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Preparation and characterization of PVA/PVP/glycerin/antibacterial agent hydrogels using γ-irradiation followed by freeze-thawing

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Abstract−Hydrogels for wound dressings from a mixture of poly(vinyl alcohol) (PVA), poly(*N*-vinylpyrrolidone) (PVP), glycerin and an antibacterial agent were obtained by a γ-irradiation combined with freeze-thawing. The physical properties such as the gelation and swelling degree of the hydrogels were examined. When the PVP/PVA ratio was 6 : 4 (wt%) and prepared by combined irradiation and freeze-thawing, it showed an excellent swelling capacity (>1,200%). The antibacterial effect of the hydrogels containing the antibacterial agents was observed to be effective as the concentration of antibacterial agents increased. The results demonstrated that hydrogel in a proper blending ratio could be used as a wound dressing that can accelerate wound healing with an antibacterial effect.

Key words: Hydrogels, Radiation, Antibacterial, Freeze-thawing

INTRODUCTION

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing a large amount of water or biological fluid [1]. The networks are composed of a homopolymer or copolymer, and are insoluble due to the presence of chemical crosslinks (tie-points, junctions), or physical crosslinks such as entanglements or crystallites [2]. The hydrogels resemble natural living tissue more than any other classes of synthetic biomaterials due to their high water content and soft consistency, which is similar to natural tissue. The chemical structures of the polymer affect the swelling ratio of the hydrogels. The hydrogels containing a hydrophilic group swell to a higher degree compared to those containing hydrophobic groups. The interaction responsible for the water sorption by hydrogels includes the process of hydration, which is connected with the presence of such chemical groups as -OH, -COOH, -$CONH_2$, -CONH-, -SO_3H, and the existence of capillary areas and differences in the osmotic pressure. The force that makes it impossible for the hydrogel to dissolve is connected with the existence of the covalent bonds between the individual polymer chains, although they may also have a character of electrostatic or hydrophobic interactions.

Hydrogels used as wound burn dressings were invented by [3] and they have many interesting properties: immediate pain control, easy replacement, transparency to allow healing follow up, absorbance and prevention of loss of body fluids, barrier against bacteria, good adhesion, good handling, oxygen permeability, control of drug dosage and so on. They usually show a good biocompatibility in contact with blood, body fluids and tissues [3]. Hence, they are often used for contact lenses, burn wound dressings, artificial cartilages or membranes as well as the coating materials being applied in the contact with living organism, e.g., coating of the surface of catheters, electrodes, vascular prostheses etc. Because of their ability to swell as well as to release the trapped particles into the surrounding medium, hydrogels are often used as drug delivery systems. Gamma or electron beam irradiation, especially if combined with simultaneous sterilization of the product, is a very convenient tool for the preparation of hydrogels [4].

PVA is frequently used in the preparation of various membranes and hydrogels [5,6]. The PVA can generate a physical hydrogel by freeze-thaw cycles [7]. The PVA hydrogels have received increasing attention in biomedical and biochemical applications because of their permeability, biocompatibility and biodegradability [1,8,9]. They have excellent transparency and biocompatibility. The PVP is used for a main component of temporary skin covers or wound dressings [10].

Glycerin is a colorless, transparent, and odorless sweet syrupy liquid. It is a humectant, i.e., "draws moisture." It is used in creams, lotions, facial treatments, masks, and other body care products.

Gamma-ray irradiation is recognized as a very suitable tool for the formation of 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 [11].

We are exposed to harmful microorganisms such as bacteria and molds every day. Antibacterial agents are added to plastics, papers and fibers products to kill or to prevent them from growing bacteria and molds.

If the wound's pollution is heavy, a natural antibacterial agent such as chitosan has limitation. Hence the addition of antibacterial agents such as silver sulfadiazine, chlorohexidine digluconate, and

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zinc oxide is needed.

In this work, attempts were made to prepare the hydrogels for wound dressing that consisted of PVA, PVP, glycerin, and an antibacterial agent. The physical properties, such as the gelation and swelling, were examined to evaluate the usefulness of the hydrogels for wound dressings. The antibacterial effect of the hydrogels before and after irradiation was observed through the antibacterial experiment.

