คุณสมบัติของเจลพอลิแซ็กคาไรด์จากทุเรียนในการเตรียมแผ่นแปะแผลและผลของผลิตภัณฑ์ต่อการหายของบาดแผลผิวหนังของสุนัข

นางสาว ระวีวรรณ ศิริโภคทรัพย์กุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาชีวเวชเคมี ภาควิชาชีวเคมี
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2547
ISBN 974-53-2131-1

PROPERTY OF POLYSACCHARIDE GEL FROM DURIAN AS DRESSING PREPARATIONS AND ITS EFFECT ON WOUND HEALING IN DOG SKIN

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Biomedicinal Chemistry

Department of Biochemistry
Faculty of Pharmaceutical Sciences
Chulalongkorn University

Academic Year 2004
ISBN 974-53-2131-1

Thesis Title	Property of Polysaccharide Gel from Durian as Dressing Preparations and its Effect on Wound Healing in Dog Skin
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Field of Study	Biomedicinal chemistry
Thesis Advisor	Associate Professor Sunanta Pongsamart, Ph.D.
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ระวีวรรณ ศิริโภคทรัพย์กุล: คุณสมบัติของเจลพอลิแซ็กคาไรด์จากทุเรียนในการเตรียมแผ่นแปะแผลและผลของผลิตภัณฑ์ต่อการหายของบาดแผลผิวหนังของสุนัข (PROPERTY OF POLYSACCHARIDE GEL FROM DURIAN AS DRESSING PREPARATIONS AND ITS EFFECT ON WOUND HEALING IN DOG SKIN) อาจารย์ที่ปรึกษาวิทยานิพนธ์ : รศ. ดร. สุนันท์ พงษ์สามารถ, อาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ.สพ.ญ. ดร.ปิยะรัตน์ จันทร์ศิริพรชัย, 109 หน้า, ISBN 974-53-2131-1

