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Development and in vitro evaluation of chitosan–polysaccharides composite wound dressings

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Abstract

This study focuses on the design and evaluation of chitosan-based films intended for wound dressing application. Films of chitosan and their blends with cornstarch and dextran were developed to improve the films' physical strength. Polypropylene glycol at concentrations of 0.5, 1.0 and 1.5% (w/v) was added to improve the films' flexibility. Some properties required for successful wound dressing, such as liquid adsorption, vapor and oxygen penetration, bioadhesiveness, and film elasticity, were examined. Chitosan films showed the highest liquid adsorption and the adsorption tended to decrease with addition of cornstarch and dextran. Moisture vapor and oxygen were found to be able to penetrate through all film formulations, and those films with cornstarch and dextran showed increased penetration rates through the films. The bioadhesiveness test using a pig gut model did not show significantly different bioadhesive properties with the addition of cornstarch and dextran. The film elasticity of the formulation containing only chitosan exhibited the lowest elongation of the film at a force of 2 N, but increased with the addition of cornstarch and dextran, respectively. In conclusion, the design and development of chitosan as a wound dressing can be improved by the addition of cornstarch or dextran, and propylene glycol to obtain the films with optimal properties for wound management.

Keywords: Wound dressing; Chitosan hydrogels; Cornstarch; Dextran; In vitro evaluation

1. Introduction

Unlike conventional wound dressing which passively provides wound protection, effective wound dressing nowadays requires not only protection of the wound from surrounding environments but also to effectively promote the healing process by providing an optimum microenvironment for healing, to remove excess of wound exudate, and allowing continuous tissue reconstruction processes (Yanna and Burke, 1980; Biagini et al., 1991; Matsuda et al., 1993; Su et al., 1997; Mi et al., 2001). The ideal wound dressing, therefore, should have the following properties: (1) able to protect the wound from secondary infection, (2) provide a moisturized wound healing environment, (3) provide thermal insulation, (4) removable without causing trauma to the wound, (5) remove drainage and debris, (6) free from particulate and toxic products, and (7) promote tissue reconstruction processes.

Chitosan has received great attention for medical and pharmaceutical applications due to its beneficial intrinsic properties. It is one of the natural polymers that has a high potential on wound healing application. Chitosan is a copolymer of β-(1-4)-linked 2-acetamido-2-deoxy-D-glucopyranase and 1-amino-2-deoxy-D-glucopyranase. This polycationic polymer is generally obtained by alkaline deacetylation of chitin, which is an extracted component of the crustacean exoskeleton. Both chitin and chitosan possess many properties that are advantageous for wound dressing, namely biocompatibility, biodegradability (Tomihada and Ikada, 1997), hemostatic activity (Abhay, 1998), anti-infection and wound healing acceleration properties (Ueno et al., 2001; Suzuki et al., 1994). However, pure chitosan films have a poor tensile strength and elasticity due to their brittleness. Hence, addition of other polymers is necessary to achieve films with improved strength and elasticity.

Cornstarch has been widely used to produce biodegradable films because of its low cost and renewability. However,

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a wide application of cornstarch film is limited by its low water solubility and brittleness (Xu et al., 2005). Dextran, a polysaccharide synthesized by microorganisms, is composed of glucose residues combined mainly by α-1 → 6 bonds with different degrees of branching. Dextran is a naturally biodegradable hydrophilic polymer, which shows enzymatic degradation and possesses relatively good biocompatibility. Addition of these polysaccharides to chitosan films may improve the physical properties of the films (Viyoch et al., 2003).

In this study, composite wound dressings were developed based on two main requirements: its wound healing performance and simple production. Chitosan-based composite films were prepared by adding cornstarch and dextran as composite polymers, glutaraldehyde as a crosslinker and polyethylene glycol as a plasticizer. The developed films were tested in vitro for properties required for wound dressing applications including water vapor penetration, water uptake, oxygen penetration, bioadhesive properties, film elasticity, bacterial penetration, and residual glutaraldehyde content.

2. Materials and methods

2.1. Materials

Chemicals were obtained from commercial suppliers and were used as received: High molecular weight chitosan (MW 474 kDa) with a deacetylation degree of 96% was purchased from Aqua Premier Co., Ltd., Thailand. Cornstarch was purchased from Taiwan Chemical, Thailand. Dextran MW 100k was purchased from Sigma–Aldrich, Inc., USA. Analytical grade of lactic acid and glutaraldehyde (25%, v/v) were purchased from Riedel deHaen, Germany.