EXPERIMENT

1. Materials

Poly(vinyl alcohol) (PVA) (M_w=8.5×10^4-1.46×10^5) and poly(*N*-vinylpyrrolidone) (M_w=1.3×10^6) were supplied by the Aldrich Chemical Company (WI, USA). Glycerin was supplied by the Showa Company (Japan). Silver nitrate and silver sulfadiazine were supplied by the Aldrich Chemical Company (USA). Chloramine-T and sulfadiazine sodium salt were supplied by the Sigma Company (MO, USA). These polymers were used without further purification. Distilled water was used as a solvent in all the experiments.

2. Preparation of Hydrogels

PVA/PVP (6 : 4, wt%) was dissolved in distilled water at 90 °C, and then mixed with an antibacterial agent and glycerin by a mechanical stirrer at room temperature to give a PVA/PVP/glycerin/antibacterial agent solution. The weight ratio of PVA to PVP was based on our previously reported paper [12,13] and the glycerin concentration was 3 wt%. The antibacterial agent concentrations were 0.5, 1, 1.5, 2 wt%, and the total concentration of the PVA/PVP/glycerin/antibacterial agent was 15 wt%. The solution was poured into a petri dish at room temperature. The solution was kept at room temperature for 24 h in order to remove any air bubbles. Hydrogels from a mixture of an antibacterial agent, glycerin, and PVA/PVP were made by freeze-thawing or ^{60}Co γ-ray irradiation after freeze-thawing. The number of repeated freeze-thawing was 2 times to crosslink the PVA/PVP/glycerin/antibacterial agent solution physically. Each freeze-thawing involved lowering the temperature to −70 °C, standing at this temperature for 1 h, and then raising the temperature to room temperature. Gamma irradiation dose was 25 kGy at dose rate of 10 kGy/hr.

3. Gel Content

The gel content of the hydrogels was measured by extraction in hot distilled water at 50 °C for 48 h and drying in vacuum at 50 °C for 48 h until they reached constant weight. The gel content was defined as in Eq. (1), where W_d is the dried gel weight after extraction, and W_i the initial weight of the polymer.

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

4. Degree of Swelling

The degree of swelling could be described as the water absorptivity of the hydrogels. The gel samples were immersed in distilled water for 48 h at room temperature until the gel reached the equilibrium state of swelling. After the water on the surface of the swollen gels was removed with cellulose paper, the mass was determined. The dried gels were obtained by drying at 50 °C until they reached a constant weight.

The degree of swelling was defined as in Eq. (2), where W_s is the weight of the swollen gels and W_d is the dried gel weight.

$$\text{Water absorptivity } (\%) = \frac{W_s - W_d}{W_d} \times 100 \quad (2)$$

5. Antibacterial Test

Escherichia coli (*E. coli*), *Staphylococcus aureus* (*S. aureus*), and *Shudomonas aeruginosa* (*S. aeruginosa*) were used. These strains, in their original dehydrated forms, were reconstituted in Luria-Bertani (LB) broth at 37 °C for 1 h. From this reconstituted bacterial broth, single colonies were obtained by sub-culturing the three strains onto Tryptic Soy Agar (TSA) via the 16-streak dilution technique. Single colonies obtained were sub-cultured back into the LB broth and incubated at 37 °C overnight on a rotary shaker. Single colony cultures of the broth strains obtained through this technique were kept in the LB broth at 4 °C prior to use. When the confluent agar cultures were required, 100 μL of the single colony broth cultures was inoculated into a 10.0 mL LB broth and incubated at 37 °C overnight on a rotary shaker. A 10×dilution of this initial culture, carried out with the LB broth, was plated onto TSA plates. The culture was grown to confluence at 37 °C overnight.

The antibacterial effect of the antibacterial agent-incorporated wound dressing (11 mm diameter disc) was tested on agar plates inoculated with *E. coli*, *S. aureus*, and *S. aeruginosa* by using the disk diffusion test. Inhibition zones around the antibacterial agent-incorporated wound dressing were compared after 24 h incubation at 37 °C.

RESULTS AND DISCUSSION

1. Gel Content

Crosslinking by radiation transforms a linear polymer into a three-dimensional molecule, resulting in a significant increase in the molecular mass, lower solubility in organic solvents, and improved mechanical properties. Degradation results in a decrease in the molecular mass, and has the opposite effect on the physical properties of the polymer. Crosslinking and degradation occur simultaneously. However, the ratio of their rates depends on the chemical structure of the polymer, its physical state, and the irradiation state. Polymers are generally divided into those that predominantly crosslink and those that predominantly degrade. PVA and PVP are easily crosslinked in their homogeneous mixture with water.