ผลิตภัณฑ์แผ่นแปะแผลที่เตรียมจากสารเจลพอลิแซ็กคาไรด์สกัดจากเปลือกแห้งของผลทุเรียนในรูปแบบของแผ่นฟิล์มและแผ่นใยแห้งแปะแผล ในสูตรตำรับแผ่นฟิล์มแปะแผลประกอบด้วยด้วยโพรไพลีน ไกลคอล 15 เปอร์เซนต์ของน้ำหนักผงแห้งเจลพอลิแซ็กคาไรด์เป็นพลาสติไซเซอร์ เตรียมเป็นแผ่นฟิล์มโดยวิธี casting/solvent evaporation แผ่นฟิล์มที่เตรียมได้มีลักษณะบางใส ไม่มีสีถึงมีสีส้มอมชมพูจาง การทดสอบคุณสมบัติเชิงกลของแผ่นฟิล์มที่เตรียมได้ พบว่าแผ่นฟิล์มเจลพอลิแซ็กคาไรด์ที่เติมพลาสติไซเซอร์ มีความอ่อนตัว เหนียว และยืดหยุ่น น่าพอใจกว่าแผ่นฟิล์มเจลพอลิแซ็กคาไรด์ที่ไม่เติมพลาสติไซเซอร์ นอกจากนี้พบว่าแผ่นฟิล์มแปะแผลเจลพอลิแซ็กคาไรด์สามารถพองตัวและมีคุณสมบัติในการยึดติดกับเนื้อเยื่อได้ แผ่นใยแห้งแปะแผลเตรียมได้จากการใช้เครื่อง freeze-dryer แผ่นใยแห้งแปะแผลที่เตรียมจาก 1 เปอร์เซนต์ของสารละลาย PG ในน้ำ ให้ผลิตภัณฑ์แผ่นใยแห้งที่น่าพอใจที่สุด แผ่นใยแห้งที่เตรียมได้ มีลักษณะ อ่อนนุ่ม เหนียวและหนา มีสีขาว จากการศึกษาคุณสมบัติของแผ่นฟิล์มและแผ่นใยแห้งแปะแผล พบว่าผลิตภัณฑ์ทั้งสองรูปแบบมีความชื้นและสามารถดูดความชื้นจากภายนอกได้ การศึกษาผลของผลิตภัณฑ์แปะแผลที่เตรียมจากสารเจลพอลิแซ็กคาไรด์ต่อการหายของบาดแผลเปิดบนผิวหนังของสุนัข โดยทดลองในสุนัขเพศเมียโตเต็มที่สุขภาพแข็งแรง น้ำหนักประมาณ 12-15 กิโลกรัม จำนวน 4 ตัว ทำการผ่าตัดเปิดแผลผิวหนังสุนัขในแนวกลางหลัง ขนาดเส้นผ่าศูนย์กลางของแผล 2 เซนติเมตร จำนวน 8 แผล ทำการรักษาบาดแผลแบบสุ่มโดยแบ่งเป็น 4 กลุ่ม คือ บาดแผลที่รักษาด้วย 1% โพวิโดนไอโอดีน (กลุ่มควบคุม) บาดแผลที่รักษาด้วยแผ่นฟิล์มแปะแผลเจลพอลิแซ็กคาไรด์ (กลุ่มทดลองที่ 1) บาดแผลที่รักษาด้วยแผ่นใยแห้งแปะแผลเจลพอลิแซ็กคาไรด์ (กลุ่มทดลองที่ 2) บาดแผลที่รักษาด้วย 1% โพวิโดนไอโอดีนและปิดด้วยแผ่นฟิล์มแปะแผล Opsite® Flexigrid (กลุ่มทดลองที่ 3) ตามลำดับ ทุกแผลปิดทับด้วยผ้าก๊อส ประเมินการหายของบาดแผลโดยดูจากลักษณะทางมหพยาธิวิทยาของบาดแผลทุก 3 วัน คือวันที่ 3 6 9 12 15 18 และ 21 พื้นที่ของบาดแผลคำนวณโดยใช้โปรแกรมคอมพิวเตอร์ ผลการศึกษาแสดงให้เห็นว่า มีอัตราการหายของแผลได้รวดเร็วในกลุ่มทดลองที่ 2 โดยในวันที่ 12 และ 15 ของการทดลอง บาดแผลที่รักษาด้วยวิธีในกลุ่มทดลองที่ 2 มีผลทำให้แผลปิดเร็วกว่าและมีขนาดของบาดแผลเหลืออยู่เล็กกว่าแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) เทียบกับบาดแผลในกลุ่มควบคุม อย่างไรก็ตาม ในวันที่ 18 และ 21 ของการทดลอง บาดแผลที่รักษาด้วยวิธีในกลุ่มทดลองที่ 1 และ 2 มีขนาดของแผลเหลืออยู่เล็กกว่าแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) เทียบกับแผลที่รักษาด้วยวิธีในกลุ่มควบคุม และกลุ่มทดลองที่ 3 บาดแผลที่รักษาด้วยแผ่นใยแห้งแปะแผลเจลพอลิแซ็กคาไรด์ (กลุ่มทดลองที่ 2) แสดงการปิดของทุกแผลอย่างสมบูรณ์ 100% ในวันที่ 21 ของการทดลอง ในขณะที่บาดแผลที่รักษาด้วย 1% โพวิโดนไอโอดีน (กลุ่มควบคุม) ที่รักษาด้วยแผ่นฟิล์มแปะแผลเจลพอลิแซ็กคาไรด์ (กลุ่มทดลองที่ 1) และ ที่รักษาด้วย1% โพวิโดนไอโอดีนร่วมกับปิดด้วยแผ่นฟิล์มแปะแผล Opsite® Flexigrid (กลุ่มทดลองที่ 3) ให้ผลมีการปิดของแผลอย่างสมบูรณ์ได้ 50.0 % 87.5% และ 37.5 % ตามลำดับ การประเมินผลทางจุลพยาธิวิทยาของแผลในวันที่ 21 ผลการศึกษาพบว่าแผลที่รักษาด้วยผลิตภัณฑ์แปะแผลที่เตรียมจากเจลพอลิแซ็กคาไรด์ พบการเจริญแบ่งตัวของเซลล์เยื่อบุผิวหนังในระดับเบาบาง พบจำนวนเซลล์ไฟโบรบลาสท์อยู่ในระดับเบาบาง พบเกิดการอักเสบน้อยโดยพิจารณาจากที่ไม่พบการอักเสบแบบกึ่งเฉียบพลันในชั้นผิวหนังแท้ และการเกิดแกรนูโลมาในชั้นผิวหนังแท้ พบได้น้อยกว่าบาดแผลในกลุ่มควบคุมและการทดลองที่ 3 จากผลการศึกษาชี้ให้เห็นว่า ผลิตภัณฑ์แปะแผลที่เตรียมจากเจลพอลิแซ็กคาไรด์แสดงคุณสมบัติในอุดมคติที่ดีของแผ่นแปะแผลโดยรักษาความชื้นของบาดแผลทำให้บาดแผลหายเร็ว ส่งเสริมการหายของบาดแผลโดยลดการอักเสบและปฏิกิริยาของเนื้อเยื่อ ส่งเสริมการเจริญแบ่งตัวของเซลล์เยื่อบุผิวหนังและการเกิดไฟโบรซิส จากผลการศึกษาแนะนำว่าผลิตภัณฑ์แปะแผลที่เตรียมจากเจลพอลิแซ็กคาไรด์ ทั้งในรูปแบบแผ่นฟิล์มและแผ่นใยแห้งแปะแผล สามารถนำมาใช้รักษาบาดแผลเปิดในสุนัขได้อย่างมีประสิทธิภาพ ดีกว่าการรักษาด้วยวิธีที่มักนิยมใช้และการใช้แผ่นฟิล์มแปะแผลที่ขายทั่วไป

