Evaluation of Radiation Synthesized β-Glucan Hydrogel Wound Dressing using Rat Models

Hui J. Gwon, Youn M. Lim, Jong S. Park, and Young C. Nho

Abstract**—**In this study, hydrogels consisted of polyvinyl alcohol, propylene glycol and β-glucan were developed by radiation technique for wound dressing. The prepared hydrogels were characterized by examining of physical properties such as gel fraction and absorption ratio. The gel fraction and absorption ratio were dependent on the crosslinking density. On observing the wound healing of rat skin, the resulting hydrogels accelerated the wound healing comparing to cotton gauze. Therefore, the PVA/propylene glycol/β-glucan blended hydrogels can greatly accelerate the healing without causing irritation.

Keywords**—**β-Glucan, poly(vinyl alcohol), propylene glycol, radiation, wound dressing.

I. INTRODUCTION

HYDROGELS are three dimensional, hydrophilic, polymeric networks, which can absorb large amounts of water or a biological fluid without dissolution due to the presence of chemical crosslinks, or physical crosslinks [1], [2]. It has been reported that a hydrogel can absorb an excess of wound exudates, protect a wound from secondary infection, and effectively promote the healing process by providing a moisturized wound healing environment [3]. An ideal wound dressing should absorb body fluids effectively, be painless for a removal, have a high elasticity, good adhesion and easy replacement, and act as a barrier against bacteria [4], [5]. Many commercially available synthetic polymers, such as polyvinyl alcohol (PVA), show physicochemical and mechanical properties comparable to those biological tissues to be substituted [6]. These PVA based on hydrogels have received increasing attention in biomedical and biochemical applications, because of their permeability, biocompatibility and biodegradability [7]. Therefore, PVA hydrogels have been developed for repair of wounds and promotion of a wound healing [8]. Propylene glycol is a colorless, nearly odorless, clear, viscous, sweet, and hydroscopic liquid. In the skin and hair, the propylene glycol works as a humescent, which causes retention of moisture content of skin or cosmetic products by preventing the escape of moisture or water [9].

β-Glucan is composed of glucose units linked together to form a long polymer chain and is a fiber-type homopolysaccharide obtained from the cell walls of yeast, oats, barley, and from many medicinal mushrooms [10]. It has immune-enhancing properties, which nutritionally potentiate and modulate an immune response [11]. Also, the β-glucan is effective against allogenic, syngenic, and autochthonous tumors due to its antibacterial and antiviral effects [12].

Recently, γ-irradiation is recognized as a very suitable tool for the formation of those hydrogels. The radiation process has various advantages such as easy process control, possibility of combining the hydrogel formation and sterilization in one technological step, no necessity to add any initiators and crosslinkers possibly harmful and difficult to remove [13].

In this study, the poly(vinyl alcohol) (PVA) hydrogel containing β-glucan (Glu) was prepared by using γ-ray irradiation to simplify the crosslinking process, and propylene glycol (PG) was used as a moisturizer to improve the skin adherence when the PVA hydrogels were applied. The effects of the irradiation dose and the contents of PVA and PG on the gel fraction and absorption ratio were investigated to create the desired hydrogels for an advanced wound dressing.

II. EXPERIMENT

A. Materials

β-Glucan (Mw = 3.5×10^5) was supplied by Quegen biotech Inc. (Siheung, Korea). Poly(vinyl alcohol) (PVA) (Mw of about 79 kDa, 98.0–99.5% hydrolyzed) was purchased from DC Chemical Co., Ltd. (Iksan, Korea). Propylene glycol (PG) was supplied by SHOWA Chemical Co., Ltd. (Tokyo, Japan). All reagents were used without further purification. Distilled water (DW) was used as a solvent in all experiments. Animal experiments were approved by the Korean Laboratory Animal Care and Use Committee. All procedures were conducted in accordance with the ethical guidelines of the institute (KAERI), and for the care and use of laboratory animals (Institute for Laboratory Animal Research 1996).

B. Preparation of PVA/PG/Glu hydrogels

PVA hydrogel was prepared with previously reported procedure [14]. The mixture of PVA and PG was dissolved in

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distilled water at 120 °C for 20 min by using an autoclave. The solution was blended with Glu (1.0 wt%). To obtain a homogenous mixture, the PVA/PG/Glu solution was stirred and then kept in a water bath at 75 °C for 12 h. Thereafter, the solutions were then poured into Petri-dishes and γ-irradiation was performed by ^{60}Co γ-ray and its dose rate was 10 kGy/h. The PVA/PG ratio was in the range of 9:1–7:3 wt%, and the solid concentration of the total PVA/PG solution was 25 wt%.

