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# 방사선 이용 상처치료용 β-Glucan 하이드로젤 제조 및 특성 분석

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# Preparation and Characterization of Radiation Fabricated β-Glucan Hydrogels for Wound Dressing

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**초록:** 화학가교제의 사용없이 감마선을 이용하여 β-glucan, poly(vinyl alcohol)(PVA), poly(vinyl pyrrolidone)(PVP), κ-carrageenan(κC), glycerin으로 구성된 고분자 하이드로젤을 제조하였다. 25, 50, 75 kGy로 감마선 조사선량을 달리하여 하이드로젤을 제조한 후, 젤화율, 팽윤도, 압축강도의 측정을 통해 제조된 하이드로젤의 물리적 특성을 확인하였다. 조사선량의 증가에 따라 하이드로젤의 가교밀도가 증가되어 젤화율과 압축강도는 증가하였고, 팽윤도는 감소되었다. 제조된 베타글루칸 하이드로젤로 손상된 조직의 재생 특성을 평가해 보기 위해 동물실험을 수행하였고, 그 결과 베타글루칸이 함유된 하이드로젤이 빠른 상처 치유능을 나타내었고, 상용제품과 비교 시에도 동등한 치료효과를 관찰할 수 있었다. 따라서 감마선으로 제조된 β-glucan/PVA/PVP/κC/glycerin 하이드로젤은 화학가교제 독성의 우려 없이 β-glucan에 의한 향상된 치료효과와 더불어 향후 조직재생용 소재로서 유용하게 사용될 수 있을 것으로 사료된다.

**Abstract:** Hydrogels consisted of β-glucan, poly(vinyl alcohol) (PVA), poly(vinyl pyrrolidone) (PVP), κ-carrageenan (κC) and glycerin were prepared by gamma-ray irradiation for damaged tissue regeneration. Irradiation doses of 25, 50, and 75 kGy were exposed, respectively, to the β-glucan hydrogel to evaluate the effect of irradiation dose on physical properties. The physical properties were examined such as gel fraction, absorption ratio, and compressive strength. It was found that the gel fraction and the compressive strength increased with increasing the irradiation dose. This is due to the fact that the crosslinking density increases with increasing the irradiation dose, whereas the absorption ratio decreased with increasing the irradiation dose. On observing the wound healing of rat skin, the resulting hydrogels accelerated the wound repair, which can be attributed to the release of β-glucan from the hydrogel. Therefore, radiation fabricated β-glucan/PVA/PVP/κC/glycerin blended hydrogel was suitable for wound healing and could be considered as good tissue regeneration biomaterials without chemical toxicity.

**Keywords:** gamma-ray, irradiation, hydrogels, tissue regeneration, wound dressing.

## Introduction

As it is known, dressings are used to cover wounds to accelerate healing. There are two kinds of dressings; dry and wet. It has been reported that healing under a wet environment is faster than in a dry environment.[1] This is due to the fact that renewed skin, no formation of eschar, takes place during healing in a wet environment.[2]

Hydrogels are three dimensional, hydrophilic, polymeric networks capable of imbibing a large amount of water or bio-

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logical fluid without dissolution due to the presence of chemical cross-links or physical cross-links.[3] The hydrogel networks are formed by crosslinking polymer chains via covalent ionic, hydrogen bonds, or via physical entanglement.[4] The hydrogels have many special properties such as transparency to allow healing follow up, absorb, and prevent loss of body fluids, barrier against bacteria, good handling, and oxygen permeability.[5]

β-Glucan is composed of glucose units linked together to form a long polymer chain and is a fiber-type homopoly-saccharide obtained from the cell walls of yeast, oats, barley, and from many medicinal mushrooms.[6] The β-glucan has immune-enhancing properties, which nutritionally potentiate and modulate an immune response.[7-9] It shows antibacterial and antiviral effects and exhibits wound healing activity.[10,11] κ-Carrageenan (κC) is a viscosifying polysaccharide obtained commercially by extraction of certain species of red seaweeds and it is mainly used in the food industry as gelling, thickening, and stabilizing agents.[12] Other major applications are in cosmetics, pharmaceuticals, and personal care industries.[13] However, natural polymers are considered to be a limitation in their applications for a wound dressing material because of their shortage of processing and mechanical properties. The combination of natural and synthetic polymers can endow optimal properties for wound repair.[14] Poly(vinyl alcohol) (PVA) is frequently used in the preparation of various membranes and hydrogels.[15] Other synthetic polymer, poly(vinyl pyrrolidone) (PVP) is used as a main component of temporary skin covers or wound dressing because of their excellent transparency and biocompatibility.[16]

