CHAPTER III

RESULTS

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

Polysaccharide gel (PG) which was isolated from dried fruit- hulls of durian and purified by modifying the method of Pongsamart and Panmaung (1998). A dried crude extract of PG was a light brown or beige powder. After PG crude extract was further purified, a purified extract was light brown to pale beige color powder. The product of purified PG was blended to a fine powder and passed through 60 mesh sieve as shown in Figure 6.

2. Physical Properties of Polysaccharide Gel (PG)

2.1 Viscosity of PG

Aqueous solution PG at 2 % concentration and a mixture of 2% PG with plasticizer propylene glycol at concentration of 15 % w/w based on PG were prepared. The viscosity was measured repeatedly 3 times in each preparation mixture. The mean values of 3 measurement were calculated and solution mixtures were compared. The results demonstrated that adding of plasticizer was not effect the viscosity of 2% PG. The data are shown in Table 5.

2.2 pH of Aqueous Solution PG and Mixture of PG with Plasticizer

The 2 % PG aqueous solution and a mixture of 2% PG solution with propylene glycol 15 % w/w based on PG were prepared and the pH was measured by using pH meter. The results showed that pH of the mixture of 2% PG with propylene glycol and 2% PG solution were about 2.5. The data are shown in Table 5.

Figure 6. Polysaccharide gel (PG) powder isolated from dried fruit-hulls of durian. Purified extract of PG was a light brown to pale beige powder, blended product was passed through 60 mesh sieve.

Table 5. The viscosity and pH properties of PG aqueous mixture and mixture of PG with plasticizer.

Parameter	2%PG in DW (mean ± SD)	2%PG with15%w/w propylene glycol base on PG (mean ± SD)
pH	2.501 ±0.003	2.498±0.017
Viscosity (mPa·s)	156.667±3.215	158.067±0.017

3. Preparation of PG Dressing Films and PG Dressing Gel

3.1 Preparation of PG Dressing Films

The PG dressing films were prepared. A thin, colorless to pale beige color, transparent film of PG dressing film product was obtained (Figure 7).

3.2 Preparation of PG Gel

The PG dressing gel was prepared, a pale beige color of PG dressing gel preparation was obtained (Figure 8).

4. Analysis of Physical Properties of PG Dressing Films

4.1 Thickness of PG Dressing Films

The thickness of PG dressing films were measured by micrometer at five different points on each film. The results demonstrated that PG films and PG dressing films with plasticizer had thickness of 0.029 ± 0.002 and 0.027 ± 0.004 mm, respectively (Table 6).

4.2 Mechanical Properties of PG Dressing Films

The mechanical properties of nonsteriled of PG films without plasticizer and PG dressing films with plasticizer were illustrated in Table 6. The values of Young's modulas, stress at break (Mpa), % strain at break and toughness were evaluated, respectively.

Young's modulas or modulas of elasticity is a measurement of stiffness and rigidity of a film. Stress at break or tensile strength (Mpa) is a measurement of an ultimate strength and strain at break or elongation (%) is a measurement of a ductility of a film (O' Donnell and Mc Ginity, 1997; Gerddit, 2002). The results demonstrated that PG dressing film with plasticizer showed lower

Young's modulas and stress at break than that of PG film without plasticizer, the results indicated that PG dressing film with plasticizer was lower strength, the film was solfter or less brittle than that of PG film. However, PG dressing film with plasticizer showed a higher % strain at break and toughness than that of PG film without plasticizer, the results indicated that PG dressing film with plasticizer was higher in elongation, elasticity and toughness than PG film without plasticizer. These results was correlated with the results reported by Gerddit, 2002.

An ideal dressing film with respect to retaining its physical continuity should be soft, tough and not too brittle. The results in this study indicated that PG dressing film showed a satisfactory film in term of softness, elasticity and toughness.

In comparison the mechanical properties between sterilization techniques of PG film and PG dressing film demonstrated that % strain at break, stress at break and toughness of PG dressing film after sterilization by using hot air oven at 120 °C for 1 hr were higher, whereas, Young's modulas was lower than that of PG film. This results indicated that PG dressing film had softness, strengthen and toughness more increased, but brittleness more decreased than that of PG film, as shown in Table 7.

The mechanical properties of steriled PG film and PG dressing film by using autoclave at 121 °C, 15 lb/in^2 for15 min showed that PG dressing film after sterilization produced higher strength, softness and toughness, but lower brittleness than that of PG film, as illustrated in Table 8.

A comparison of mechanical properties of PG film and PG dressing film before and after sterilization in hot air oven, and autoclave showed that nonsteriled PG dressing film with plasticizer was softer and tougher, and less brittle than that of PG film after strerilization with hot air oven and autoclave, respectively.

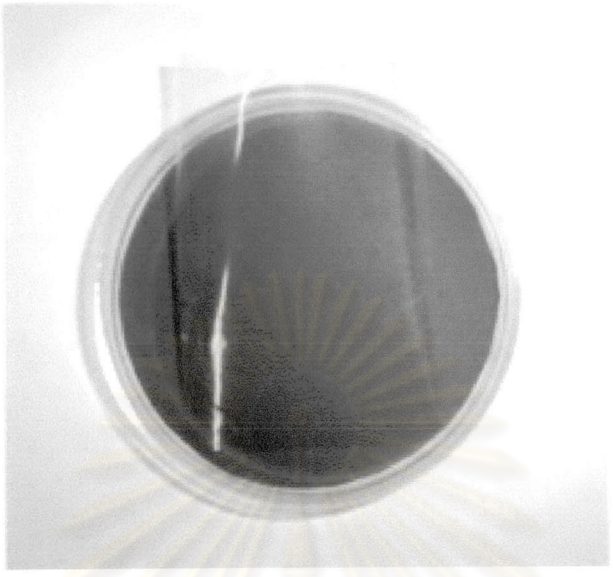

Figure 7. PG dressing film prepared from polysaccharide gel isolated from fruit-hulls of durian.

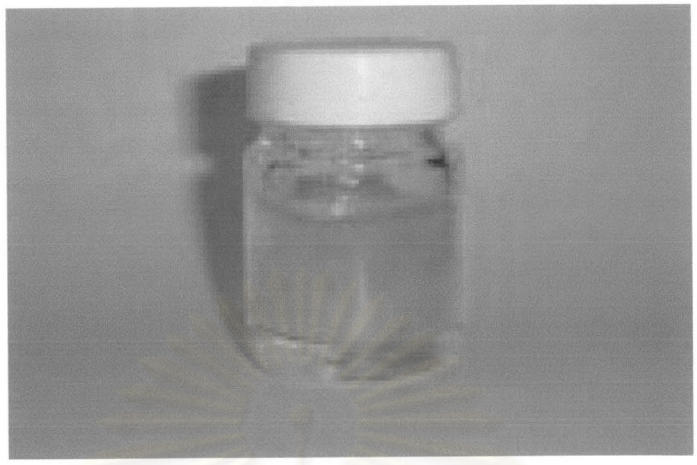

Figure 8. PG dressing gel prepared from polysaccharide gel isolated from fruit-hulls of durian.