C. Gel fraction ratio of PVA/PG/Glu hydrogels

The gel fraction of the hydrogels was measured by extraction in a hot DW of 70 °C for 48 h and vacuum dried at 50 °C for 48 h until they reached constant weight. The gel fraction (G_c) was defined gravimetrically by G_c (%) = (W_d/W_i) × 100, where W_d is the oven-dried gel weight after the washing for 48 h, and W_i is the initial weight of the dried hydrogels.

D. Absorption ratio of PVA/PG/Glu hydrogels

The hydrogel samples were immersed in distilled water for different times at room temperature until an equilibrium state of absorption was achieved. After the excessive surface water was removed with filter paper, the weight of the swollen gel was measured at various time intervals. The procedure was repeated until there was no further weight increase. The swollen gels were then dried at 70 °C for 48 h to a constant weight. The absorption ratio (D_s) was given by D_s (%) = [(W_s–W_d)/ W_i] × 100, where W_s is the weight of the swollen gels, W_d is the oven-dried gel weight after the swelling, and W_i is the initial weight of the dried hydrogels.

E. Animal test

In order to examine the therapeutic effect of the hydrogel on wounds, the following experiments were conducted. Male Sprague-Dawley rats, aged 7 weeks and weighing 200–220 g, were purchased from Orient Bio Inc. (Korea). Prior to the test, the rats were anesthetized with diethyl ether. After removing the dorsal hair of the rats with an electric razor, 10% aqueous povidone-iodine and 70% ethylalcohol 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 created from the back. Each wound was covered with equal size of the PVA/PG/Glu hydrogels, PVA/PG hydrogel, and cotton gauze. The hydrogel treated rats were placed in individual cages and the wound healing progress was observed using a digital camera.

III. RESULTS AND DISCUSSION

A. Gel fraction ratio and absorption ratio of PVA/PG/Glu hydrogels

The radiation technique is a very convenient tool for the improvement or modification of polymer materials through crosslinking, grafting, or degradation. The PVA/PG/Glu hydrogels were successfully prepared by using γ-irradiation.

Gel fraction (G_c) and absorption (Ds) are very important properties to evaluate application of the hydrogels for a wound dressing. Fig. 1 shows the G_c of the PVA/PG/Glu hydrogels.

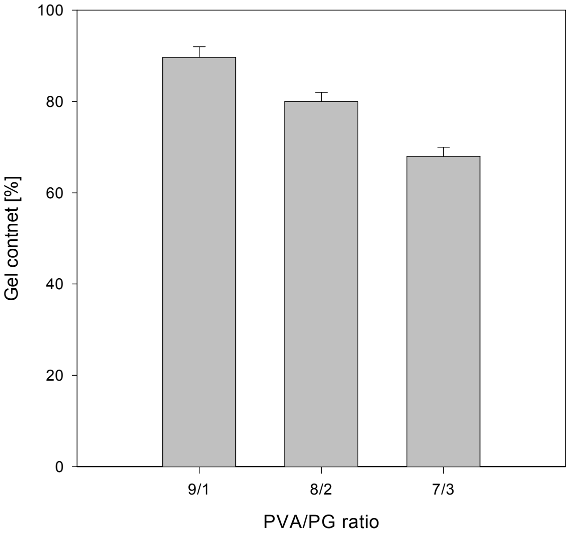

Fig. 1 Gel content of PVAL/PG/Glu hydrogels as a function of PVA/PG ratio

When the PVA/PG contents was 9/1 wt% and the irradiation dose at 25 kGy, the hydrogels showed an excellent gel fraction ratio (85%). The G_c was continuously decreased as the amount of PG in PVA/PG increased because the PG is not crosslinked by an irradiation. Therefore, the crosslinking density decreased with increasing PG concentration. The crosslinking density is one of the most important factors that affect gel fraction and absorption ratio of hydrogels.

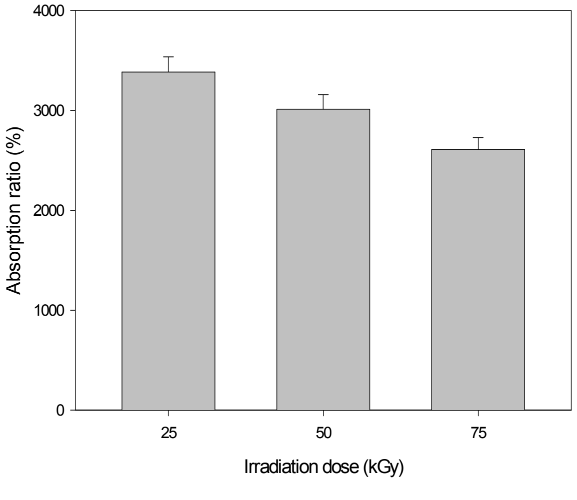

Fig. 2 Absorption ratio as a function of the irradiation dose

Highly crosslinked hydrogels have a tighter structure so that they hinder the mobility of the polymer chains, hence lowering their absorption ratio. These hydrogel should have large water absorption capacity in order to absorb wound exudates and this absorption property makes those hydrogels a suitable material as a wound dressing.