Irradiation is recognized as a very suitable tool for the formation of hydrogels. Radiation process has various advantages such as easy process control, possibility of joining hydrogel formation, and sterilization in one technological step, no necessity to add any initiators and crosslinkers possibly harmful and difficult to remove. They make irradiation the method of choice in the synthesis of hydrogels.[17]

In this work, hydrogels composed of β-glucan, PVA, PVP, κC and glycerin were prepared by $^{60}$Co gamma-ray irradiation doses of 25, 50, and 75 kGy, respectively. Gel fraction, absorption ratio, and compressive strength of the resulting hydrogels were measured to evaluate the effect of irradiation dose. In animal study, the resulting hydrogels were compared to commercial products for healing effect. The results of this work would demonstrate the applicability of hydrogels as a wound dressing.

## Experimental

**Materials.** β-glucan (M.W. $3.5\times10^5$) was supplied by Quegenbiotech Inc. (Siheung, Korea). PVA (M.W. $8.5\times10^4$-$1.2\times10^5$) and glycerin (F.W. 92.1) were supplied by DC Chemical Co. (Iksan, Korea). PVP (M.W. $1.2\times10^6$-$2.0\times10^6$) and κC (M.W. $1.0\times10^5$-$8.0\times10^5$) were purchased from BASF Co. (Germany) and MSC Co. (Yangsan, Korea), respectively. All reagents were used without further purification. Distilled water (DW) was used as a solvent in all experiments.

**Radiation Fabrication of Hydrogels.** Firstly, aqueous solution of 2 wt% PVA was heated in an autoclave at 120 °C for 20 min. The solution was blended with β-glucan (4.0 wt%), PVP (15 wt%), κC (1.5 wt%), and glycerin (2.0 wt%). The mixture was homogeneously stirred, kept in a water bath at 75 °C for 12 h and then poured into petri dishes to form a layer having a thickness of 2 or 9 mm. Finally, the β-glucan containing hydrogels were prepared by $^{60}$Co gamma-ray irradiation doses of 25, 50, and 75 kGy (dose rate: 10 kGy/h), respectively, at room temperature and evaluated the effect of irradiation dose on the physical properties.

**Gel Fraction.** The gel fraction of the hydrogels was measured by extraction in warm DW at 37 °C for 48 h and dried in an oven at 60 °C for 48 h, until they reached constant weight. The gel content was calculated as follows:

$$\text{Gel fraction}(\%) = (W_d/W_i) \times 100 \quad (1)$$

where $W_i$ is the initial weight of the polymer, and $W_d$ is the dried gel weight after extraction.

**Absorption Ratio.** To measure the absorption ratio of the hydrogels, specimens of $1.3\times1.3\times2$ cm$^3$ were dried in an oven at 60 °C for 48 h and then immersed in DW. Before weighing, the specimen's surface DW was removed with filter paper. The weight of swollen specimen was measured at various time intervals up to 48 h. The absorption ratio was calculated as follows:

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

where $W_d$ and $W_s$ represents the weight of the dried gel and the maximum weight of swollen state, respectively.

**Mechanical Property.** To evaluate the mechanical property, the compressive strength tests were performed on cylindrical hydrogel samples with 9 mm thickness and 34 mm diameter using a universal mechanical tester (Instron, model 5569,

USA) with a 5 kN load cell. It was recorded at a cross-head speed of 2 mm/min and compressive strain of 50%. All results were the mean value of five measured specimens.

**Chemical Analysis.** To identify the chemical interactions in β-glucan blended hydrogels, the ATR-FTIR spectra were recorded on a Bruker Tensor37 (Bruker AXS. Inc., Germany) in the range of 400-4000 $cm^{-1}$. Also, the NMR was recorded on a 500 MHz $^1H$ NMR (ECA 500 MHz spectrometer, JEOL, Tokyo, Japan) with averaged over 32 scans in $D_2O$ solution.