Table 6. A comparison of mechanical properties of PG film and PG dressing film with plasticizer. Solid content of PG = 4.42 mg/cm^2.; data are expressed as means (SD) in parentheses; n=5

Sample	Physical properties				
	Young's modulas (Mpa)	Stress at break (Mpa)	% Strain at break	Toughness (Mpa)	Thickness (mm)
PG film	2702 (127)	63.172 (9.087)	5.003 (1.045)	1.317 (0.456)	0.029 (0.002)
PG dressing film with 15% propylene glycol based on weight of PG	2552 (525)	60.898 (6.873)	5.043 (1.991)	1.909 (0.962)	0.027 (0.004)

Table 7. A comparison of mechanical properties of PG film and PG dressing film with plasticizer after sterilized by hot air oven at 120°C for 1 hr. Solid content of PG = 4.42 mg/cm^2 ; data are expressed as means (SD) in parentheses; n=5

Sample	Physical properties				
	Young's modulas (Mpa)	Stress at break (Mpa)	% Strain at break	Toughness (Mpa)	Thickness (mm)
Sterile PG film	2933 (483)	46.230 (8.888)	2.669 (0.490)	0.433 (0.078)	0.029 (0.002)
Sterile PG dressing film with 15%propylene glycol based on weight of PG	2526 (552)	49.820 (8.787)	3.502 (0.378)	0.929 (0.230)	0.028 (0.007)

Table 8. A comparison of mechanical properties of PG film and PG dressing film with plasticizer after sterilized by autoclave at 121 °C, 15 lb/in^2 for 15 min. Solid content of PG = 4.42 mg/cm^2 ; data are expressed as means (SD) in parentheses; n=5

Sample	Physical properties				
	Young's modulas (Mpa)	Stress at break (Mpa)	% Strain at break	Toughness (Mpa)	Thickness (mm)
Sterile PG film	3087 (650)	35.830 (16.305)	1.628 (1.154)	0.306 (0.256)	0.028 (0.007)
Sterile PG dressing film with 15%propylene glycol based on weight of PG	3424 (333)	40.140 (5.982)	1.802 (0.453)	0.283 (0.136)	0.031 (0.005)

4.3 Swelling Property of PG Dressing Film

The swelling property of PG dressing film was determined by using the film size 1 x 1 cm^2, impregnated in deionized water. The dimension of PG dressing film was measured every 1 hr for 24 hrs. The result demonstrated that PG film was swollen 1.5 fold compared to its original size as illustrated in Figure 9.

5. Wound Healing Evaluation

5.1 Gross Pathology Evaluation

5.1.1 Wound Lesion

Wound lesions were observed every 3 POD at days 3, 6, 9, 12, 15 and 18.

On POD 3, all wounds in each treatment became hyperremia (red in color), moderate serosanguineous exudate appeared on wound surface with wound swelling. These indicated that all wounds were in the acute inflammatory phase of wound healing process. The wound appearances were illustrated in Figure 10.

On POD 6, the wound treated with 1% povidone iodine (control) showed red color, severe exudate and scab was covered on wound surface. The wound was reduced in size and slightly firm in consistency, this condition could be due to dermal fibrosis. Some wounds showed inflammatory lesion and swelling. Wound treated with PG dressing film (T_1), 1% povidone iodine and covered with PG dressing film (T_2) and PG dressing gel (T_3) were red color and moderate exudate and scab was appeared. The granulation tissue was more increased. However, one wound in T_2 showed a sign resemble acute infection which was characterized by swelling and sero-purulent exudate covered on wound surface. The wound appearances were illustrated in Figure 11.

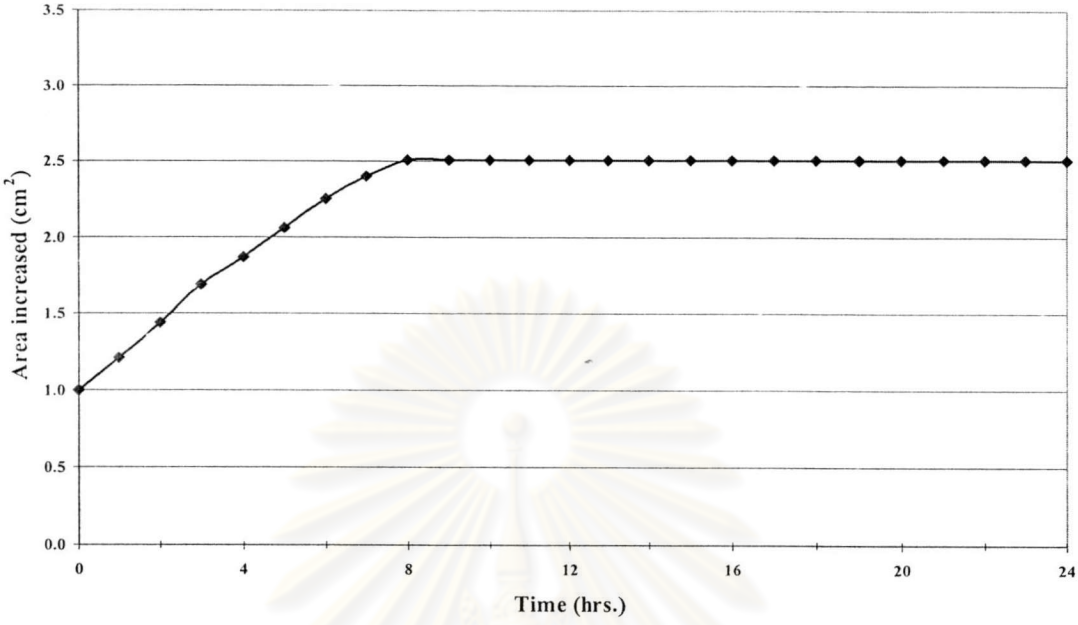

Figure 9. Swelling property of PG film in distilled water, n =3

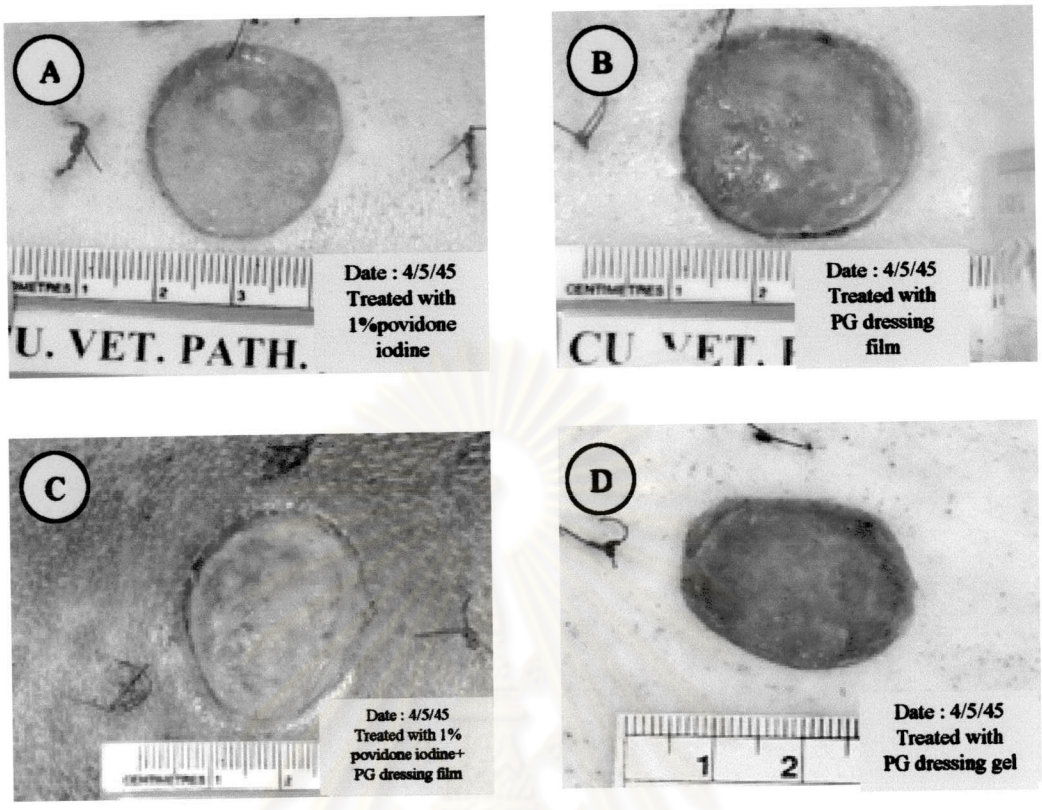

Figure 10. The wound appearance on day 3. A) control : a wound treated with 1% povidone iodine. B) T_1 : a wound treated with PG dressing film. C) T_2 : a wound treated with 1% povidone iodine and cover with PG dressing film. D) T_3 : a wound treated with PG dressing gel.

