The Efficiency of Polysaccharide Gel Extracted from Fruit-Hulls of Durian (*Durio zibethinus* L.) for Wound Healing in Pig Skin

Piyarat Chansiriropnchhai, Churee Pramatwinai and Anudep Rungsipipat  
Faculty of Veterinary Science  
Chulalongkorn University  
Bangkok 10330  
Thailand

Sunanta Ponsamart and Oranuch Nakchat  
Faculty of Pharmaceutical Sciences  
Chulalongkorn University  
Bangkok 10330  
Thailand

**Keywords:** durian dressing film, durian fruit-hull extract, polysaccharide gel, pig, wound healing

**Abstract**

Polysaccharide gel (PG) extracted from fruit-hulls of durian (*Durio zibethinus* L.) was prepared as a preparation of dressing film. Efficacy of PG film for the treatment of full-thickness excisional wounds was performed in swine. Six young-female, cross-bred pigs, weighing 18-20 kg were used. Three full-thickness excisional wounds 2.45 cm in diameter were operated along both sides of dorsal midline area in each pig. Thirty-six wounds were randomly divided into 3 groups of 12 wounds. All wounds in group 1 were applied 1% Lugol’s solution and covered with sterile gauze dressing (control). The wounds in group 2 and 3 were treated with PG dressing film (treatment 1) and applied 1% Lugol’s solution and covered with PG dressing film (treatment 2), respectively. The wounds were examined for performance of wound healing on day 3, 6, 9, 12 and 15 postoperation and each group were re-treated with the same procedure. On day 18, skin samples from each wound were taken for histopathological study. The results demonstrated that PG dressing film treated wounds (treatment 1) showed more rapid wound closure, slightly and less tissue reactions than those in the control and the treatment 2 groups. The results suggest that PG dressing film seems to have beneficial property on wound healing in pig skin in this study.

**INTRODUCTION**

Plants having structural substance associated with their cellulose structures usually contain commercially valuable pectin supplies for food and pharmaceutical industries (Whistler and BeMiller, 1959). In Thailand, there are many reports on the utilization of plant waste as a source of valuable materials of commercial importance. Durian (*Durio zibethinus* L.) is one of the most favorite fruit of Thailand. The polysaccharide gel (PG) is isolated from fruit-hulls of durian by deriving the process of Pongsamart and Panmaung (1998). PG is a water soluble component of five sugars such as glucose, rhamnose, fructose, arabinose and galacturonic acid and it is useful in preparation of food and pharmaceutical products such as jelly, tablet, suspension and emulsion (Griddit et al., 2001). Toxicity test of PG was determined, a high oral dose (2 g/kg) did not induced severe toxicity in male mice and rats (Pongsamart et al., 2001). Subchronic toxicity test also has not induced toxic effect in male and female mice after longterm feeding at 0.5 g/kg/d for 60-100 days (Pongsamart et al., 2002). Furthermore, Griddit et al. (2001) found that PG can be use as a good film forming agent and used to prepare as a satisfactory film dressing. In a recent study, PG showed antibacterial properties against *Staphylococcus aureus* and *Staphylococcus epidermidis*, which found on skin and caused wound infection (Nantawanit et al., 2001). According to the properties of film forming and antibacterial...
activity of PG, it is expected to be applied for treatment of wounds. Therefore, the purpose of this study was to evaluate the efficiency of PG dressing film on wound healing compared with conventional treatment using 1% Lugol’s solution and treatment with 1% Lugol’s solution plus PG dressing film.

MATERIALS AND METHODS

Preparation of PG Dressing Film

PG was isolated from dried fruit-hulls of durian (Durio zibethinus L.) (Pongsamart and Panmaung, 1998). The PG dressing film was prepared by casting/solvent evaporation method (Remunan-Lopez and Bodmeier, 1996). The formulation of PG dressing films composed of 2% PG powder were dissolved in deionized water at room temperature and 15% propylene glycol was added as plasticizer (Girddit et al., 2001). The solutions were warmed and stirred until homogeneously mixed and poured into a plastic casting area 10.2 x 24.8 cm², solid content of PG as 4.42 mg/cm². The films were dried for 3 hrs at room temperature and oven dried for 8 hrs at 50°C. The PG dressing films were cut into 3x3 cm² and were sterilized as follows; (1) moist sterile by autoclave at 121°C, 15 min.; (2) dry sterile at 121°C, 45 min. (Autoclave, Model HA-300MD, HIRAYA Corp, Japan). The PG dressing films were kept in desiccator until use.