**Animal Study.** Male Sprague-Dawley rats, aged 7 weeks and weighing 200-220 g, were purchased from Orient Bio Inc. (Korea). Firstly, the rats were anesthetized with diethyl ether and removed the dorsal hair of the rats with an electric razor. 10% aqueous povidone-iodine and 70% alcohol were employed to sterilize the dorsal area of the animals. Then two full thickness wounds with a surface area of about 1 cm (diameter) were symmetrically created from the back. Each wound was covered with equal size of the hydrogels irradiated to 50 kGy or the commercial products (the trade name of Ridoar gauze, and Mediform) for comparison of wound healing. On top of the covered wound, transparent film, Tegaderm (3M, USA) was applied and bandaged with adhesive plaster (FIX ROLL, Young Chemical. Co., Ltd, Korea) to fix the treated wound. Treated rats were placed in individual cages and the healing wounds were observed on the 1st, 3rd, 5th, 8th, 10th, 12th, 15th, 17th and 19th days using a digital camera.

**Histological Study.** Wound tissue was dissected after 22 days of treatment, fixed with 10% buffered formalin and stained with hematoxylin and eosin (H&E) reagents for histological observations.

**Statistical Analysis.** The results were expressed as means± standard deviation (SD) for $n \geq 3$. A student's t-test (Excel, Microsoft) was used to assess statistical significance of the results ($p<0.05$).

## Result and Discussion

Radiation reactions utilize gamma-ray to excite a polymer and produce a crosslinked structure.[14] The crosslinked polymer chains provide the network structure and physical integrity.[18,19] Gel fraction and absorption ratio are important properties to evaluate application of the hydrogels for a wound dressing. Figure 1 shows the gel fraction of the hydrogels which were synthesized by gamma-ray irradiation. The gel fraction was in the range of 33-72% and increased with increasing irradiation dose. The 50 kGy irradiated hydrogel was selected for animal study considering physical properties with a gel fraction greater than 50%. As shown in Figure 2, the absorption ratio was in the range of 2609-3381% and decreased with increasing irradiation dose. The absorption percent was inversely proportional to the gel fraction percent. This is due to the fact that the crosslinking density increases with increasing irradiation dose. The crosslinking density is one of the most important factors that affect gelation and absorption behavior of hydrogels. Highly crosslinked hydrogels have a tighter structure so that they hinder the mobility of the polymer chains, hence lowering their degree of swelling.[3] Figure 3 shows the absorption ratio of the hydrogels *vs*. immersion times. It could be seen that the degree of swelling was sharply higher at the immersion time of less than 10 h, whereas the degree of swelling slowly decreased at the immersion time of more than 10 h. These crosslinking structures restrict extensibility of the polymer chains induced by swelling of the water and thus counter