ภาควิชา……ชีวเคมี…… ลายมือชื่อนิสิต……

สาขาวิชา……..ชีวเวชเคมี…… ลายมือชื่ออาจารย์ที่ปรึกษา……

ปีการศึกษา……2547…….. ลายมือชื่ออาจารย์ที่ปรึกษาร่วม……

4676589633 : MAJOR BIOMEDICINAL CHEMISTRY

KEY WORDS : *Durio zibethinus* L. / DURIAN POLYSACCHARIDE GEL / DRESSING FILM / FIBER DRESSING PATCH / WOUND HEALING / DOG

RAVEEWAN SIRIPOKASUPKUL : PROPERTY OF POLYSACCHARIDE GEL FROM DURIAN AS DRESSING PREPARATIONS AND ITS EFFECT ON WOUND HEALING IN DOG SKIN. THESIS ADVISOR : ASSOC. PROF. SUNANTA PONGSAMART, Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. PIYARAT CHANSIRIPORNCHAI, Ph.D., 109 pp. ISBN 974-53-2131-1

The dressing preparations of polysaccharide gel (PG) extracted from fruit-hulls of durian (*Durio zibethinus* L.) were prepared as a PG dressing film and fiber dressing patch. PG dressing film with 15% w/w propylene glycol based on PG as a plasticizer was prepared by casting/solvent evaporation method. The PG dressing films were a transparent thin film, colorless to pale beige in color. The mechanical properties of PG dressing films were investigated. The results indicated that PG dressing film with plasticizer was softer, tougher and more flexible than those without plasticizer. The PG dressing film also showed swelling and bioadhesive properties. PG fiber dressing patch was prepared by using freeze-dryer. The PG fiber dressing patch prepared by freeze-drying, using 1% PG aqueous solution, provided the most satisfactory dried fiber product. The resulting product was a soft, tough and thick fiber, white in color. Study of the properties of PG dressing film and fiber dressing patch were found that both forms of PG dressing contain moisture and also absorbed moisture from environment. An effect of the PG preparations on wound healing in open excisional wounds was performed in dog skin. Four adult and healthy female dogs, weighing 12-15 kg, were used in this experiment. Eight full-thickness wounds of 2 cm in diameter were operated on both side of vernal midline. All wounds in each dog were randomly treated by 4 treatments. Wounds in group 1 treated with 1% povidone iodine (control), group 2 treated with PG dressing film (T_1), group 3 treated with PG fiber dressing patch (T_2) and group 4 treated with 1% povidone iodine and covered with commercial dressing film -Opsite® Flexigrid (T_3). All wounds were covered with sterile gauze. Subsequently, the performance of wound healing was evaluated by gross pathology on days 3, 6, 9, 12, 15, 18 and 21 postoperative days. Each wound area was calculated using a computer program. The results demonstrated that a rapid healing rate was obtained in T_2, the PG fiber dressing patch treated wounds clearly showed statistic significantly faster wound closure and smaller wound area ($p < 0.05$) than that of control on days 12 and 15. However, a significant smaller wound area ($p \leq 0.05$) was obtained in wounds treated with PG dressing film and PG fiber dressing patch than that of wounds in control and T_3 on days 18 and 21. Wounds treated with PG fiber dressing patch (T_2) represented 100% complete wound healing on days 21 whereas wounds treated with 1% povidone iodine (C), PG dressing film (T_1) and 1% povidone iodine and covered with Opsite® Flexigrid film (T_3) were 50.0 %, 87.5% and 37.5 %, respectively. Histopathological study of tissue reaction was examined on days 21. The results demonstrated that both PG dressing preparations treated wounds showed mild epidermal regeneration, mild dermal fibrosis and less inflammation represented by no remarkable lesion of subacute suppurative dermatitis and less of pyogranuloma formation than those wounds treated with 1% povidone iodine (C) and 1% povidone iodine and covered with commercial film -Opsite® Flexigrid (T_3). The results indicated that PG dressing preparations represented the properties of ideal wound dressing by maintaining a moist environment, rapidly healing wounds, and promoting wound healing by reducing the inflammation and tissue reaction, promoting epithelalization and dermal fibrosis. The results suggest that PG dressing preparations, PG dressing film and PG fiber dressing patch, were effectively used as a wound dressing for healing open excisional wounds in dog skin better than those of traditional treatment and treatment with commercial available dressing film.