Operation and Treatment of Wound

Six young female, cross-bred pigs, weighing 18-20 kg were used. The animals were kept separately in each cage and fed a standard swine diet (ad libitum). All pigs were acclimatized for at least 7 days before the experiment started. On experiment day (day 0), all pigs were sedated by intramuscular injection of 4 mg/kg azaperone (Stresnil®, Janssen Pharmaceutica, Belgium) and anesthetized with 20 mg/kg pentobarbital sodium (Nembutal®, Sanofi, France) by intravenous injection. The dorsal aspects (back) of all pigs were clipped and cleansed with povidone iodine and 70% alcohol solution. Three full-thickness excisional wounds 2.45 cm in diameter were operated along both sides of dorsal midline area in each pig. Thirty-six wounds were randomly divided into 3 groups of 12 wounds. All wounds in group 1 were treated with applied 1% Lugol’s solution (control), while the wounds in group 2 and 3 were treated with PG dressing film (treatment 1) and 1% Lugol’s solution plus PG dressing film (treatment 2), respectively. All wounds were covered with sterile gauze dressing on the top and fixed into the skin at the edges by nylon sutures. On day 3, 6, 9, 12 and 15 postoperation, all animals were sedated and anesthetized by the same protocol. The wounds in each treatment were observed and the areas of wound closure were measured by tracing the wound boundaries using sterile transparent sheets with permanent marker. Wound were cleaned with sterile normal saline and treated by the same treatment as day 0.

On day 18 postoperation, the animals were euthanized and the skin tissues in the area of operation were removed size 1x1 in² deep in epidermal and dermal layers. Some selected tissues were fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned at 5 µm, and stained with hematoxylin and eosin. Histopathological lesions in each treatment were evaluated by veterinarian pathologist.

Histopathology Evaluation

Histopathological lesions were evaluated by the criteria as follows; (1) epidermal hyperkeratinosis and epidermal hyperplasia; (2) subacute dermatitis; (3) chronic dermatitis; (4) dermal fibrosis; and (5) dermal granuloma. They were given a score ranging from 0 (no remarkable lesions), 1 (mild), 2 (moderate) and 3 (severe).

The values of the histopathological scores were averaged and expressed as the mean ± standard deviation. All statistical evaluations were performed by ANOVA. The results were considered significant at p < 0.05.
Statistical Analysis
The areas of wounds were calculated by image analysis program provided by Department of Computer Engineering, Faculty of Engineering, Chulalongkorn University. The statistical comparison was made with ANOVA. The results were considered significant at $p < 0.05$.

RESULTS

Rate of Wound Closure
The results on the rate of wound closure demonstrated that treatment 1 and treatment 2 were shown statistic significant decreasing wound area difference from the control on day 12, ($p = 0.010$ and $p = 0.049$, respectively), as represent in Table 1. In comparison of wound closure rates, calculated as the percentage of closure, the result illustrated that treatment 1 and treatment 2 showed statistic significant more rapidly wound closure rates than that in the control group on day 12, ($p = 0.010$ and $p = 0.049$, respectively), as illustrated in Fig. 1. However, there was no statistic significant difference of the decreasing of wound area and the wound closure rate between the pigs in treatment 1 and 2. On days 18, the wounds in treatment 1 showed 100% complete closure (Fig. 2), while the wounds in treatment 2 and in the control groups showed complete closure in 91.67% and 83.33%, respectively, as shown in Table 2.

Histopathology Evaluations
On the basis of histopathology study, epidermal hyperkeratosis and epidermal hyperplasia were determined by proliferation of epithelial with increased keratin production in stratum corneum. From this study, the wounds in treatment 1 showed more epithelial proliferation and hyperkeratosis than those in the treatment 2 and the control groups, as shown in Table 3. Subacute dermatitis was characterized by a number of PMNs and some macrophages aggregated in the lesions. Chronic dermatitis was characterized by vascularization with chronic inflammatory cells (lymphocytes and plasma cells) aggregation in dermal layer. In this study, we found that a mild subacute and chronic dermatitis in the control and the treatment 1 need prolonged inflammation reaction as compared to the treatment 2. Dermal fibrosis was determined by increasing a number of reactive fibroblasts with increased collagen fiber in dermal layer. The evidence of dermal fibrosis was mild to moderate in all groups but the dermal granuloma formations characterized by central necrosis and proliferative zone surrounded by macrophages, lymphocytes and plasma cells with occasionally foreign-body giant cells throughout the deep layer of dermis were obviously observed in the groups of control and the treatment 2. Whereas, the wounds treated with PG dressing film (treatment 1) showed the less dermal granuloma formation than that in the other groups (Fig. 3).

DISCUSSION
The PG is a new material beneficial for wound dressing. The reasons supported that PG film dressing appropriate to healed-wound such as, (1) it is a watersoluble film, can removed by washed with water and is not destroy newly epithelium; (2) it has moisture sorption property (Griddit, 2002); (3) it is extracted from natural product, without toxicity to tissues; and (4) PG film dressing inhibits S. aureus and S. epidermidis growing, which found on skin and caused wound infection (Nantawanit et al., 2001).