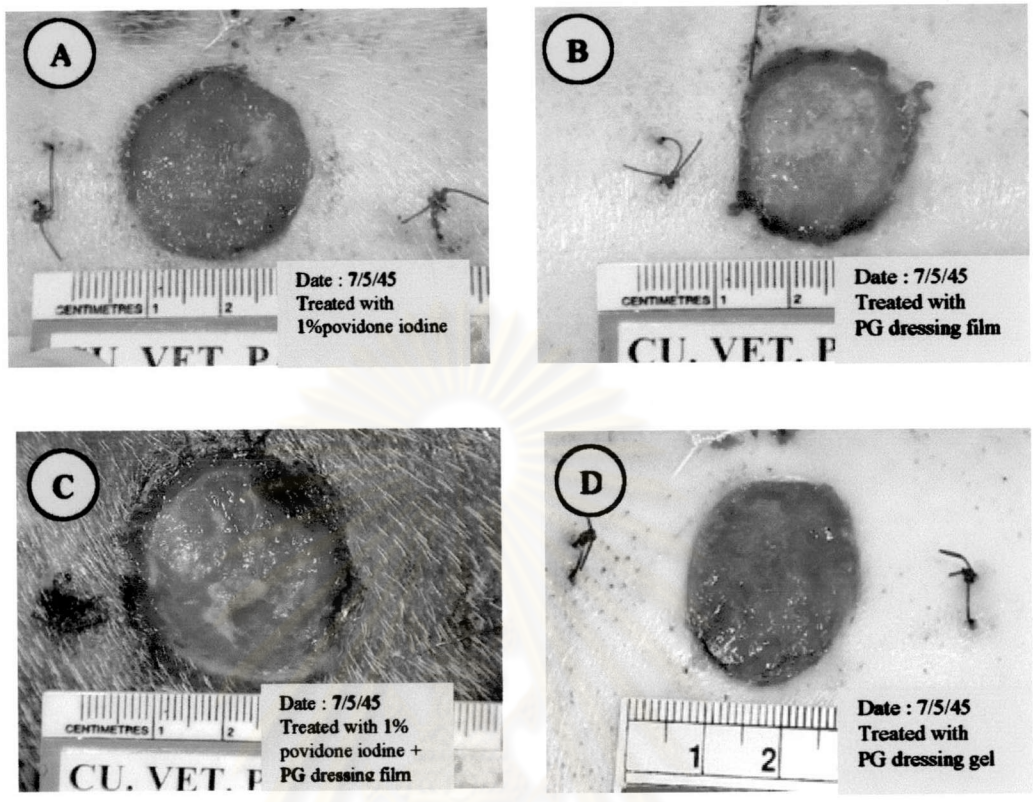

Figure 11. The wound appearance on day 6. A) control : a wound treated with 1% povidone iodine. B) T_1 : a wound treated with PG dressing film. C) T_2 : a wound treated with 1% povidone iodine and cover with PG dressing film showed infected lesions; by exudate covered on wound surface and became swelling. D) T_3 : a wound treated with PG dressing gel.

On POD 9, all wounds in each treatment showed red to pink color, dry wound surface, increase epithelial formation and wound contraction. All wounds were cleaned and scabs were removed. The wound previously showed a sing look like infected in T_2 was still inflamed. The wounds surface of T_3 was covered with moisty exudate without inflammation, this appearance could be due to a high water content of PG dressing gel. The wound appearance of all wounds were demonstrated in Figure 12.

On POD 12, all wounds in each treatment were pink without exudate, contraction and epithelialization increase, scabs were still appeared and it was removed. Most of wounds treated with PG dressing film showed the smallest in size compared with control and other treatments. A sing of infection previous saw in the wound previously look infected of T_3 was disappeared. The wounds treated with PG dressing gel were more moisty than other treatments and control. The wound appearance represented in Figure 13.

On POD 15, all wounds showed pale pink color, continuously contraction and wound areas became very small. The wound appearance illustrated in Figure 14.

On POD 18, the day of experiment ended. All wounds treated with PG dressing film showed complete wound closure, the wound surface was smooth and mild scarring appeared. However, some wounds in control, T_2 and T_3 were not completely closed, pale color, the scar formations were appeared. The wound appearance illustrated in Figure 15.

Wounds treated with PG dressing film (T_1) showed 100% complete closure on day 18, whereas wounds treated with 1% povidone iodine and covered with PG dressing film (T_2), PG dressing gel (T_3) and 1% povidine iodine (control) showed 91.67 %, 90 % and 68.75% of complete wound closure, respectively. The results showed in Table 9.

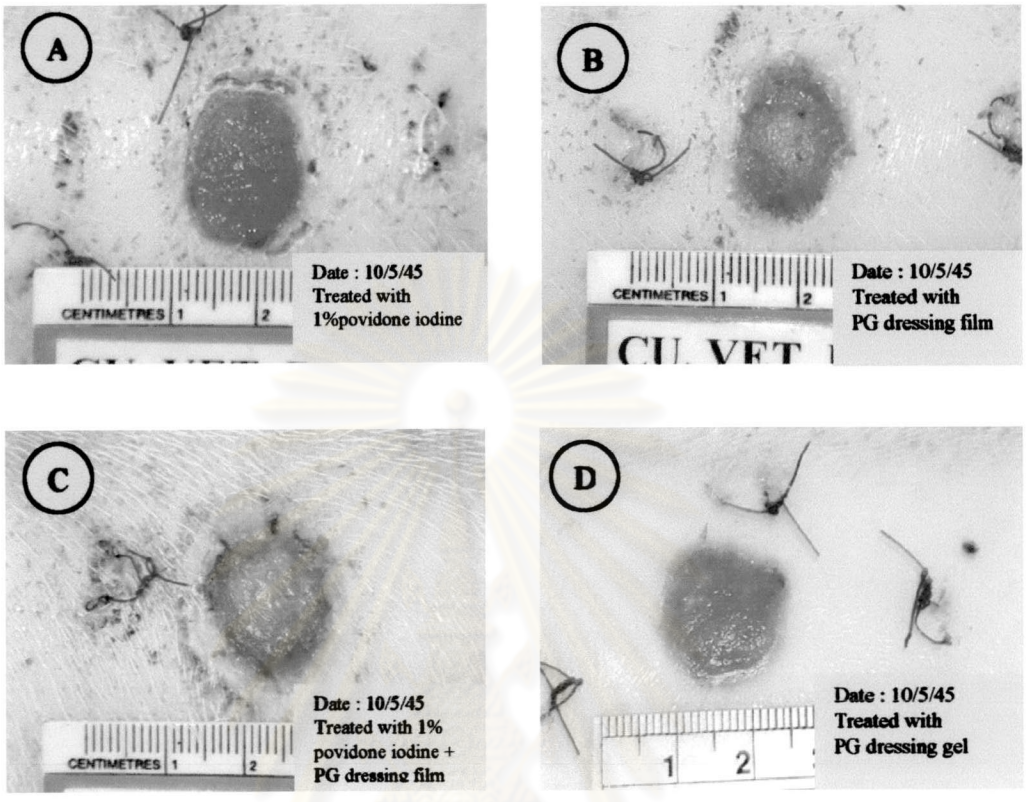

Figure 12. The wound appearance on day 9. A) control : a wound treated with 1% povidone iodine. B) T_1: a wound treated with PG dressing film. C) T_2: a wound treated with 1% povidone iodine and cover with PG dressing film. D) T_3 : a wound treated with PG dressing gel showed moisty wound.