Some researchers proposed a computationally efficient method for calculating the affinity of the silver ion for C-, H-, N-, O- and S-containing ligands as well as the silver affinities of 18 ligands and the geometries of these complexes [14]. The increase in the gelation by the addition of a silver-containing antibacterial agent can be attributable to the silver complex with PVP/PVA.

Fig. 1 shows the gelation behavior of the hydrogels containing silver nitrate, chloramine-T, silver sulfadiazine and sulfadiazine sodium salt in a PVA/PVP/glycerin/antibacterial agent. Gel content increased as the concentration of the silver nitrate increased, while the gel content decreased as the concentration of the chloramine-T increased. Gel content containing silver nitrate was in the range of 38-51%. Gel content of the hydrogels containing chloramine-T was in the range of 36-42%. Gel content of the hydrogels containing silver sulfadiazine was in the range of 56-63%. Gel content containing sulfadiazine sodium salt was in the range of 36-52%. Gel

content of the hydrogels increased regardless of the type of antibacterial agent as the concentration of the antibacterial agents increased. These results support that the affinity of silver ion and S-containing ligands increases the gelation of the PVP/PVA hydrogels as reported in a previous paper [14]. Therefore, the more silver ions or S-containing ligands were included in the PVP/PVA hydrogels, the higher gel content of PVP/PVA hydrogels showed.

2. Degree of Swelling

Fig. 2 shows the swelling behavior of the hydrogels containing silver nitrate, chloramine-T, silver sulfadiazine and sulfadiazine sodium salt in a PVA/PVP/glycerin/antibacterial agent. Degree of the swelling containing silver nitrate was in the range of 1,350-1,667%. Degree of the swelling of the hydrogels containing chloramine-T was in the range of 1,600-1,716%. Degree of the swelling of the hydrogels containing silver nitrate decreased as each concentration increased. Whereas, the degree of the swelling of the hydrogels containing chloramine-T increased as the concentration of the chloramine-T increased. Degree of the swelling of the hydrogels containing silver sulfadiazine was in the range of 1,190-1,522%. Degree of swelling of the hydrogel containing sulfadiazine sodium salt was in the range of 1,343-1,776%. Degree of the swelling of the hydrogels containing the four antibacterial agents decreased as the concentration of the antibacterial agents increased. According to Fig. 1-2, the swelling percent is inversely proportional to the gel percent [15,16]. As a result, the swelling ratio in water decreased as each concentration increased to the exclusion of chloramine-T. For this reason, an increase in ionization of each ion in Ag- or Na-containing ligands might be expected to decrease the swelling ratio during the test.

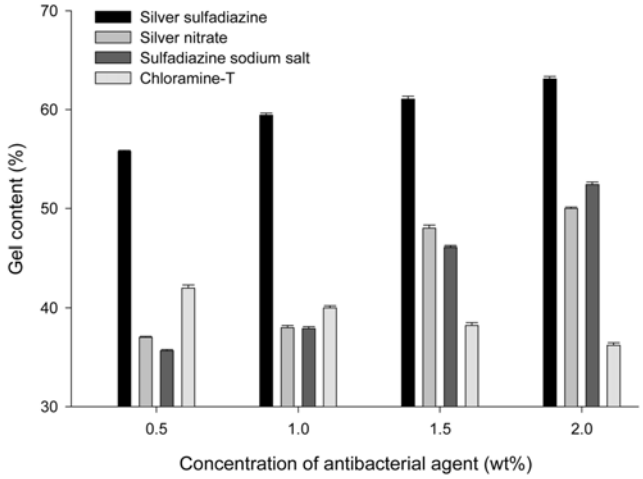

Fig. 1. Gel content of the PVA/PVP/glycerin/antibacterial agent hydrogels.

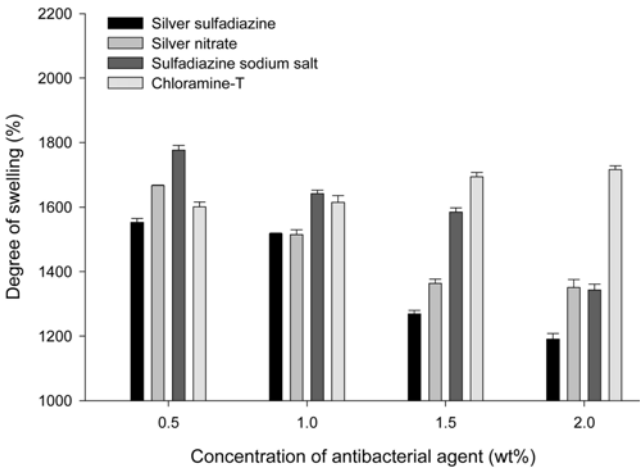

Fig. 2. Degree of the swelling of the PVA/PVP/glycerin/antibacterial agent hydrogels.