Fig. 2 and 3 shows the absorption ratio of the hydrogels as a function of the irradiation dose and immersion time. The absorption decreases with increase in irradiation dose due to an increasing of crosslinks. These crosslinks restrict extensibility of the polymer chains induced by swelling of fluids and thus counter any tendency for dissolution. Thus the swelling ratio reduces with the increase in network. The absorption curves show a similar shape but differ in size and position, and the absorption ratio sharply increases and then begins to decrease. This is probably due to a collapse of the hydrogel network.

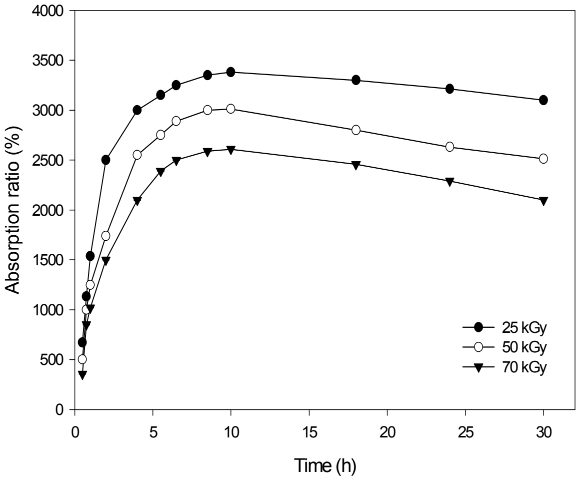

Fig. 3 Absorption ratio as a function of the immersion time

B. *Animal test of PVA/PG/Glu hydrogels*

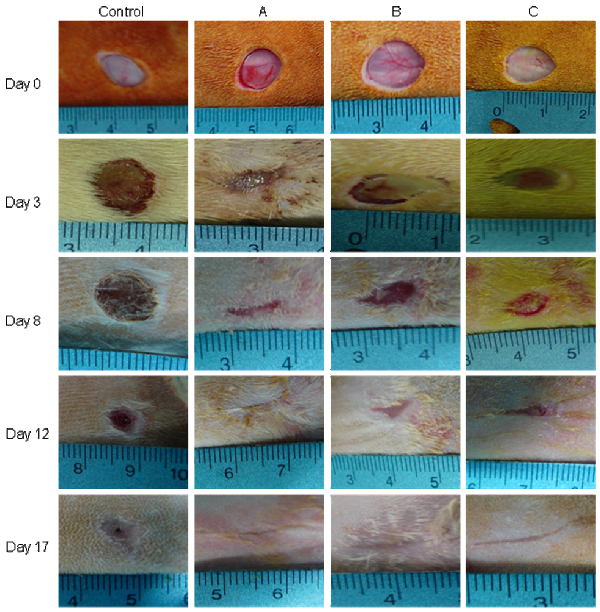

Fig. 4 Photographs of macroscopic appearance of wound repair covered with control, PVA/PG/Glu hydrogel (A), PVA/PG hydrogel (B), cotton gauze (C) at different time: day 0, day 3, day 8, day 12, and (f) day 17

Fig. 4 shows the macroscopic observations of the wound healing progress treated with different means. In case of hydorgel- and cotton gauze-treated wound, healing was faster than the non-treated wound. More precisely, substantial wound contractions in the PVA/PG/Glu-treated wounds were observed and more than 50% wound closure was achieved in the 3 days. The healing of PVA/PG treated wound was slower than Glu-treated wound. It can be accounted for the release of β-glucan from the resulting hydrogel. On the other hand, the cotton gauze-treated wounds healed slowly and about 85% wound closure was achieved only after 12 days. With the exception of the non-treated wound (control), all the wounds were fully recovered after 17 days of treatment.

Therefore the PVA/PG/Glu hydrogels were applicable as wound dressing.

IV. CONCLUSION

In this work, attempts were made to prepare hydrogels for advanced wound healing which consisted of PVA/PG and β-glucan. Gel fraction increased with increasing irradiation dose and decreasing PG concentration, whereas the absorption ratio decreased with increasing irradiation dose. On observing the wound healing of rat skin, the wound contraction ratio can reach 85% after treating with PVA/PG/Glu hydrogel for 8 days, while that was 85% when treating with cotton gauze for 12 days. This accelerating effect can be attributed to the release of β-glucan from the hydrogel. The results demonstrated that PVA/PG/Glu hydrogels has a potential for an accelerate wound healing.

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