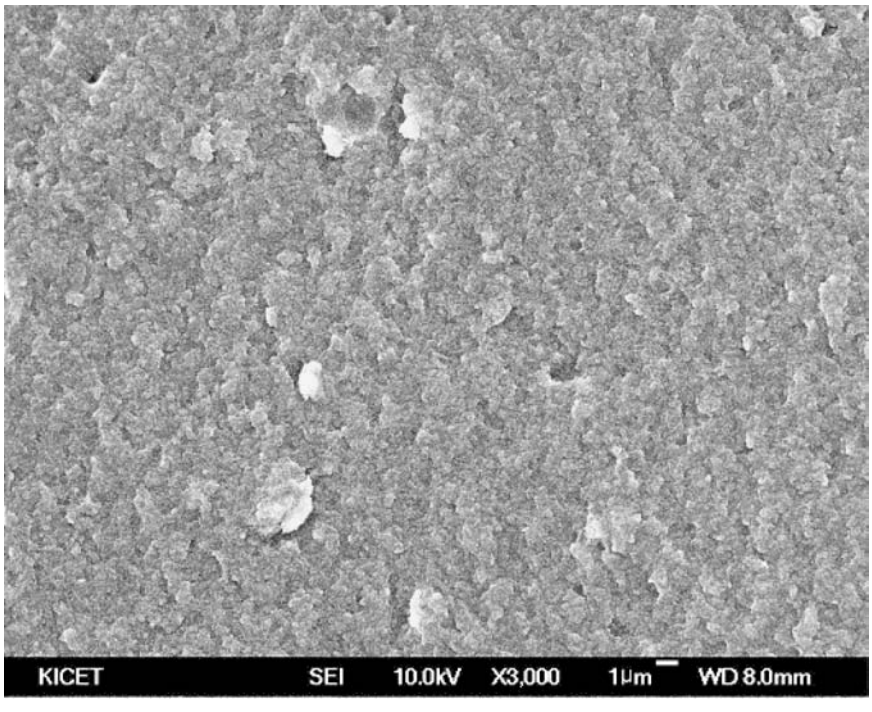


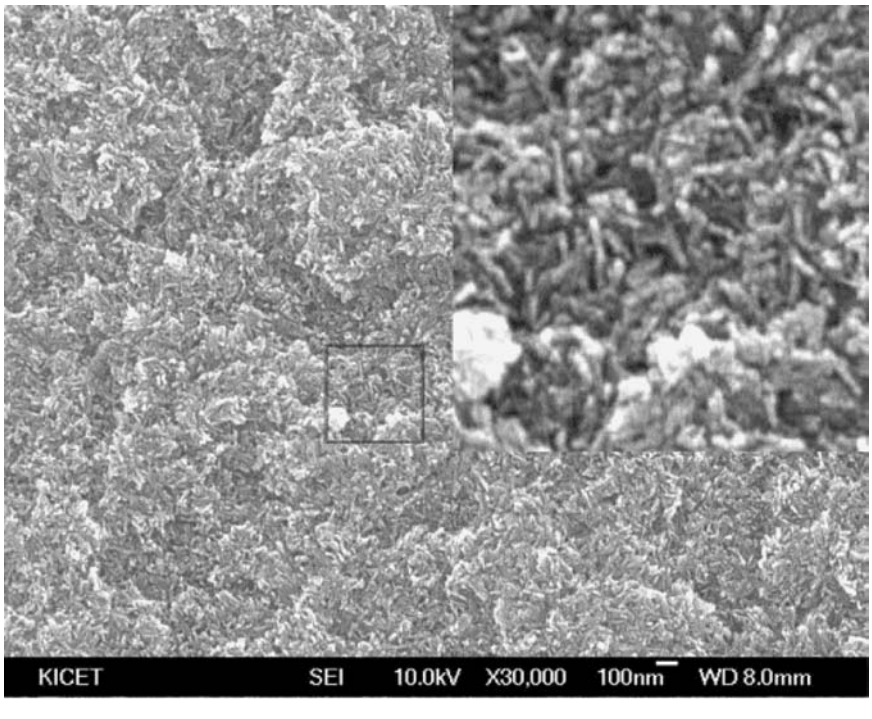
Figure 3 TEM morphology for HG3-37, indicating the needle-shaped morphology. We could observe the needle-like particles over the entire range. The scale bar indicates 20 nm.

induced nonuniform microstructure as confirmed by the elemental analysis in Fig. 4C.