Department…….Biochemistry…..…….. Student's signature……...…….

Field of study….Biomedicinal Chemistry.. Advisor's signature…….

Academic year……..2004…….. Co-advisor's signature……..…….…….……

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to my thesis advisor, Associate Professor Dr. Sunanta Pongsamart for her invaluable advice, guidance and encouragement throughout this study. Her patience and kindness are also deeply appreciated.

I wish to express my grateful thank to my thesis co-advisor, Associate Professor Dr. Piyarat Chansiripornchai, Department of Veterinary Pharmacology, Faculty of Veterinary Science, Chulalongkorn University for her kindness, valuable advice, and helpful suggestion for this research.

My special thanks to Associate Professor Dr. Anudep Rungsipipat, Department of Pathology, and Assistant Professor Dr. Churee Pramatwinai, Department of Veterinary Surgery Faculty of Veterinary Science, Chulalongkorn University, for their helpfulness and their valuable time to suggest a good idea for my thesis.

I also wish express deep appreciation to all members of the thesis committee for their suggestions and comments.

I would like to thanks to Mr. Ardong Atiptamvaree for his helpful during wounds operating.

Thanks are also due to Chulalongkorn University and the Ministry of University Affairs for granting partial financial support to fulfill this study.

Finally, I would like to express my infinite thanks and deepest gratitude to Miss. Apinya Wichan, Department of Pathology, Faculty of Veterinary Science, all staff members and my friends of Department of Biochemistry and other person whose names have not been mentioned here for their assistance and great encouragement.

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LIST OF ABBREVIATIONS

°C	degree celsius (centigrade)
cm	centimetre (s)
cps	centipoises
cv.	cultivar
d	day (s)
DW	deionized water
e.g.	exampli gratia, for example
et al.	et alii, and others
g	gram (s)
hr	hour
hrs	hours
in^2	square inch (s)
kg	kilogram (s)
KN	kilonewton (s)
L	litre (s)
lb/in^2	pound per square inch (es)
LSD	least significant difference
m^2	square metre (s)
min	minute (s)
ml	millilitre (s)
mm	millimetre (s)
mm^2	square millimetre (s)
Mpa	megapascal (s)
mPa·s	milliPascal seconds
n	number
nm	nanometre (s)
PG	polysaccharide gel
POD	postoperative days
%	percentage
pH	the negative logarithm of hydrogen ion

	concentration
PMNs	polymorphonuclear leucocytes
RH	relative humidity
SD	standard deviation
sec	second (s)
T_1	treatment 1
T_2	treatment 2
T_3	treatment 3
UV	ultraviolet
vs	versus
w/v	weight by volume
w/w	weight by weight

CHAPTER I

GENERAL BACKGROUND

1. Introduction

Nowadays, natural material has been developed for various applications. Plant extracts are normally used as a biomaterial for food, cosmetic and pharmaceutical industries. One of the more interesting material is polysaccharides, a high hydrolytic stability and ease of biodegradability, which has many variations in composition, structure and function. Some polysaccharides may possess properties which have been beneficially used in food, cosmetic and pharmaceutical products as thickeners, binders, stabilizers, film formers, gelling agents, suspending agents, lubricants and also wound management aid (Park, *et al*., 2001).