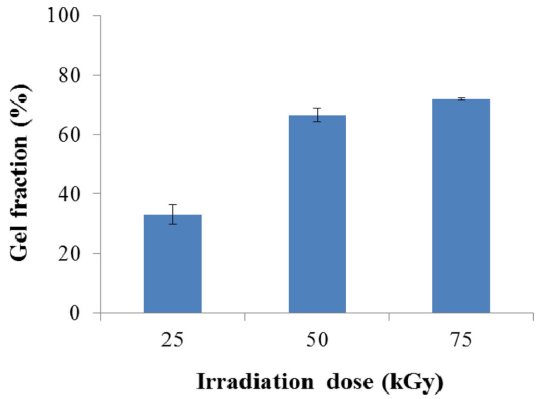


**Figure 1.** Gel fraction of β-glucan/PVA/PVP/κC/glycerin blended hydrogels *vs*. irradiation doses.

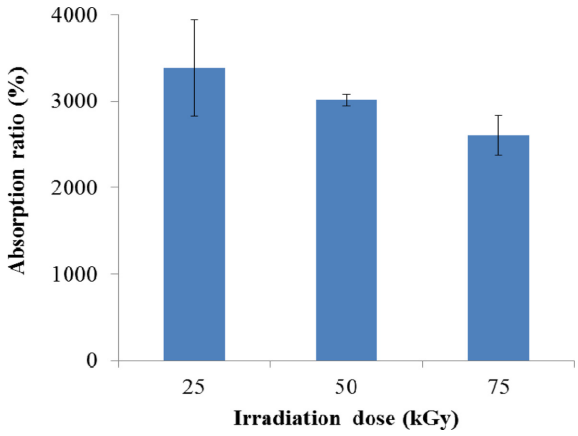


**Figure 2.** Absorption ratio of β-glucan/PVA/PVP/κC/glycerin blended hydrogels *vs.* irradiation doses.

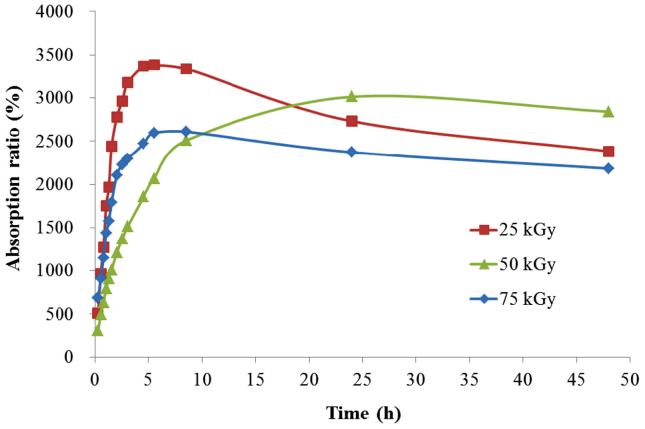


**Figure 3.** Absorption ratio of β-glucan/PVA/PVP/κC/glycerin blended hydrogels *vs*. immersion times.

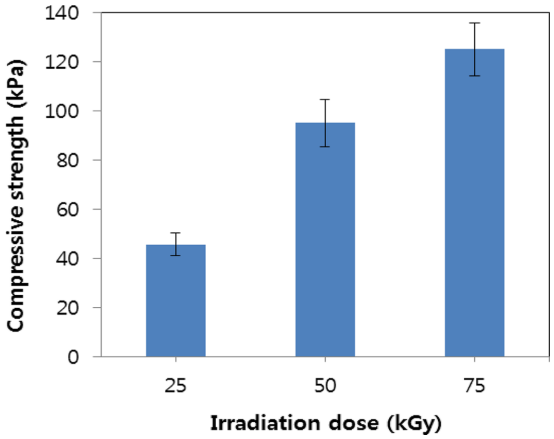


**Figure 4.** Compressive strength of β-glucan/PVA/PVP/κC/glycerin blended hydrogels versus irradiation doses.

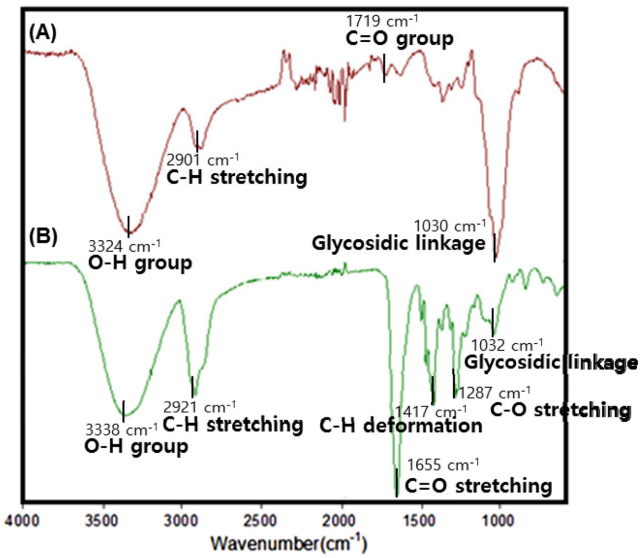


**Figure 5.** FTIR spectra of (A) β-glucan; (B) β-glucan blended hydrogels prepared by radiation.

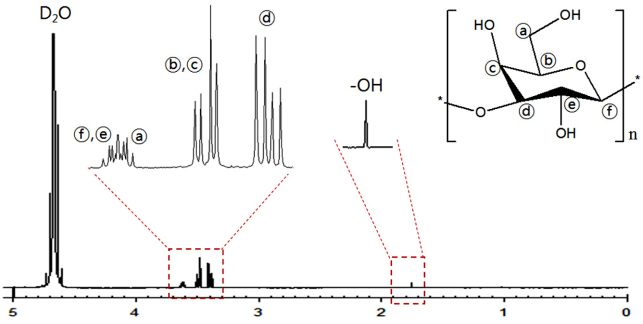


**Figure 6.** $^{1}$H NMR spectra of the released β-glucan solution from the β-glucan blended hydrogels.

any tendency for dissolution. Thus the absorption ratio reduces more than 10 h. As shown in Figure 4, the compressive strength increased with increasing irradiation dose from 46 to 125 kPa. The increase of the compressive strength was believed to be due to increased crosslinking density. It was shown that the compressive strength was proportional to the gel fraction. These values are enough to fulfill the mechanical properties required for a wound dressing. As shown in the FTIR spectrum (Figure 5) of the original β-glucan powder (a) and the radiation