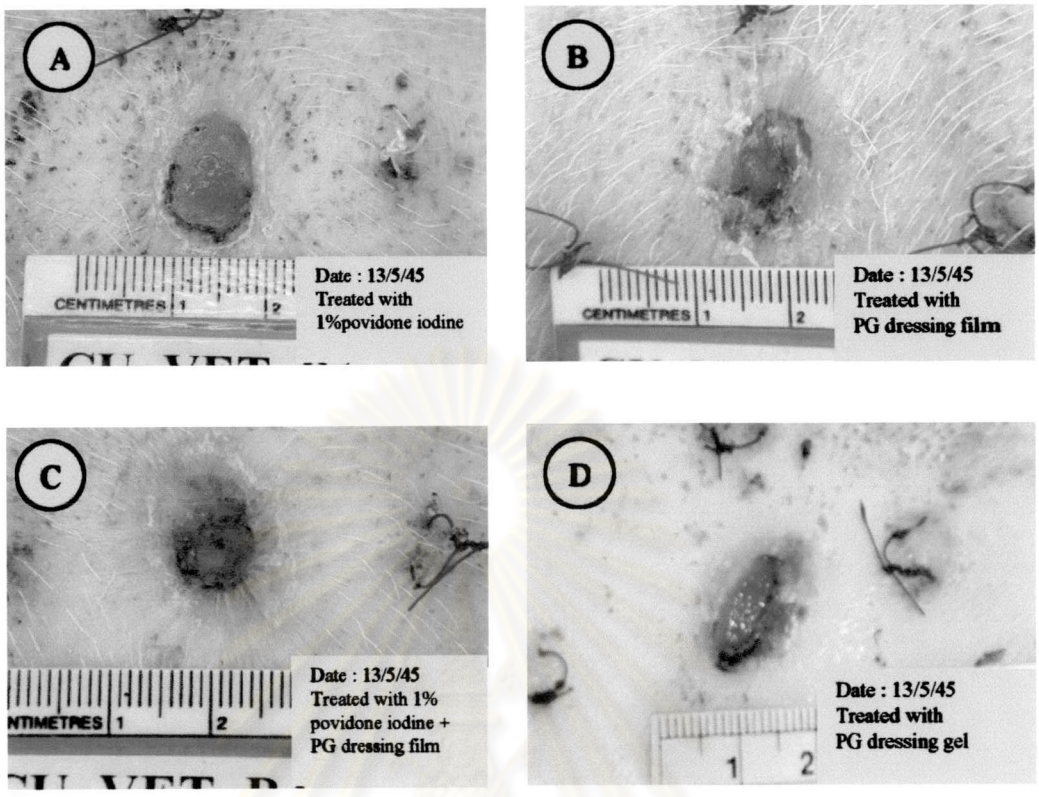

Figure 13. The wound appearance on day 12. A) control: a wound treated with 1% povidone iodine. B) T_1: a wound treated with PG dressing film. C) T_2: a wound treated with 1% povidone iodine and cover with PG dressing film. D) T_3 : a wound treated with PG dressing gel showed moisty wound.

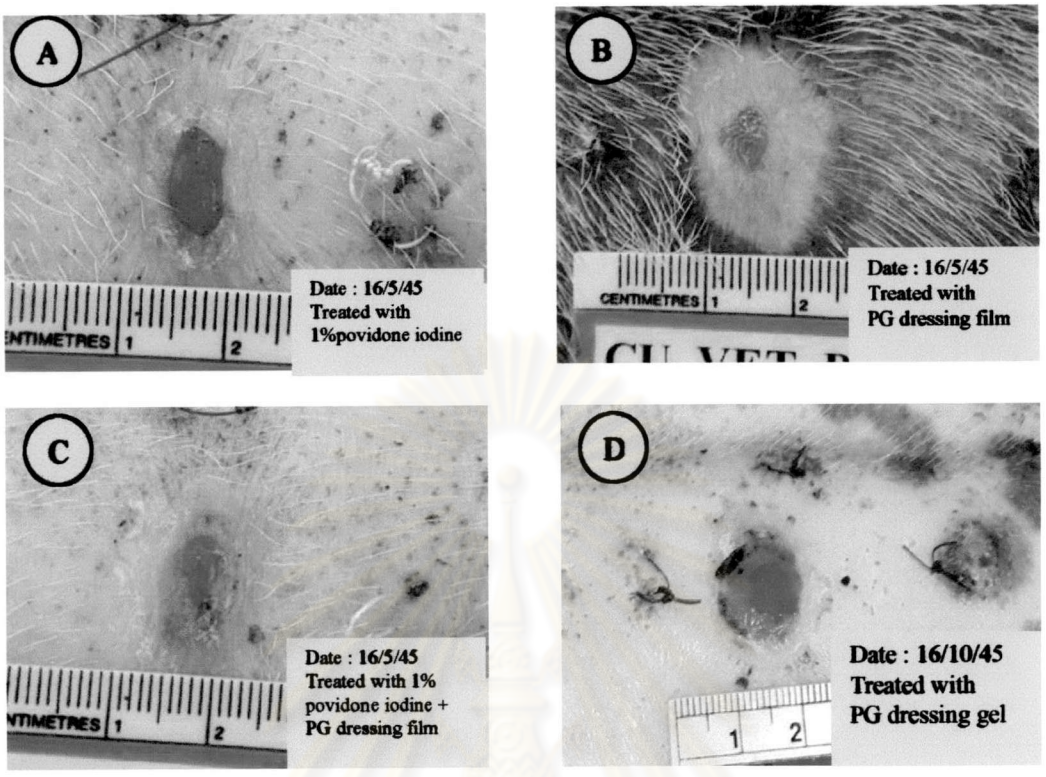

Figure 14. The wound appearance on day 15. A) control: a wound treated with 1% povidone iodine. B) T_1: a wound treated with PG dressing film. C) T_2: a wound treated with 1% povidone iodine and cover with PG dressing film. D) T_3 : a wound treated with PG dressing gel.

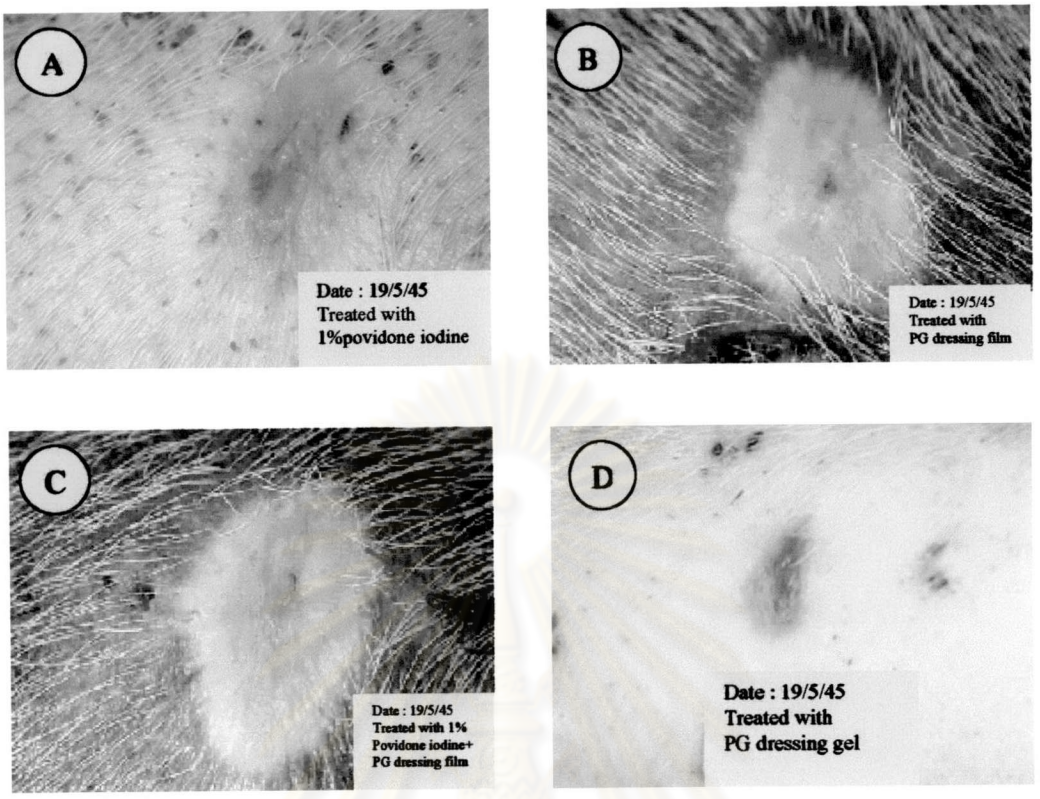

Figure 15. The wound appearance on day 18. A) control: a wound treated with 1% povidone iodine. B) T_1: a wound treated with PG dressing film. C) T_2: a wound treated with 1% povidone iodine and cover with PG dressing film. D) T_3 : a wound treated with PG dressing gel.

Table 9. Wound closure rate of full-thickness wounds of pig skins after treated with PG preparations on 18 days. Control = treated with 1% povidone iodine, T_1= treated with PG dressing film, T_2 = treated with 1% povidone iodine and covered with PG dressing film, T_3 = treated with PG dressing gel.

