CHAPTER IV

DISCUSSION AND CONCLUSION

Discussion

1. Polysaccharide Gel (PG) from Fruit-Huils of Durian

Polysaccharide gel (PG) was isolated from durian fruit-hulls, according to a procedure developed by Pongsamart and Panmauang (1998), except the process was modified by using dried hulls. The product of purified PG was 6.28% yield by weight of dry-hull. The result of purified PG obtained was about the same as previous study by Gerddit (2002), the percentage yield of purified PG was 7.3% by weight of dried fruit-hull.

2. Physical Properties of Polysaccharide Gel (PG)

The pH of 2% PG aqueous solution concentration and a mixture of 2% PG in water with plasticizer 15%w/w propylene glycol based on PG weight was about 2.5, addition of propylene glycol did not change acidity of PG aqueous solution. The viscosity of a mixture of 2% PG was not different when added plasticizer as showed in Table 5. The results in this study was slightly different from the results reported by Gerddit (2002), 2% PG aqueous solution gave 279.36±25.866 mPa·s viscosity and pH was 2.236±0.003. However, these results correlated with the condition which indicated that pH has effected on viscosity, increasing of pH resulted in decreasing viscosity (Gerddit, 2002).

3. Analysis of Physical Properties of PG Dressing Films

3.1 Thickness of PG Films

The PG film and PG dressing film with plasticizer were prepared by

casting/solvent evaporation method (Remunanlopez and Bodmeier, 1996). The dressing formula, PG film prepared from 2% PG concentration in deionized water, PG films had solid content of 4.42 mg/cm^2. PG dressing film prepared by 2% PG by weight in deionized water with platicizer using propylene glycol at concentration 15% w/w based on PG weight, PG dressing films had solid content of 4.42 mg/cm^2. The thickness of PG film and PG dressing film with plasticizer was approximately 0.03 mm which was agreed with the previous report of Gerddit (2002).

3.2 Mechanical Properties of PG Dressing Films

The mechanical properties of PG dressing film with plasticizer compared with PG film were evaluated the values of % strain at break, stress at break, Young's modulas and toughness. PG dressing film showed lower Young' modulas and stress at break, and higher % strain at break and toughness than those of PG film. The higher value of stress at break indicate the hardness of film, the high value of % strain indicate the elasticity or toughness and the lower value of Young's modulas can be the result of low value of stress at break or high value of % strain. Too high stress at break and too low % strain made the unsuitable film because of the film brittleness. The plasticizer help decreasing the value of strain at break and/or increasing the value of % strain. The results of their mean values indicated that the PG dressing film had softness, elasticity and toughness of film more than PG film without plasticizer (Table 6), the results were similar to that reported by Peh and Wong (1999), Khan, Peh and Ching (2000), and Gerddit (2002). The mechanical properties of PG dressing film with plasticiser still showed that softness, elasticity and toughness of film more than those of PG film without plasticizer after sterilized by hot air oven or autoclave (Table 7,8). This results indicated that PG dressing film with plasticizer produce a satisfactory film that can be a useful preparation for application in pharmaceuticals.

3.3 The Swelling Properties of PG Dressing Films

The PG dressing film with plasticizer showed swelling property, the films swelled 1.5 fold in distilled water (Figure 9). This results indicated the high absorbent ability of PG dressing film, that can be suitably applied as a modern

wound dressing, absorption property of PG dressing film can maintain moist wound environment, promote epithelial cell proliferation and wound healing (Winter, 1962; Dyson, 1988; Vogt, 1995; Ueno, 1999).

4. Wound Healing Evaluation

4.1 Gross Lesions Evaluation

4.1.1 Wound Lesion

The polysaccharide gel (PG) powder was used to prepared a PG dressing film and PG dressing gel. They were applied as dressing for treatment of wound on the skin of pigs *in vivo* in this study. The gross wound lesions were evaluated every 3 POD. All wounds became red and swelling on days 3 (Figure 10), which indicated that all wounds in the first phase of wound healing process as inflammatory phase. The swelling was continuously reduced on day 3 to day 6, however, one wound which treated with 1% povidone iodine and covered with PG dressing film was still swelling. On day 6, 9, 12, 15 and 18 postoperation (Figure 11-15), the wounds in all groups showed progressive decrease in size, increased wound contraction and epithelialization, which indicated that all wounds were in the second phase of wound healing process, proliferative phase. However, the wounds treated with PG dressing film appeared smaller areas than other treatments. The serosanguineous exudate was moderate during the initial days and was reduced on day 6 (Figure 11), and disappeared on day 9 (Figure 12) in all groups. The wounds were red color in the initial day and turned to peal color on the last day. Scab appeared on day 6 to day 18. Scar formation was observed in all groups on the last day, however, the wounds treated with PG dressing film seem to have slight scar. The results indicated the gross wound lesions were no different in all treatments.