fabricated hydrogels (b), the peak of β-glucan that appears in O-H groups (3324 $cm^{-1}$), C-H stretching vibrations (2901 $cm^{-1}$), carbonyl groups (1719 $cm^{-1}$) and the glycosidic linkages (1030 $cm^{-1}$), respectively. The major peak of β-glucan blended hydrogels showed in 3338, 2921, 1655, 1417, 1287, and 1042 $cm^{-1}$. The C=O stretching vibrations in the 1655 $cm^{-1}$ are related with PVP, and the C-O stretching vibrations in the 1287 $cm^{-1}$ are related with PVA. Also, the glycosidic linkages (1032 $cm^{-1}$) for the β-glucan have been confirmed. Also, the $^{1}$H NMR spectra confirmed the release of β-glucan from the blended hydrogels. In the Figure 6, the ⓐ~ⓕ peaks near 3.6 ppm display the methylene group in the glycosidic linkages with an integral of 2 and the OH group is showen at 1.75 ppm. Figure 7 shows the macroscopic observations of the wound healing effect for the five groups. The healing of the resulting hydrogel-treated wound was faster than the others up to 8 days. Since β-glucan can accelerate wound healing,[10] the acceleration of wound healing in this experiment can be attributed to the release of β-glucan from the resulting hydrogel.[20] Therefore, the results demonstrate that the resulting hydrogel can accelerate the healing process. In the literature, β-glucan is speculated to reduce the time for fibroblasts to invade wound tissue and by early synthesis of new skin tissue.[10,21] With the