2.2. Preparation of the composite films

The films were prepared, using casting and solvent evaporation technique. To prepare chitosan films, a specified amount of chitosan was dispersed in deionized water and agitated for 1 h. Lactic acid was then added to the chitosan solution, followed by glutaraldehyde under gentle agitation. The mixed solution was left to stand until air bubbles have disappeared and the solution was then poured on a dry glass petri dish in a dust-free environment and allowed to air dry at 40 °C for 24 h. The obtained films were tested for their properties.

To prepare chitosan–cornstarch films, the colloidal mixture of cornstarch was prepared by dispersing cornstarch in deionized water, and heating with constant agitation until gelatinized occured. The colloidal mixture was left to cool and added to the chitosan solution. Glutaraldehyde was added to the mixed solution under gentle agitation. The resultant mixture was left to stand until the air bubbles disappeared and was then poured on a dry glass petri dish in a dust-free environment and allowed to air dry at 40 °C for 24 h. The obtained films were tested for their properties.

To prepare chitosan–dextran films, dextran was dissolved in deionized water and added to the chitosan solution. The aqueous solution of chitosan–dextran was left to stand until the air bubbles disappeared, followed by the addition of glutaraldehyde. The air bubble-free solution was poured on a dry glass petri dish in a dust-free environment and allowed to dry at 40 °C for 24 h. The obtained films were tested for their properties.

To study the effect of propylene glycol as a plasticizer, polypropylene glycol at concentrations of 0.5, 1.0 and 1.5% (w/v) was added to each formulation before the addition of glutaraldehyde. The resultant mixture was left to stand until the air bubbles disappeared and was then poured on a dry glass petri dish in a dust-free environment and allowed to air dry at 40 °C for 24 h. The obtained films were tested for their properties.

2.3. Characterization of the composite films

2.3.1. Water vapor penetration

To measure the water vapor penetration, the films were cut and placed on top of open 2.5 cm bottles containing 5 g of silica gel and held in place with a screw lid (test area: 4.9 cm^2). The bottles were conditioned in a desiccator containing silica gel for 12 h. The bottles were then placed in a desiccator containing a saturated solution of NaCl solution at 30 °C (75% RH). The equilibrium moisture penetration was determined by weighing the bottles at 0, 12, 24, and 48 h, respectively.

2.3.2. Water uptake

The water uptake was determined gravimetrically. The weights of the completely dried films were determined directly with an analytical balance. Strips of chitosan-based films (1 cm × 2 cm) were immersed into deionized water at 37 °C in an incubator for 24 h. The resultant swollen film was gently blotted with filter paper to remove excess surface water and weighed again. The water uptake of the film is expressed as the percentage of weight increased.

2.3.3. Mechanical properties

To determine the mechanical properties of the films, a stretch test was performed on an Instron apparatus (Model 3342, Instron Corp., Canton, MA). In the stretch test, the test films (0.5 cm × 3 cm test section) were held in place to the clippers which was attached to the cell of an Instron device. The upper clipper was driven upward stretching the film at various forces. The mechanical properties of films were determined as the elongation of films under the stress of 1 and 2 N.

2.3.4. Oxygen penetration

Oxygen penetration through films was performed by placing the films on top of open 250 ml flasks containing 200 ml of deionized water and held in place with a screw lid (test area: 4.9 cm^2). The negative control was the closed flask with an airtight cap preventing oxygen to enter the flask while the positive control was the open flask allowing oxygen to enter the flask and dissolve in the water as recipient. The test flasks were placed in an open environment under constant agitation for 24 h. The collected water samples were then analyzed for dissolved oxygen according to Winkler's method (Glazer et al., 2004). In the Winkler's method, a divalent manganese solution was added into the tested solution, followed by a strong alkali (NaOH). Under such

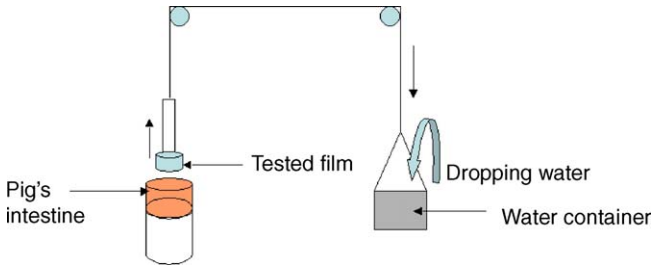

Fig. 1. Diagram of the instrument for in vitro evaluation of bioadhesive properties of the films.