Treatment groups	% area of wound closure (No. of complete wound closure /n)
Control	68.75 (11/16)
T_1	100.00 (12/12)
T_2	91.67 (11/12)
T_3	90.00 (9/10)

5.1.2 Wound Area Analysis

Wound areas were measured every 3 days starting from day 3 to day 18 by tracing the wound boundaries using sterile transparent sheets with permanent marker. The transparent sheets were scanned and the areas of each wound was computerized by using computer program of image analysis program. The results clearly demonstrated on day 3 that the mean values of wound area in each treatment were increased in comparison with its value on day 0 in all group. The expansion of wound area was the consequence of each wound swelling. Inflammatory phase of healing process can clearly explain this result. Data are showed in Table 10.

On POD 6, mean value of wound area in each treatment was reduced to almost equal to that of day 0 as showed in Table 10. Inflammatory phase or phase 1 of healing process was ended, wound swelling was stopped.

On POD 9, each treatment showed the reduction of its wound area mean values. The mean value of wound area reduced to almost half time of their wound areas previously measured on POD 6 as showed in Table 10.

On POD 12, the mean value of wound area of T_1 and T_2 were a significantly smaller than that of wounds in control ($p \leq 0.05$). However, wound area of T_1 and T_2 were not significantly different from the T_3. The wound areas on day 12 were 1.059 ± 0.130, 0.553 ± 0.082, 0.645 ± 0.116 and 0.926 ± 0.179 cm^2 in wounds treated in control, T_1, T_2, T_3, respectively. Table 10 showed not significant ($p>0.05$) wound closure between T_3 and control.

On POD 15, the wound areas of wounds treated with PG dressing gel were the 4 times markedly reduced compared with the previous measurement. Wound areas in 3 treatments were not significant difference ($p>0.05$) between treatment groups and control (Table 10).

Table 10. Rate of wound closure of full-thickness wound in skin of pigs treated with PG preparations. Control = treated with 1% povidone iodine, T_1 = treated with PG dressing film, T_2 = treated with 1% povidone iodine and covered with PG dressing film, T_3 = treated with PG dressing gel. a, b, = significant difference between groups ($p \leq 0.05$).

Days	Wound area (cm^2) mean ± SEM			
	Control (n=16)	T_1 (n= 12)	T_2 (n=12)	T_3 (n=10)
0	4.173 ± 0.000	4.173 ± 0.000	4.173 ± 0.000	4.173 ± 0.000
3	5.385 ± 0.176	5.210 ± 0.242	5.401 ± 0.287	5.299 ± 0.116
6	4.771 ± 0.152	4.323 ± 0.307	4.452 ± 0.529	4.293 ± 0.202
9	2.832 ± 0.200	2.227 ± 0.201	2.391 ± 0.240	2.388 ± 0.245
12	1.059 ± 0.130^{a}	0.553 ± 0.082^{b}	0.645 ± 0.116^{b}	0.926 ± 0.179^{ab}
15	0.468 ± 0.112	0.285 ± 0.045	0.344 ± 0.095	0.225 ± 0.057
18	0.023 ± 0.013	0.000 ± 0.000	0.005 ± 0.005	0.003 ± 0.003

On POD 18, the day of experiment ended. All of wounds treated with PG dressing film showed complete wound closure, mean values of wound area was zero, whereas the values in T_3, T_2 and control were 0.003 ± 0.003, 0.005 ± 0.005 and 0.023 ± 0.013, respectively (Table 10).

A comparison of wound closure rates in Figure 16, calculated as percentage of wound area, the result illustrated that wound closure in T_1 and T_2 showed faster healing rate, percent of wound areas clearly demonstrated significantly small wound areas ($P \leq 0.05$) were obtained on day 12 (Figure 16). The wounds treated with PG dressing gel were healed in about the same rate as wound in T_1 and T_2 as well as in control.

5.2 Histopathological Evaluation

The selected wound healing in this study was examined from 18 days wound. It was in the late stage of proliferative phase in wound healing process (inflammatory phase, proliferative phase and remodeling phase). On the basis proliferative phase; the inflammatory cells (PMNs and macrophages) were decreased; the epithelial cells were highly migration, proliferation and differentiation for promote wound closure; the fibroblast cells were increased for synthesize collagen and they were decreased in remodeling phase; the granuloma cells and foreign body giant cells were not appeared (Young *et al.,* 1991; Lawrence, 1998).

The criterias of histopathology used in this study were followed such as epidermal hyperplasia and hyperkeratosis, subacute dermatitis, chronic dermatitis, dermal fibrosis, and dermal granuloma.

Epidermal hyperkeratosis and hyperplasia were determined by proliferation of epithelial with increased keratin production in stratum corneum. From this study, the wound treated with PG dressing gel (T_3) showed statistic significantly highest epithelial proliferation and hyperkeratosis by presented higher score than those in control, T_1, and T_2, respectively, (as illustrated in Table 11 and Figure 17).

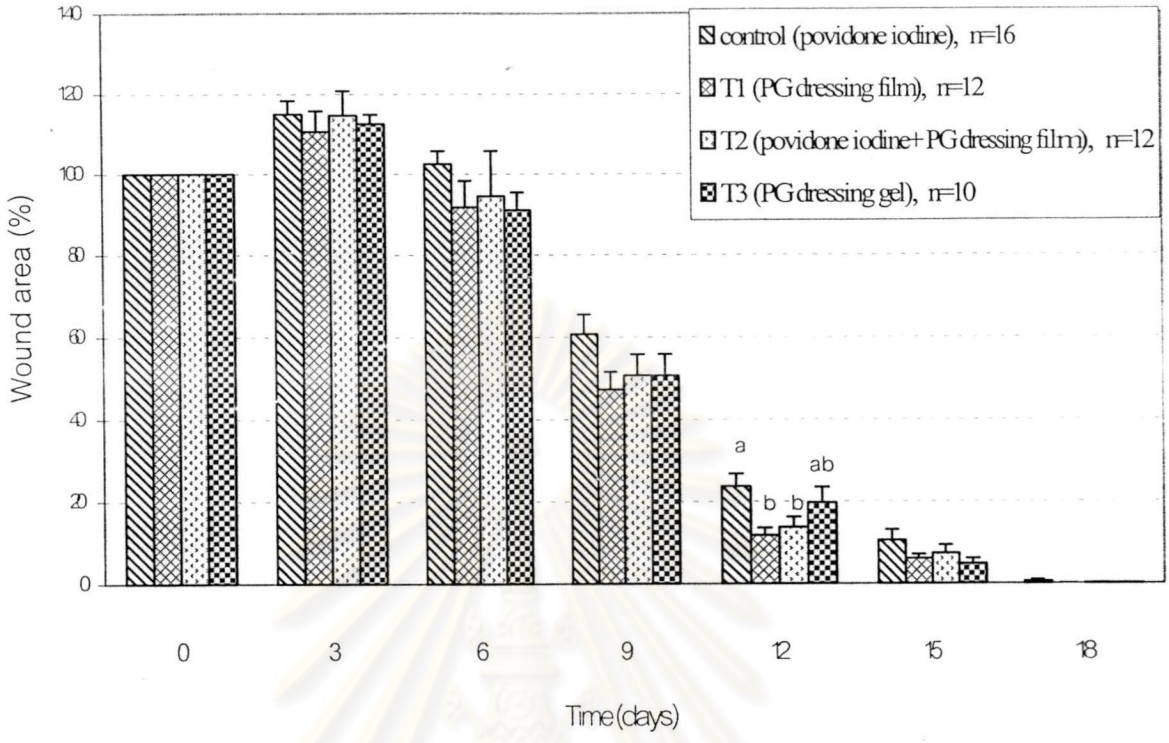

Figure 16. A comparison of wound areas (mean±SEM) of full-thickness wounds in skin of pig at every 3 days interval for 18 days of treatment with PG dressing preparation. Control = treated 1% povidone iodine, T_1 = treated with PG dressing film, T_2 = treated with 1% povidone iodine and covered with PG dressing film, T_3 = treated with PG dressing gel. a, b = significant difference between groups ($p \leq 0.05$).