Application of PG dressing film for wound healing in this study revealed that PG dressing film showed statistic significant decreasing wound area and more rapid wound closure rate than those in the control group on day 12 (Table 1 and Fig. 1) and the 100% wound closure was shown in the treatment 1 group on day 18. The data suggested that PG dressing film seems to have beneficial property on wound healing. Especially, it provides a moist wound environment which promotes healing and epidermal regeneration. This finding is consistent with previous studies that wounds re-epithelialize more rapidly under moist conditions than under dry ones (Winter and Scales, 1963; Suzuki et al., 1998).
From histopathology study, PG dressing film treated group had a higher increase of epithelial proliferation and hyperkeratosis and it caused slightly tissue reaction, and granuloma formation. These data supported that PG dressing film accelerates wound closure because it can be removed by water and does not destroy newly epithelium. Difference from covered with gauze dressing when removed, it strips away newly formed epithelium, causing bleeding and prolonged healing process. Furthermore, granuloma formation in the dermis can interfere with the wound healing time and can initiate the formation of large scar tissues.

CONCLUSIONS

The wounds treated with PG extracted from fruit-hulls of durian showed more rapid wound closure, slightly and less tissue reactions than those in the wounds treated with 1% Lugol’s solution plus PG dressing film or applied 1% Lugol’s solution. The results suggest that PG dressing film have a satisfactory for wound treatment and seems to have benefit in pharmaceutical and medical applications.

ACKNOWLEDGEMENTS

This work was supported in parts by Graduate Study Research Fund and by research budget of Faculty of Pharmaceutical Sciences, Chulalongkorn University. Thanks are also extended to Department of Computation Engineering, Chulalongkorn University for providing computer analysis program. We thank Dr. Weerasak Sada (D.V.M.) for his help in wound operating and treating throughout the experiment.

Literature Cited

### Tables

#### Table 1. Wound area (cm²) in each treatment (data shown as mean ± SD).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after wounding</th>
<th>Days after wounding</th>
<th>Days after wounding</th>
<th>Days after wounding</th>
<th>Days after wounding</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Control¹</td>
<td>5.409±0.810</td>
<td>4.704±0.617</td>
<td>2.733±0.783</td>
<td>0.912±0.256</td>
<td>0.367±0.176</td>
</tr>
<tr>
<td>Treatment 1²</td>
<td>5.210±0.837</td>
<td>4.323±1.602</td>
<td>2.227±0.696</td>
<td>0.553±0.284</td>
<td>0.285±0.155</td>
</tr>
<tr>
<td>Treatment 2³</td>
<td>5.401±0.994</td>
<td>4.452±1.817</td>
<td>2.391±0.831</td>
<td>0.645±0.402</td>
<td>0.344±0.328</td>
</tr>
</tbody>
</table>

* * significant from control (p<0.05)

¹ 1% Lugol’s solution treatment
² PG dressing film treatment
³ 1% Lugol’s solution plus PG dressing film treatment

#### Table 2. Percentage of complete wound healing in each treatment observed on day 18.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>% incompletely wound healing</th>
<th>% completely wound healing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control¹</td>
<td>16.67 (2/12)</td>
<td>83.33 (10/12)</td>
</tr>
<tr>
<td>Treatment 1²</td>
<td>-</td>
<td>100.00 (12/12)</td>
</tr>
<tr>
<td>Treatment 2³</td>
<td>8.33 (1/12)</td>
<td>91.67 (11/12)</td>
</tr>
</tbody>
</table>

¹ 1% Lugol’s solution treatment
² PG dressing film treatment
³ 1% Lugol’s solution plus PG dressing film treatment

#### Table 3. Blind analysis of histopathology lesions in each group (data shown as mean ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Control¹</th>
<th>Treatment 1²</th>
<th>Treatment 2³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal hyperkeratosis and hyperplasia</td>
<td>1.083±0.669</td>
<td>1.250±0.622</td>
<td>1.167±0.718</td>
</tr>
<tr>
<td>Subacute dermatitis</td>
<td>0.083±0.289</td>
<td>0.417±0.996</td>
<td>0.417±0.793</td>
</tr>
<tr>
<td>Chronic dermatitis</td>
<td>0.833±1.030</td>
<td>1.167±1.030</td>
<td>0.417±0.669</td>
</tr>
<tr>
<td>Dermal fibrosis</td>
<td>1.583±0.515</td>
<td>1.500±0.674</td>
<td>1.583±0.515</td>
</tr>
<tr>
<td>Dermal granuloma</td>
<td>1.250±1.288</td>
<td>0.417±0.515</td>
<td>1.000±1.206</td>
</tr>
</tbody>
</table>

¹ 1% Lugol’s solution treatment
² PG dressing film treatment
³ 1% Lugol’s solution plus PG dressing film treatment
Figures

Fig. 1. A comparison of percentage of wound areas (mean ± SD) in pig skin treated with 1% Lugol’s solution, PG dressing film and 1% Lugol’s solution plus PG film for 18 days. * = significant from control groups ($p < 0.05$).

Fig. 2. Gross lesion of wounds on days 6, 12 and 18 postoperation. C, control group; 1, treatment 1; 2, treatment 2; 6, day 6 postoperation; 12, day 12 postoperation; 18, day 18 postoperation.
Fig. 3. Histopathological finding in the dermis. C, control group; T₁, treatment 1; T₂, treatment 2. Arrow heads show dermal granulomas.