conditions, any dissolved oxygen in the tested solution rapidly oxidizes an equivalent amount of divalent manganese (Mn^{+2}) to manganese dioxide (MnO_2(s)) of a higher valence state (Mn^{+4}). The MnO_2(s) exists as a precipitate in the solution. When the solution is acidified in the presence of iodide (KI), free iodine (I_2) is produced in a concentration which is equivalent to the original concentration of dissolved oxygen in the tested solution. The sample was titrated with 0.025 N sodium thiosulfate solution with the addition of starch solution as an indicator until blue color was obtained. The results were expressed as the amount of dissolved oxygen (mg/ml).

2.3.5. Bioadhesive properties

The in vitro evaluation of the bioadhesive properties of the films was carried out using an in-house pulley system instrument (Fig. 1). The proximal portion of a pig's large intestine was used in order to represent the mucous-like texture of a fresh wound. The large intestine of freshly slaughtered pigs was washed with physiological saline at 4 °C and attached on a platform (test area: 4.9 cm^2). A pre-wetted film was taken and attached to the intestine with a weight of 100 g for 2 min with the other side attached to a platform connecting to a pulley system. The bioadhesiveness of the film was measured by adding water to a water container connected with the pulley system until the film was separated from the intestine. The weight of water needed to separate the film from the intestine was recorded and used for the calculation of the detachment force.

2.3.6. Microbial penetration

The ability of films to prevent microbial penetration was tested by placing the films on open 10 ml vials containing 5 ml of nutrient broth (Merck, Germany) and held in place with a screw lid (test area: 0.8 cm^2). The negative control was a vial closed with cotton ball while the positive control was an open vial. The tested vials were placed in an open environment for 1 week. The cloudiness of the nutrient broth in any vial was recorded as microbial contamination.

2.3.7. Residual glutaraldehyde measurement

The film of a size 0.5 cm × 0.5 cm was dissolved in 1.0% (w/v) acetic acid and the sample solution was tested to measure amount of residual glutaraldehyde, using GC–MS (GP 2010, Shimadzu, Japan) with the injection temperature of 200 °C, column temperature of 150–250 °C, split injection mode and 100 kPa pressure.

2.4. Statistical analysis

All experiments for the characterization of the composite film were done in triplicate. One-way analysis of variance (ANOVA) was performed to determine the significant difference in each property among the formulated films. The differences were considered to be significant at a level of $P < 0.05$.

3. Results and discussion

From preliminary tests, non-crosslinked chitosan films were transparent, smooth and water-soluble. However, the films broke apart shortly after rehydration. Glutaraldehyde (3%, w/v) was added to weakly crosslink the films. During crosslinking, glutaraldehyde diffused into the polymeric solution forming either intermolecular or intramolecular linkages (Nakatsuka and Andrady, 1992; Thacharodi and Rao, 1993). The crosslinked films were not soluble in water and showed substantially strong membrane properties both before and after the rehydration process. Higher glutaraldehyde concentrations from 4 to 30% (w/v), while keeping the chitosan concentration constant at 3% (w/v) resulted in dark brown and insoluble films. Moreover, water absorption of the films decreased with increasing glutaraldehyde concentration due to the lower water solubility of chitosan after crosslinking. Increasing the total solid contents resulted in less flexibility and poor strength of the films, whereas lower concentrations resulted in poor structural integrity of the films, i.e., uneven and hard to remove from petri dish. The formulation of 3% (w/v) total polymers concentration and 3% (w/v) glutaraldehyde concentration was found to be the optimal concentrations with good overall basic properties of the films. These concentrations were used for the following experiments. The composition and physical properties of the films are summarized in Table 1. The chitosan–cornstarch films, whose solution appeared slightly cloudy during preparation, were less transparent than the other formulations.