From the understanding of the wound healing process, wound management aids are important in promoting wound healing. Wound management is performed by initial cleansing and debridement after antiseptic procedures have been completed the wound was covered with dressing to stop bleeding and preventing wound infection (Foster, *et al*., 1995). In the early 1962s, Winter demonstrated that wound covered with moist dressing is healed better than those exposed to the air. So wound dressing is essential in wound management. Traditional management is simple applying antiseptic and covering with dressing such as gauze dressing which give a wound protection from the external environment during the formation of the scab. However, gauze dressing is a dry dressing which give a wound dry and interfere growing epithelial cells on a wound when open it. Wound management aids have seen, in recent years, a transition to specialized high technology materials which are produced from both synthetic and natural polymers. Much of the development has resulted from a greater understanding of the processes involved in wound healing coupled with advances in technology to produce biocompatible materials with the necessary physical, chemical and biological properties for enhancement of the healing process. Polysaccharides have been an obvious choice for investigation as beneficial wound management aids. In recent years it was recognized that not only can polysaccharides

be produced with the required physical characteristics for a wound management products but also the biological properties of polysaccharides which enable them to participate actively in the wound healing process. According to literature review, there are various types of polysaccharides being used as wound management aids (Figure 1) such as:

Dextran, consisting of linear chains of (1-6)-linked α-D-glucopyranose residues with (1-3), (1-4) and less frequently (1-2) branch points off the main polysaccharide backbone (Figure 1a). Dextran has shown to accelerates the polymerization of fibrin and also influence the structure of the fibrin clot with the diameter of the fibrin fibers being broader (Lloyd *et al*., 1998)

Alginic acid / alginate, is obtained from the cell walls of brown algae (seaweed). It is a linear copolymer composed of two hexuronic acid monosaccharide residues, D-mannuronic acid and L-guluronic acid (Figure 1b). Alginate has been reported that calcium alginate dressing has beneficial effects on wound healing by providing a moist wound environment and reduces cytotoxicity to fibroblast cells and has shown rapid wound closure (Lloyd *et al*., 1998 and Suzuki *et al.*, 1998).

Hyaluronic acid / hyaluronate, a (1-3)-β-linked linear polysaccharide consisting of a disaccharide repeat unit of D-glucuronic acid and 2-acetamido-2-deoxy-D-glucose (Figure 1c). It has been shown modulate the inflammation and recognized by receptors on a variety of cells which are associated with tissue repair and regeneration (Lloyd *et al*., 1998).

Chitosan, a (1-4)-β-linked glycan composed of 2–amino–2–deoxy–D–glucose (glucosamine) and 2-acetamido-2-deoxy-D-glucose (N-acetylglucosamine) (Figure 1d), partially deacetylated from chitin. Chitosan has been prepared to a wound dressing and having a function in the acceleration of migration of PMN cells and macrophages at the early stage of wound healing (Ueno *et al*, 1999). Chitosan has also stimulated secreting of interleukin (Nishimura et al, 1984 and Mori *et al*, 1997)