Table 11. Blind analysis of histopathological section in skin of pigs after 18 days treatment with PG dressing preparations. Values represent validity score range between 0 (no remarkable lesion) to 3 (severe). Control = treated with 1% povidone iodine, T_1 = treated with PG dressing film, T_2 = treated with 1% povidone iodine and covered with PG dressing film, T_3 = treated with PG dressing gel. Data are mean $\pm$ SEM. a, b, c = significant difference between groups ($p \leq 0.05$).

Histopathology criteria	Control (n=16)	T_1 (n=12)	T_2 (n=12)	T_3 (n=10)
Epidermal hyperkeratosis and hyperplasia	1.250 ± 0.138^{b}	1.250 ± 0.179^{b}	1.167 ± 0.207^{b}	1.892 ± 0.127^{a}
Subacute dermatitis	0.269 ± 0.162	0.333 ± 0.224	0.417 ± 0.229	0.568 ± 0.126
Chronic dermatitis	1.259 ± 0.196^{b}	1.167 ± 0.297^{b}	0.417 ± 0.193^{c}	1.684 ± 0.114^{a}
Dermal fibrosis	1.821 ± 0.074^{b}	1.500 ± 0.195^{c}	1.583 ± 0.149^{bc}	2.132 ± 0.086^{a}
Dermal granuloma	1.667 ± 0.239^{a}	0.417 ± 0.149^{b}	1.000 ± 0.348^{ab}	1.367 ± 0.175^{a}

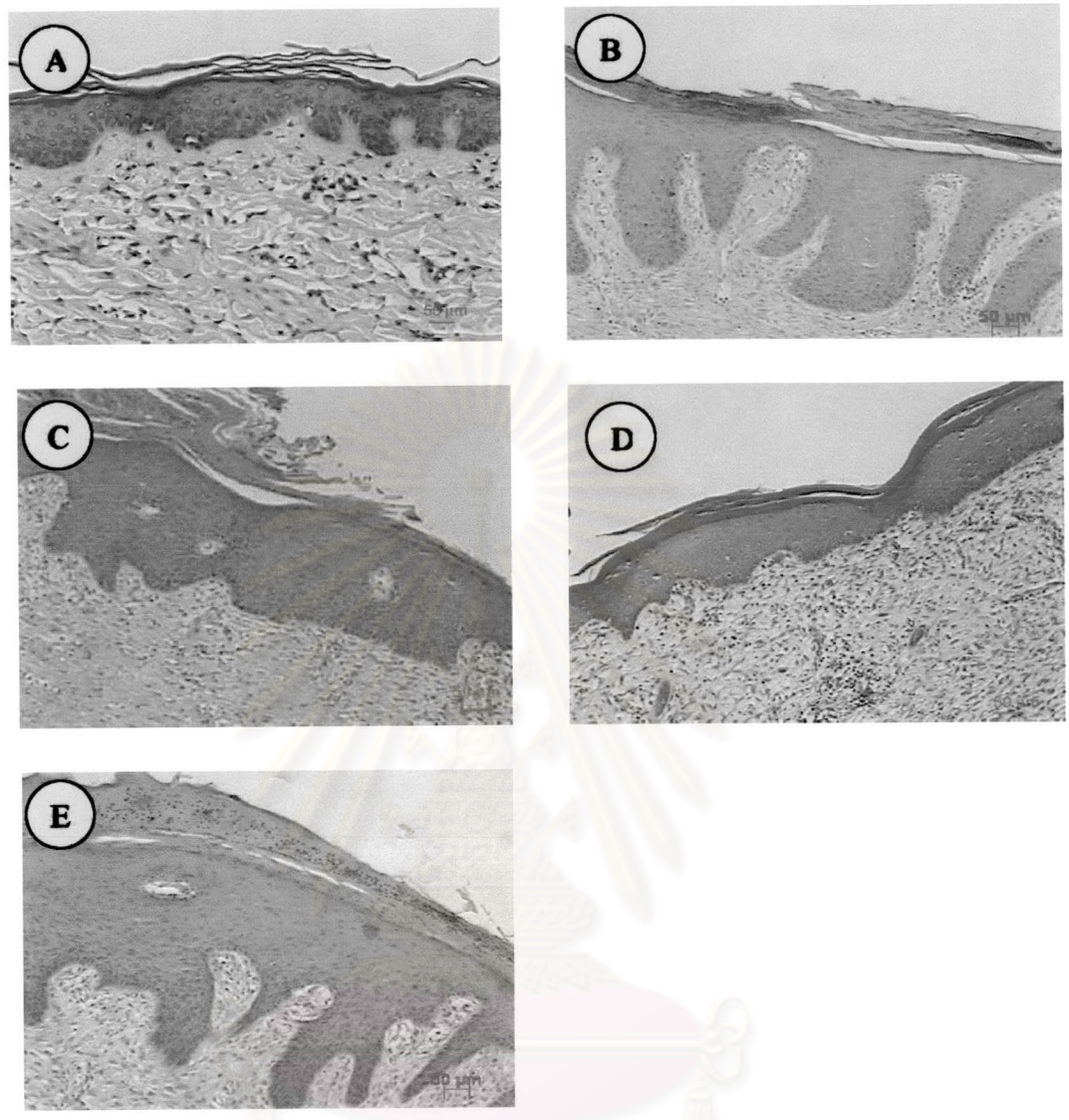

Figure 17. Photomicrographs of epidermal hyperkeratosis and hyperplasia of the wounds. A) normal skin. B) control : a wound treated with 1% povidone iodine. C) T_1: a wound treated with PG dressing film. B and C showed moderate epidermal hyperkeratosis and hyperplasia. D) T_2: a wound treated with 1%povidone iodine and covered with PG dressing film showed mild epidermal hyperkeratosis and hyperplasia. E) T_3 : a wound treated with PG dressing gel showed severe epidermal hyperkeratosis and hyperplasia.

Subacute dermatitis was characterized by a number of PMNs and some macrophages aggregated in the lesions. The results illustrated that the wound in all treatment groups showed no remarkable lesion to mild number of PMNs and macrophages aggregated in the lesions. Some congested and hemorrhagic areas were observed. The data was presented in Table 11 and Figure 18.

Chronic dermatitis was characterized by vascularization with chronic inflammatory cells (lymphocytes and plasma cells) aggregated in dermal layer. The results demonstrated that the wounds in control and T_1 groups showed the same mild chronic inflammatory cells aggregated in dermal layer by presented mild score. The wounds in T_2 presented no remarkable lesion to mild degree which is significant different from other groups. Whereas, the wound in T_3 showed moderate lesion and significant the different from other groups. The data demonstrated in Table 11 and Figure 19.

Dermal fibrosis was determined by increasing a number of reactive fibroblasts and increased collagen fiber in dermal layer. Wounds treated with PG dressing gel showed statistic significantly highest fibrosis score than control, T_2 and T_1, respectively. While, the wounds treated with PG dressing film showed statistic significantly a least fibroblasts (as illustrated in Table 11 and Figure 20).

Dermal granuloma was 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. The results represented that the wound treated with 1% povidone iodine had the highest tissue reaction score by presented mild to moderate score of dermal granuloma formation in dermal layer. Whereas, wound treated with 1% povidone iodine and covered with PG dressing film, and PG dressing gel showed mild dermal granuloma formation, respectively. In the opposite, PG dressing film showed statistic significantly a least tissue reaction by presented no remarkable lesions granuloma formation in dermal layer. The data was demonstrated in Table 11 and Figure 21.