3.1. Water vapor penetration

The water vapor penetration across the films at 6, 12, 24 and 48 h was measured and expressed as % weight increased of the dried silica gel. All composite films (chitosan, chitosan–cornstarch and chitosan–dextran) showed a similar water penetration profile as a function of time (Fig. 2). The vapor transmission was measured under steady state conditions. Therefore, the contribution of the moisture absorbed by the film can be considered negligible. Increasing the concentration of propylene glycol (0.5, 1.0, and 1.5%, w/v) as a plasticizer is expected to result in increased water vapor penetration because propylene glycol is a hydrophilic molecule that possesses water adsorbing properties. Higher propylene glycol concentrations in the film should adsorb more moisture from the atmosphere into the films. Thus a hydrated film should be able to facilitate vapor transfer from a moisture rich environment to a dry environment. However, this could only be found in chitosan–dextran films, but was not statistically different in pure chitosan and chitosan–cornstarch films (Fig. 3).

Table 1
The composition and basic physical properties of the films

Composite films	Chitosan (g)	Copolymer (g)	Lactic acid (ml)	Glutaraldehyde 25% (w/v) (g)	PG (g)	Physical features of solutions	Physical features of films
Chitosan 3%							
No PEG	3	–	3	0.09	0	Translucent , pale yellowish viscous solutions	Films are translucent, smooth, not flexible, and have poor adhesion
PG 0.5%	3	–	3	0.09	0.5		
PG 1%	3	–	3	0.09	1		
PG 1.5%	3	–	3	0.09	1.5		
Chitosan + cornstarch							
No PG	2.5	0.5	3	0.09	0	Cloudy white viscous solutions	Films are turbid, smooth, flexible, and have good adhesion
PG 0.5%	2.5	0.5	3	0.09	0.5		
PG 1%	2.5	0.5	3	0.09	1		
PG 1.5 %	2.5	0.5	3	0.09	1.5		
Chitosan + dextran							
No PG	2.5	0.5	3	0.09	0	Translucent , pale yellowish viscous solutions	Films are translucent, smooth, flexible, and have good adhesion
PG 0.5%	2.5	0.5	3	0.09	0.5		
PG 1%	2.5	0.5	3	0.09	1		
PG 1.5%	2.5	0.5	3	0.09	1.5		

3.2. Water uptake

Fig. 3 shows the equilibrium water uptake of chitosan, chitosan–cornstarch and chitosan–dextran films with different concentration of propylene glycol as a plasticizer. Chitosan films showed the highest increase in equilibrium water uptake, followed by composite films with cornstarch and dextran, respectively. Addition of gelatinized cornstarch and dextran resulted in less swollen films, due to lower water absorption of the less hydrophilic polysaccharides. Increasing concentration of propylene glycol from 0.5 to 1.5% (w/v) resulted in more hydrophilic films with increased average equilibrium water uptake.

3.3. Mechanical properties

The mechanical properties of the dry films were measured by a stretch test. As shown in Fig. 4, composite films with dextran showed the highest elongation under the force of 2 N, followed by the composite film with dextran and chitosan, respectively. These results imply that the formation of miscible, biodegradable composite film of chitosan with other hydrophilic biopolymers is a good technique to improve the mechanical properties of pure chitosan films. Xu et al. (2005) reported that an increasing tensile strength of chitosan–cornstarch films was attributable to the formation of intermolecular hydrogen bonds between NH_3^+ groups of the chitosan backbone and OH groups of the starch. The amino groups (NH_2) of the chitosan were protonated to form NH_3^+ in the presence of lactic acid in the solution, whereas the ordered crystalline structures of the starch molecules were destroyed by the gelatinization process, resulting in the OH groups being exposed readily to form hydrogen bonds with NH_3^+ of the chitosan. Composite films with increasing starch ratio, i.e., increased number of hydroxyl groups, therefore, show greater mechanical properties. On the other hand, the dextran chains exhibited high flexibility due to a greater possibility of internal rotation of α-1 → 6 bonds, compared to those of 1 → 4,

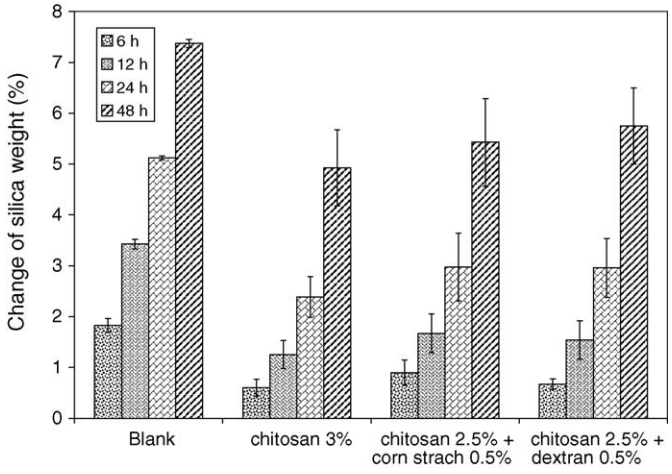

Fig. 2. The vapor penetration through films at 6, 12, 24, and 48 h.