4.1.2 Wound Area

Wound areas were measured every 3 POD. The result demonstrated that the wounds treated with PG dressing film showed progressive in size than other treatments. On day 12 (Table 10), the PG dressing film treated in T_1

showed significantly different the fastest healing rate and complete wound closure obtained on day 18 (Table 9, 10). Wounds in T_1 show significant rapid rate ($p<0.05$) of wound healing and the fastest complete wound closure compared to control that treated with 1% povidone iodine. However, T_1 did not show significant different of healing rate on day 12 (Table 10) from T_2 and T_3 . These results indicated that PG dressing film can be use successfully for healing wound better than the traditional treatment, it healed wound as fast as using 1% povidone iodine and covered with PG dressing film (T_2) and PG dressing gel (T_3) (Table 10 and Figure 16).

The wounds treated with 1% povidone iodine and covered with PG dressing film (T_2) were observed for the investigation that its might be help increasing efficiency on wound healing compared to control, T_1 and T_3. The results showed that the wounds in T_2 were significantly different faster rate of healing and complete wound closure than control. But the rate of wound healing was not significantly different ($p>0.05$) from T_1 and T_3. These results indicated that wound in T_2 can be healed faster than that of control. However, wound closure in T_2 and T_3 was delay compared to T_1 as showed in Table 9. All 3 treatments can heal wound to complete closure faster than control (Table 9).

The wounds treated with PG dressing gel (T_3) were compared with other treatments. The results showed on day 12 that the wounds treated with PG dressing gel showed healing rate as fast as T_1, T_2 and control (Table 10, Figure 16). Dry wound environment in control showed delay wound closure compared to the wounds in T_1, T_2 and T_3 which were treated with PG dressing preparations that can supply moisture wound environment in the condition of either film dressing preparation or gel dressing preparation. The rate of wound healing on day 12, T_3 showed the slow rate of wound healing, until day 15 wound area reduced to 0.225 ± 0.057 cm^2 which was about 24% of its wound area of 0.926 ± 0.179 cm^2 on day 12 (Table 10). The result indicated that on day 15 PG dressing gel helped fast healing in the smaller wound that has not much exudate or in the wound that almost healed. In this case the gel preparation can probable give more moisture than that of the film preparation. In Table 10 indicated between day 12 and 15 that PG gel preparation can markedly reduce wound area.

4.2 Histopathological Evaluation

The basic of wound healing process respect into 3 overlapping phases as inflammatory, proliferative and remodeling phases. In this study, on day 18 the process of wound healing was in the end of proliferative phase. The histopathological study indicated that wound treated with PG dressing film showed satisfactory wound healing such as

(a) PG dressing film showed mild inflammatory cells and mild chronic inflammatory cells aggregated in the dermal layer. These results correlated with the previous reported of Young *et al.*, (1991). They reported that the inflammatory cells (PMNs and macrophages) were infiltrated into the dermal layer of the wound for killing microorganisms. Its were increased on 0 to 5 day and progressive decreasing on 5 to 60 day. If the presence of inflammatory cells prolonged, the process of wound healing was delay (Sawyer, 2002).

(b) PG dressing film showed mild to moderate epidermal regeneration that provided rapid wound closure, these results correlated with the reported of Bertone *et al.* (1985), Young *et al.* (1991), and Foster *et al.*,(1995), they reported that increasing of epidermal regeneration was promoted rapid wound healing.

(c) PG dressing film showed mild fibroblasts in dermal layer, while PG dressing gel and 1% povidone iodine showed moderate fibroblasts in dermal layer. This results correlate with the previous research of Young *et al.* (1991), Ueno *et al.* (1999), and Sugihara *et al.* (2000), reported that the fibroblasts were decreased in remodeling phase, if fibroblast still high, it implicated in wound scar (Risbud, Hardishar and Bhonde, 2000).