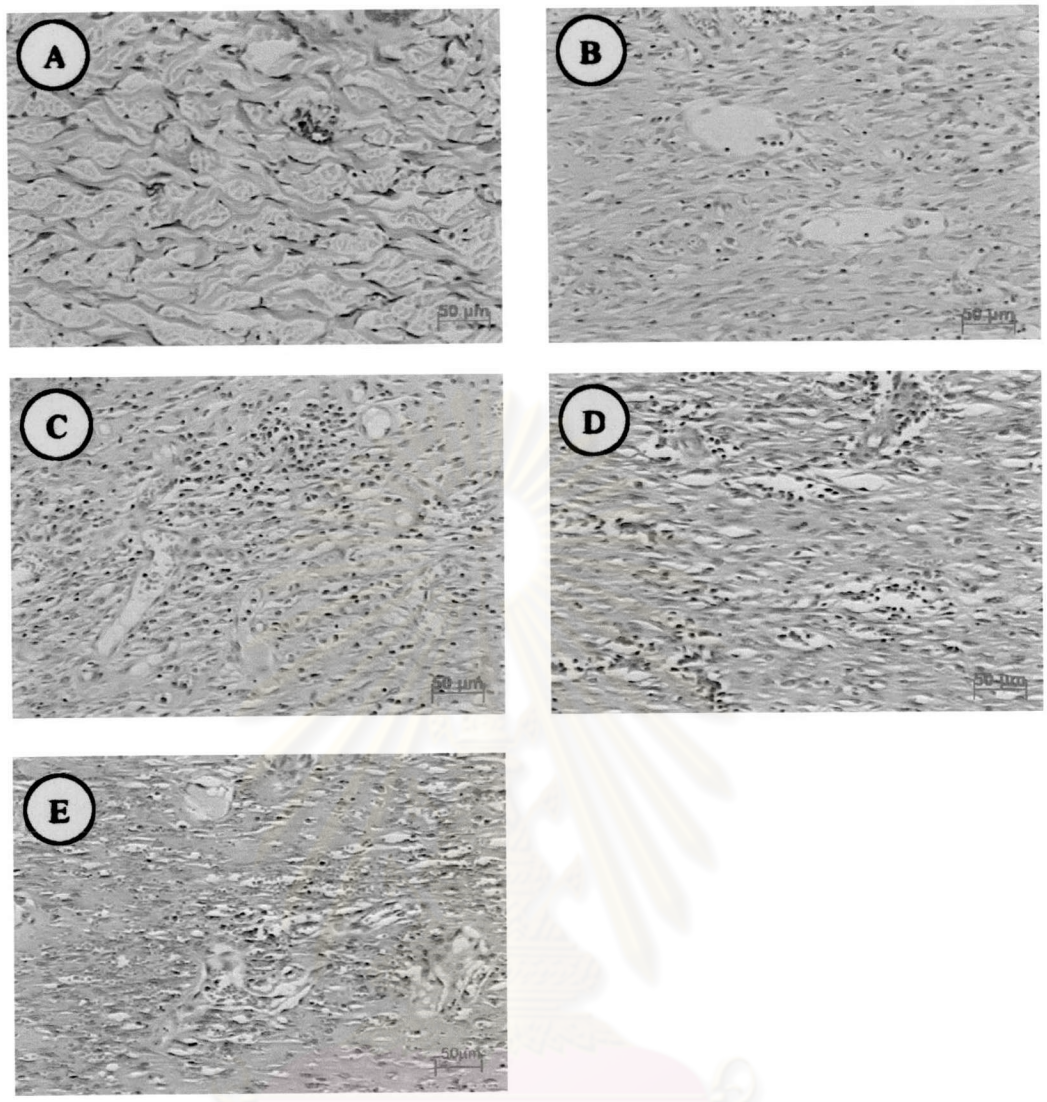

Figure 18. Photomicrographs of subacute dermatitis of the wounds. A) normal skin. B) control : a wound treated with 1%povidone iodine. C) T_1: a wound treated with PG dressing film. D) T_2: a wound treated with1 % povidone iodine and covered with PG dressing film. E) T_3 : a wound treated with PG dressing gel. B, C, D and E were mild degree of inflammatory cells aggregated in the lesion.

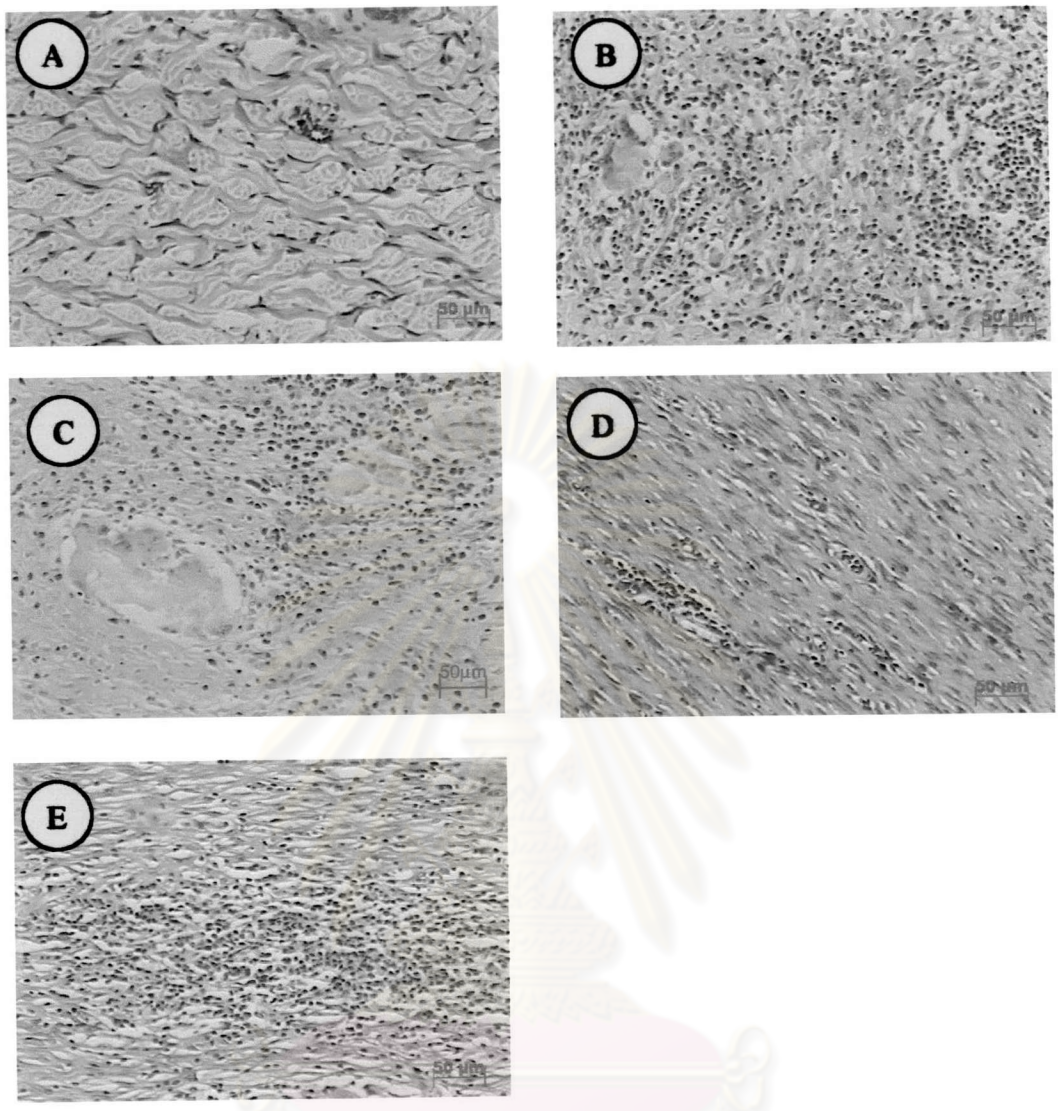

Figure 19. Photomicrographs of chronic dermatitis of the wounds. A) normal skin. B) control: a wound treated with 1% povidone iodine showed mild score of chronic inflammatory cells. C) T_1 : a wound treated with PG dressing film showed mild score of chronic inflammatory cells. D) T_2 : a wound treated with 1% povidone iodine and covered with PG dressing film showed no remarkable to mild score of chronic inflammatory cells. E) T_3: a wound treated with PG dressing gel showed moderate score of chronic inflammatory cells in the lesion.