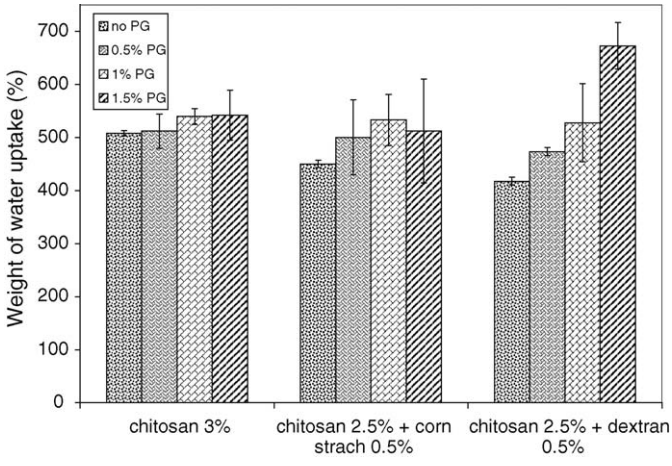

Fig. 3. The equilibrium water adsorption of the films as a function of polypropylene glycol concentration.

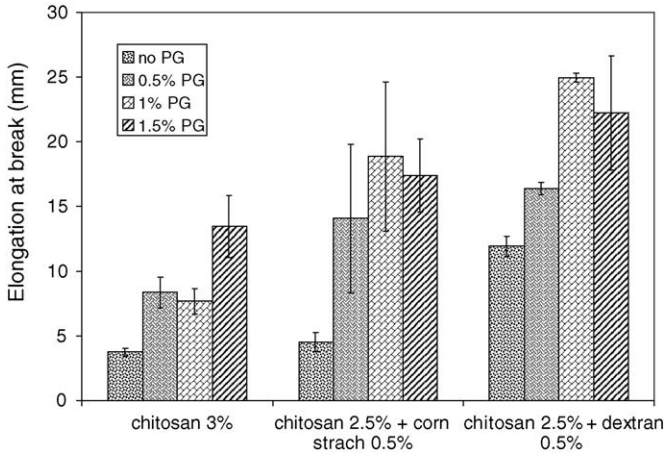

Fig. 4. The elongation of the films under the stretch test at force 2 N as a function of propylene glycol concentration.

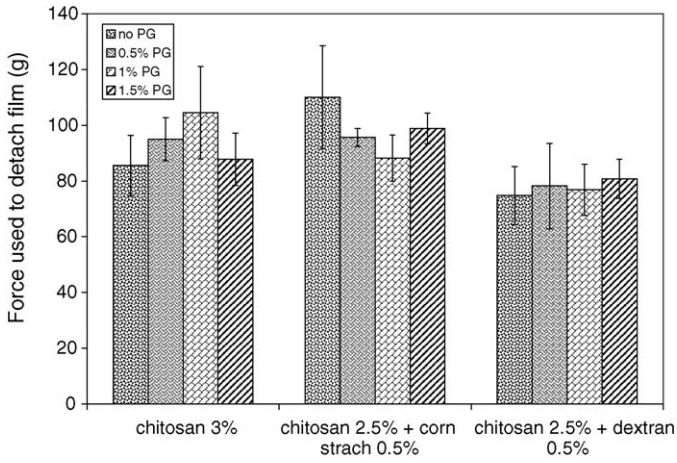

Fig. 5. The weight required to detach the attached films from the pig's intestine as a function of propylene glycol.

$1 \rightarrow 3$, $1 \rightarrow 2$ polysaccharides (Burton and Brant, 1983). Therefore, chitosan-composites film with dextran showed the highest elongation at 2 N.