Through thermal analysis and FT-IR spectra we could confirm the good chemical bond formation between PVA and GEL molecules of HAp/GEL composite through the cross-linkage reaction using GA. The primary purpose of PVA modification is based on the improvement of the toughness of the resultant composite. In order to achieve this goal it is important to uniformly disperse PVA molecules into the HAp/GEL composite and then make strong chemical bridges between PVA and GEL. From the thermal analysis results it seems that the addition of 1 g of PVA for the sample of HAp10 g/GEL3 g is sufficient to get a uniform distribution on HAp/GEL composite slurries and more PVA addition increases the amount of free PVA, which may not participate in the chemical bond formation with the composite. One factor is the molecular weight of PVA,

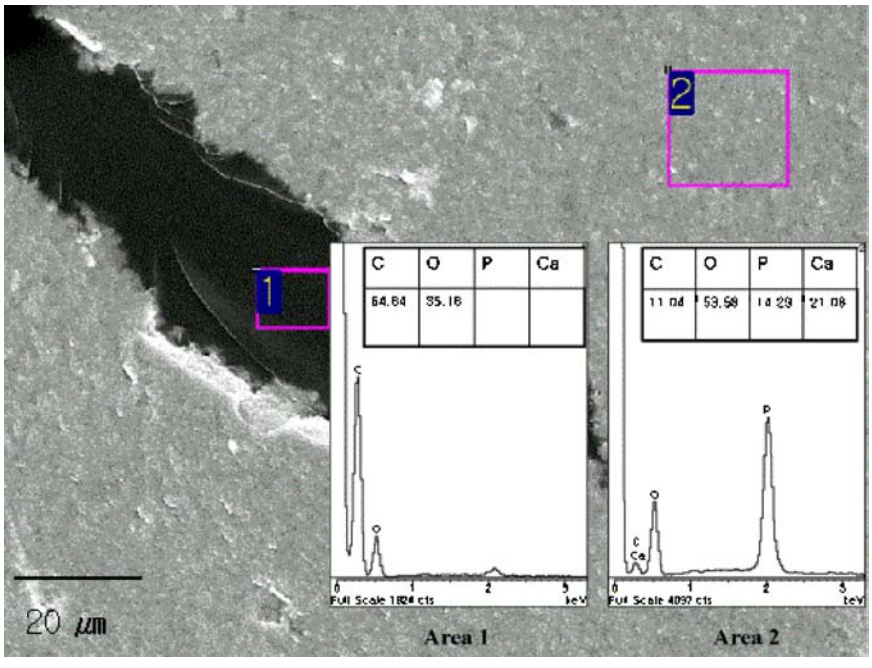


(A)



(B)

Figure 4 SEM morphology for HG3-PVA1 (A) and HG3-PVA2 (B). (B) The inset-magnified region on the upper right shows the needle-shaped particles. The grain-like region in SEM picture consists of the many needle-shaped particles. (C) shows the electron image and the estimated composition. Area 1 and area 2 correspond to PVA only region and PVA-modified HAp/GEL composite, respectively. The higher amount of PVA addition into HAp/GEL slurries often resulted in the region of PVA only. (*Continued*)



(C)

Figure 4 (*Continued*)

used for the modification of the composites, using the higher molecular weight PVA precursor we expect to produce highly tough HAp/GEL composites. Of course the amount of GA can be another factor to enhance the toughness. GEL source has been well proven as an excellent molecular template for the biomineralization, but it was hard to get uniform and tough physicochemical property because of the reaction complexity. One of most desired properties is the mechanical toughness corresponding to that of real bone (∼200 MPa). Until now we could attain only 40–50 MPa and so the modification of GEL by using polymeric source is considered as one of the most preferable technologies. The present experimental results showed that PVA was effective in giving more uniformity and toughness to HAp/GEL nanocomposite. The next experiment will focus on the use of a higher molecular weight PVA source, a limited increase of GA addition, and the application of a nontoxic cross-linkage agent.

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