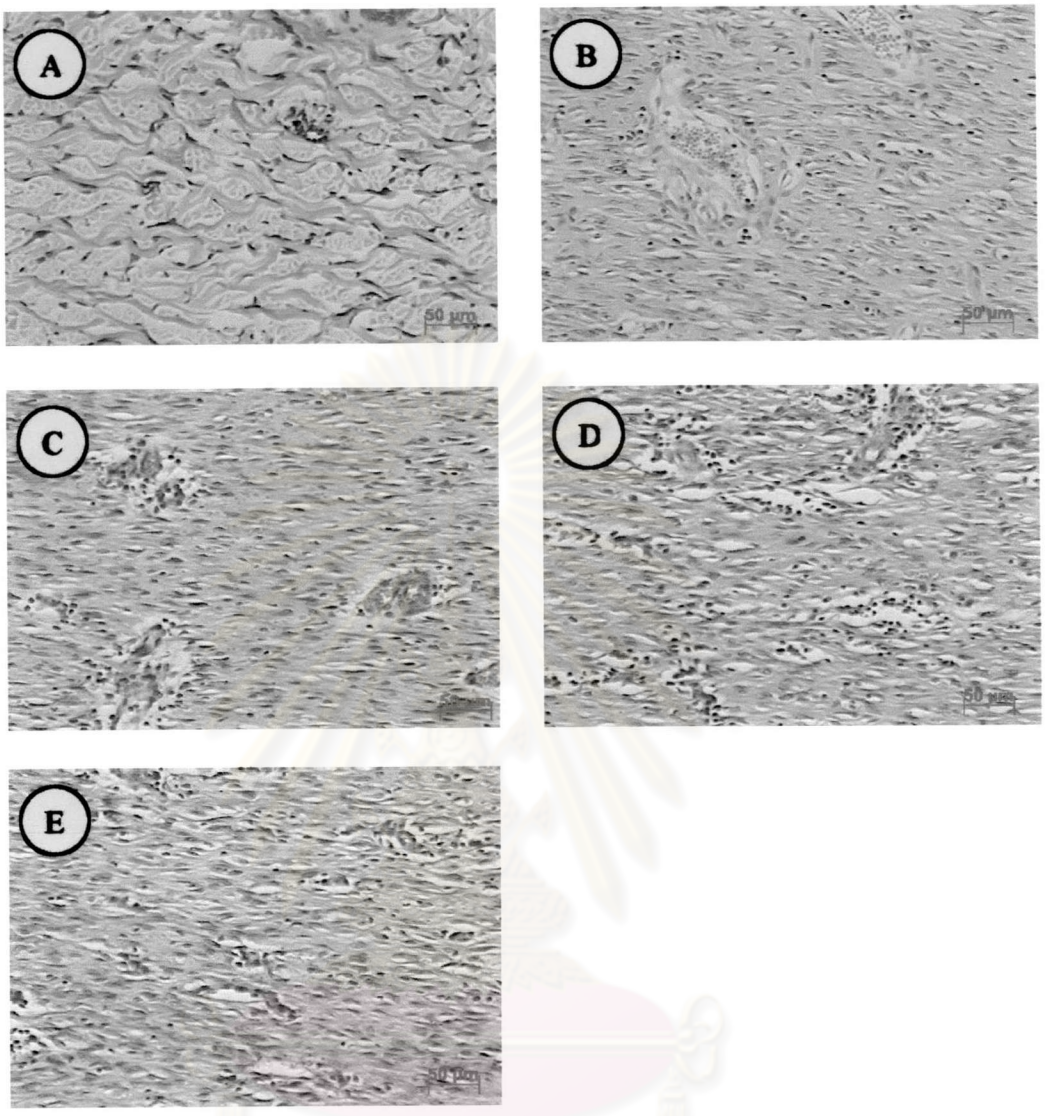

Figure 20. Photomicrograph of dermal fibrosis of the wounds. A) normal skin. B) control: a wound treated with 1% povidone iodine. C) T_1: a wound treated with PG dressing film. D)T_2: a wound treated with 1%povidone iodine and covered with PG dressing film. B, C, and D were showed mild score of fibroblasts in dermal layer. E) T_3: a wound treated with PG dressing gel showed moderate score of fibroblasts in dermal layer.

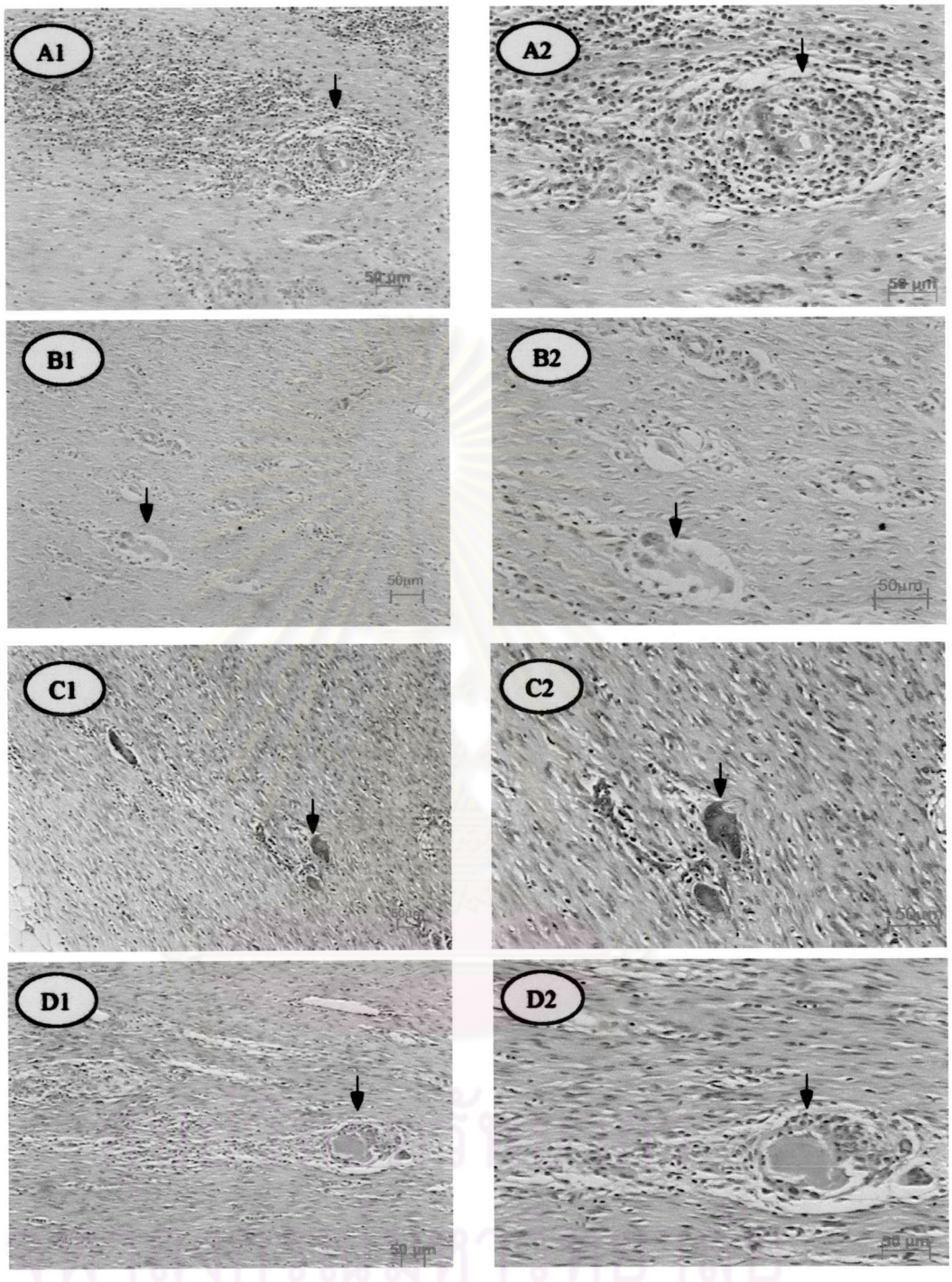

Figure 21. Photomicrograph of dermal granuloma of the wounds. A_1 ,A_2 showed moderate score of granuloma formation in wound treated with 1% povidone iodine. B_1, B_2 showed no remarkable to mild o f granuloma formation in wound treated with PG dressing film. C_1,C_2 showed mild score of granuloma formation in wound treated with 1% povidone iodine and covered with PG dressing film. D_1,D_2 showed mild score of granuloma formation in wound treated with PG dressing gel. A_2, B_2, C_2, and D_2 were high magnification of A_1, B_1, C_1, respectively. Arrow showed granuloma formation.