The flexibility of chitosan-composite films can be further improved by incorporating propylene glycol as a plasticizer. The results revealed that most stretch values significantly increased with increasing propylene glycol concentrations. Since chitosan, chitosan–cornstarch and chitosan–dextran are all hydrophilic polymers, the relative affinity of the hydrophilic propylene glycol as a plasticizer was found to be significant (Gutierrez-Rocca and McGinity, 1994; Lecomte et al., 2004). Propylene glycol is a plasticizer containing –O– ether groups and several OH groups, which can form intermolecular bonds with the excess of NH_3^+ groups in the chitosan polymer.

3.4. Oxygen penetration

The oxygen penetration across the chitosan-based films was carried out by measuring the dissolved oxygen in the purified water as recipient using the Winkler's method. The Winkler's method was used to estimate the concentration of dissolved oxygen in aqueous solution. The dissolved oxygen was expressed in the unit of milligram per milliliter. Under normal circumstance, purified water has dissolved oxygen value in the range of 7.0–14.6 mg/ml at 0–35 °C.

The tested solutions from airtight flask (negative control) and opened flask (positive control) had dissolved oxygen 7.70 ± 0.20 and 8.85 ± 0.48 mg/ml, respectively, whereas those from flasks covered with chitosan, chitosan–cornstarch and chitosan–dextran had dissolved oxygen 8.10 ± 0.20, 8.26 ± 0.15, and 8.18 ± 0.07 mg/ml, respectively. The oxygen penetrations, when compared among all composite films, however, are not significantly different. With an increasing concentration of propylene glycol from 0.5 to 1.5% (w/v), the measured oxygen penetration tended to increase for all films. As mentioned earlier, propylene glycol is a hydrophilic molecule with an ability to attract moisture. This can result in a more permeable films both for water and oxygen.

3.5. Bioadhesive properties

In this study, an in-house instrument pulley system was developed to measure the bioadhesive properties of the films (Fig. 1). The weight of water required to detach the attached film from the pig's intestine was used to represent the relative magnitude of bioadhesive force of the tested film. Chitosan–dextran composite films with added propylene glycol showed the lowest and very homogeneous detachment forces in comparison to the pure chitosan films and the chitosan–cornstarch films with increasing propylene glycol concentrations. However, the results do not show a real trend and the differences are not statistically significant (Fig. 5).

3.6. Microbial penetration

In the microbial penetration tests, positive control tubes were tested to ensure that the nutrient broth was suitable for bacterial growth, while the negative control tubes were tested to represent the condition that is free from intrinsic bacterial contamination. The results showed that only the positive control tubes had bacterial contamination whereas all formulations of chitosan in all propylene glycol concentrations investigated showed clear solutions, i.e., no visible microbial contamination. This indicates a good potential of the developed composite films for being used as wound dressing due to their capability to bind the negatively charged bacteria to the positively charged amino groups of the chitosan polymer by reducing the primary wound contamination and due to their protection ability of the wound from secondary bacterial infection.

3.7. Residual glutaraldehyde

As mentioned earlier, glutaraldehyde was utilized as a crosslinker to form either intermolecular or intramolecular linkages. Excess of unreacted glutaraldehyde from the crosslinking process can potentially cause toxicity to the wound. Since the optimal concentration of crosslinking agent (glutaraldehyde) was found at a concentration of 3% (w/v), the residual glutaralde-

hyde in the film of this concentration was further analyzed with GC–MS. The test results showed that no residual glutaraldehyde in the film prepared with 3% (w/v) glutaraldehyde could be detected by GC–MS as compared to the standard glutaraldehyde solution. Therefore, these weakly crosslinked composite films without any residual glutaraldehyde could be safely used, while sufficiently maintaining the film integrity.

4. Conclusion

Composite films of chitosan with cornstarch and dextran intended for wound dressing applications were developed and examined. The in vitro evaluation revealed that cornstarch and dextran can be incorporated into chitosan film to improve its mechanical properties while substantially maintaining good vapor penetration, water uptake, and oxygen penetration properties. Addition of propylene glycol as a plasticizer can improve the film elasticity and all other properties evaluated, except bioadhesive properties. The films showed good protection against microbial penetration, indicating a good potential for wound dressing application. The crosslinked films with 3% (w/v) glutaraldehyde developed in this study did not contain measurable residual glutaraldehyde . Hence the films can be safely used as good wound dressing systems.

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