(d) PG dressing film showed the least of granuloma formation than those wound treated with 1% povidone iodine and covered with PG dressing film, treated with PG dressing gel, and treated with 1% povidone iodine, respectively. These results suggested that PG dressing film was a satisfactory dressing and was beneficial for wound treatment. However, wound treated with 1% povidone iodine and covered with PG dressing film showed the least inflammatory cells (PMN and macrophage) infiltrated in the dermis layer. This result supported that the application of 1% povidone iodine with PG dressing film showed coeffectiveness in reducing of inflammatory reaction.

PG dressing gel showed mild inflammatory cells, higher score of chronic inflammatory cells than other treatments. It might be prolonged wound healing. Otherwise, it showed the highest score of dermal fibrosis, it might be implicate in wound scar. However, PG dressing film showed less granuloma formation than control. This results indicated that PG dressing gel less satisfactory for wound treatment than PG dressing film. However, PG dressing gel interesting to apply to treated other wounds such as burn wound. Because it is a gel compose of water about 97% which may be allows a cooling effect and reduces pain.

Conclusions

Since 1962s, after Winter reported that wound was rapid healing under moist environment. The dressing was developed to promote wound healing. A good dressing should be; (a) protect the wound ; (b) promote wound healing; (c) prevent dehydration, desiccation and maintain a moist environment; (d) allows gas exchange; (e) allows removal without trauma and reduce pain; (f) be non-toxic, non-allergenic and non-sensitizing. Many materials were developed for wound treatment. Some dressing made from synthetic materials such a polyurethane, methylcellulose. Biological dressing is produced from biological materials and used for wound treatment such as alginate derived from seaweed (Suzuki et al.,1998), chitosan a partially deacetylated product of chitin (Risbud et al., 2000) and silk film derived from silk protein (Sugihara et al., 2000). They retain moisture property, promote wound healing by induce inflammatory cells get rid of foreign material or microorganisms, accelerate fibroblast synthesis collagen matrix, and non-toxicity on tissue. Recently, a new polysaccharide was extracted from fruit-hulls of durian called polysaccharide gel (PG) (Pongsamart and Panmauang, 1998). The properties of PG was investigated such as **(a)** PG has gelling property and film forming property, it can be prepared to make a satisfactory film (Gerddit, 2002); **(b)** PG solution and PG dressing film show antibacterial property against *Staphylococcus aureus*, *Staphylococcus epidermidis, Micrococcus luteus, Bacillus subtilis* and *Escherichia coli* (Nantawanit, 2001); **(c)** PG dressing film prevent dehydration, it has moisture sorption property (Gerddit, 2002); **(d)** in this study, the PG dressing film showed swelling property can absorb water and exudate that provide wound moisture; **(e)** PG dressing film can be dissolve in water, it can be remove from wound without trauma;

and **(f)** PG dressing film showed wound healing property by treatment of PG dressing film in full-thickness open wound on skin of pigs and PG dressing film accelerated wound healing in this study. PG dressing film showed rapid healing rate, promoted epithelial proliferation and produced the least tissue reaction.

The results demonstrated that PG dressing film showed properties of ideal dressing (Linda, 1998; Biopol, 2002) such as (a) promote reepithelalization (Dyson *et al.*, 1988; Vogt *et al.*, 1995; Claus *et al.*, 1998), (b) increase wound healing rate by showing rapid and 100% complete wound closure (c) reduce inflammatory cells, (d) mild to moderate dermal fibrosis, and (e) less cytotoxicity, it produced the least tissues reaction by showing no remarkable lesion granuloma formation in dermis layer. Moreover, PG dressing film is a water-soluble film that can be remove by washing with saline solution, then wound cleaning would not disturb newly formed epithelial cells or produce trauma (Nemeth *et al*, 1991; Field and Kerstein, 1994). All of those properties indicated that PG dressing film prepared from PG extract from fruit-hulls of durian can be use as an ideal dressing for full-thickness open wounds. PG is a new interesting plant materials that can be used successfully in a film preparation of wound dressing and it seem to be useful for pharmaceutical and medical applications.

For future research, the others properties of PG dressing film on promote wound healing such as accelerate inflammatory cells in inflammatory phase, accumulate collagen synthesis by fibroblast in proliferative phase and modulate fibroblast synthesize collagen is implicated scar formation in remodeling phase, wound be interesting to study by comparing with the commercial modern wound dressing (transparent film, hydrocolloid or hydrogel dressing)