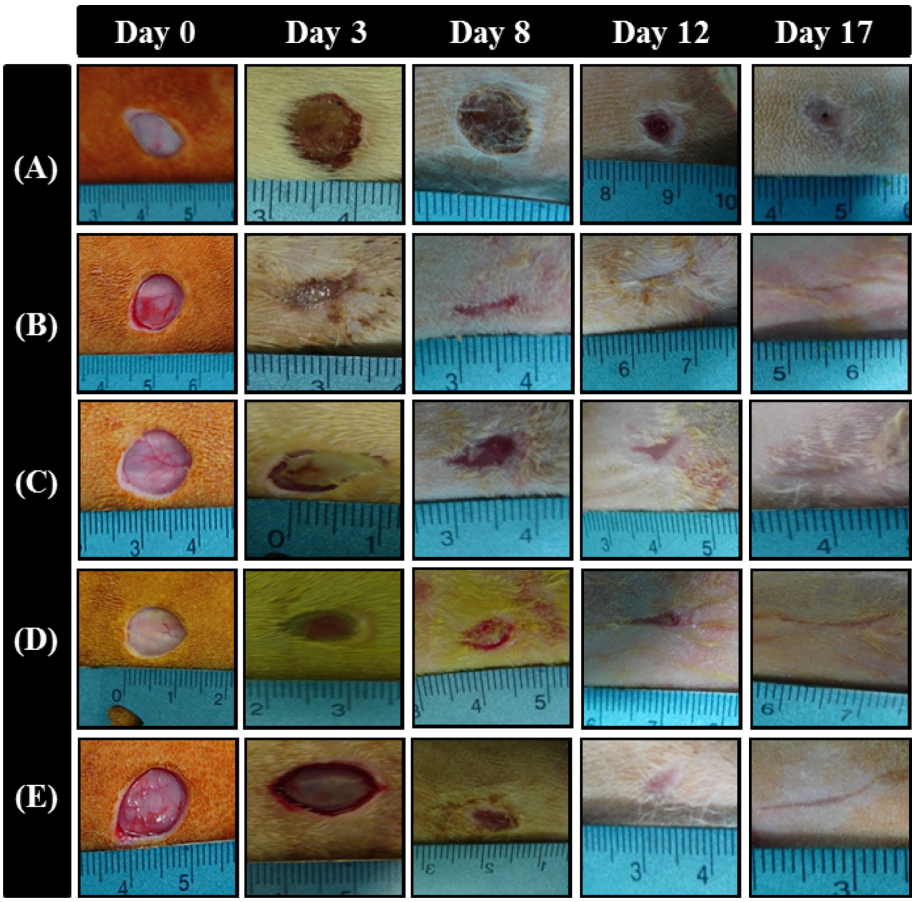


**Figure 7.** Photographs of macroscopic appearance of wound repair covered with (A) control (non–treated wound); (B) β-glucan/PVA/PVP/κC/glycerin blended hydrogel; (C) PVA/PVP/κC/glycerin blended hydrogel; (D) Ridoar gauze; (E) Mediform.

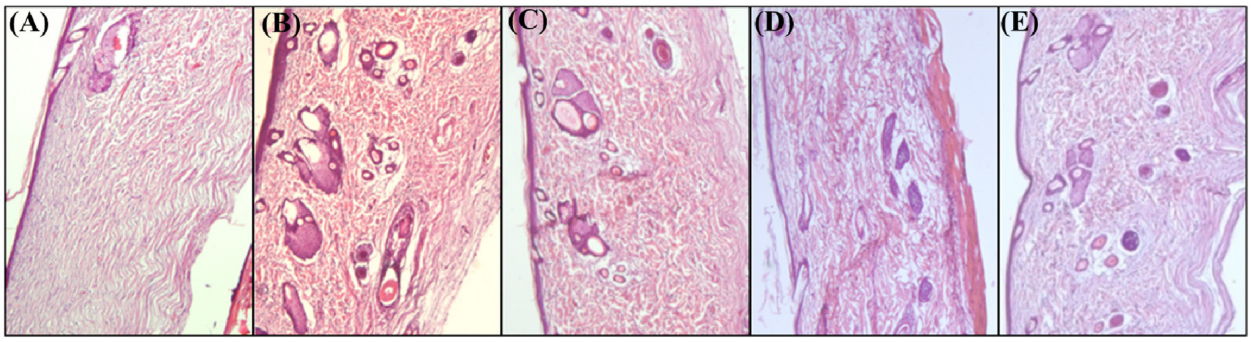


**Figure 8.** Histology of wound after 22 days of treatment with (A) control (non–treated wound); (B) β-glucan/PVA/PVP/κC/glycerin blended hydrogel; (C) PVA/PVP/κC/glycerin blended hydrogel; (D) Ridoar gauze; (E) Mediform. Magnification 10×5 (ocular lens×objective lens).

exception of the non-treated wound (Control), all the wounds were fully recovered after 17 days of treatment and there were no significant differences on the time of complete recovering of the wounds. Figure 8 shows the histological appearances of wounds after 22 days of treatment. In the epidermis, the epithelia were orderly arranged and stratum corneum was observed. The dermis was filled with collagen fibers and fibroblasts and observed angiogenesis. In addition, vasodilatation occurred that increased the permeability of blood vessels to deliver neutrophils and monocytes that differentiated into mac-

rophages to phagocytize microbes.[20] All the wounds were no significant differences on the histological appearances, but a reduction in capillary density was noted in the non-treated wound (Control).

## Conclusions

In this study, hydrogels which consisted of β-glucan, PVA, PVP, κC and glycerin were prepared by gamma-ray irradiation. Physical properties such as gel fraction, absorption ratio, and compressive strength were conducted to evaluate the effect of irradiation dose. The gel fraction and the compressive strength increased with increasing irradiation dose, whereas the absorption ratio decreased with increasing irradiation dose. The β-glucan and its release from hydrogels were confirmed by the chemical analysis data. Finally, the animal test and histological examination showed that β-glucan/PVA/PVP/κC/glycerin blended hydrogels were suitable for wound healing. Therefore, the resulting hydrogels can be considered to be a promising wound dressing materials.

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