3. Antibacterial Effect

Inhibition zones around the antibacterial agent-incorporated wound dressing were measured after 24 h incubation at 37 °C.

Table 1 shows the measured inhibition zones. PVA/PVP/Glycerin/antibacterial agent hydrogels show an antibacterial effect before and after the irradiation of hydrogels for the three strains. Concen-

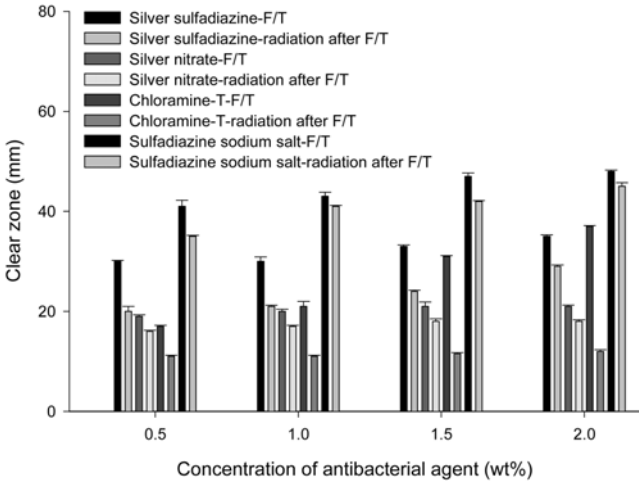

Fig. 3. Comparison of the Clear zone of the PVA/PVP/glycerin/antibacterial agent hydrogels prepared at 2 times of freeze-thawing (F/T) and irradiated at 25 kGy after 2 times of freeze-thawing against the *E. coli*.

Table 1. Clear zone of PVA/PVP/glycerin/antibacterial agent hydrogels irradiated after freeze-thawing (F/T) or F/T vs. different bacteria (antibacterial agent 1 wt%)

Strains	*E. coli*		*S. aureus*		*S. aeruginosa*	
Procedure for hydrogel	F/T	Irradiation after F/T	F/T	Irradiation after F/T	F/T	Irradiation after F/T
Silver nitrate	20 mm	17 mm	20 mm	19 mm	23 mm	21 mm
Silver sulfadiazine	30 mm	21 mm	29 mm	20 mm	35 mm	26 mm
Sulfadiazine sodium salt	43 mm	41 mm	40 mm	37 mm	46 mm	43 mm
Chloramine-T	21 mm	11 mm	20 mm	11 mm	15 mm	11 mm

tration of the added antibacterial agents was 1 wt%, and the hydrogel disc was 11 mm diameter.

Fig. 3 shows the effect of the gamma irradiation on the antibacterial effect of the hydrogels. The antibacterial effect generally decreased after irradiation. Especially, the antibacterial effect of the chloramine-T largely decreased much after gamma irradiation. It can be explained that chloramine-T does not have a radiation-resistant property. Radiolysis products may be formed which might affect the quality of the drug. Even small changes in the structure of drug molecules may lead to the formation of biologically inactive derivatives [17,18]. The hydrogels containing other antibacterial agents were partially affected by gamma-irradiation. It means that sulfadiazine sodium salt has a good radiation-resistant property.

CONCLUSION

Attempts were made to prepare hydrogels for wound dressing which consisted of PVA, PVP, glycerin, and an antibacterial agent. The hydrogels were made by ^{60}Co γ-ray irradiation after freeze-thawing. The physical properties of the hydrogels, such as the gelation and degree of swelling, and the antibacterial effect were evaluated. Gel content of the hydrogels containing silver sulfadiazine, silver nitrate and sulfadiazine sodium salt increased as their concentrations increased. Gel content of the hydrogels containing chloramine-T decreased as its concentration increased. Swelling degree of the hydrogels was inversely proportional to the gel content. Antibacterial effect of the hydrogels containing the antibacterial agents was observed to be effective as the concentration of antibacterial agents increased. Antibacterial effect decreased generally after irradiation. In particular, the antibacterial effect of the chloramine-T largely decreased after gamma irradiation. However, gamma irradiation had almost no influence on the antibacterial properties of the hydrogels containing sulfadiazine sodium salt.

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