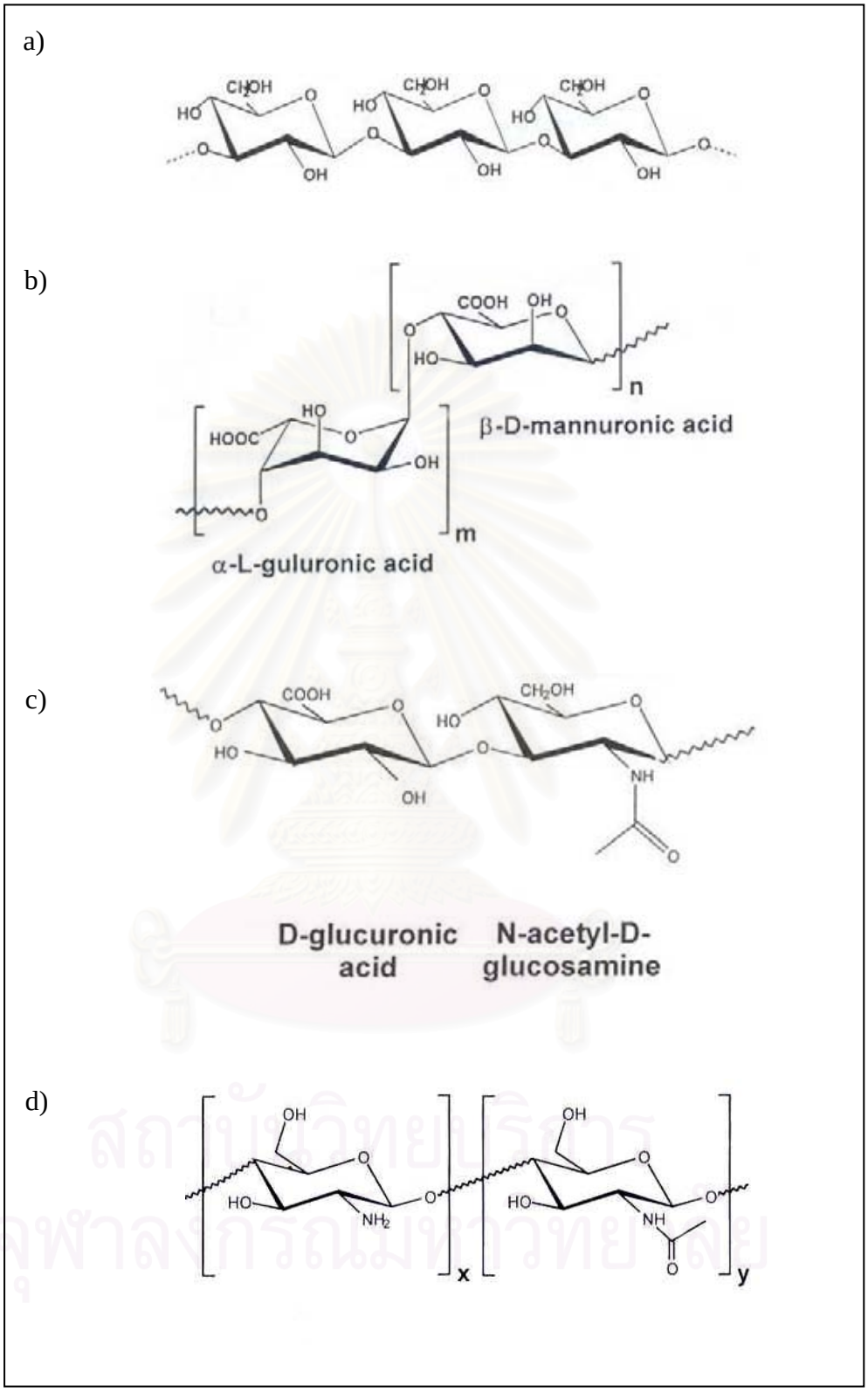

Figure 1. Structure of polysaccharide being used as wound management.

a = dextran, b = alginate, c = hyaluronate and d = chitosan

In Thailand, durian (*Durio zibethinus* Linn.) is one of the most favorite fruit. Wastes of durian fruit-hulls become a big burden to scavenge. Using durian fruit-hulls waste as a source of plant material for isolation of polysaccharide has been recently studied. The polysaccharide gel (PG) was first isolated from fruit-hulls of durian by Pongsamart and Panmaung in 1998. PG is a water soluble polysaccharide, composes of long chain polygalacturonan with branch chain neutral sugars such as galactose, glucose, rhamnose, fructose and arabinose (Hokputsa, *et al*., 2004 and Girddit *et al*., 2001). Durian polysaccharide gel seems like other natural polysaccharide from plants that can be widely use in preparation of food and pharmaceutical products such as jelly, tablet, suspension and emulsion (Pongsamart, S., and Panmaung, T. 1998 and Umprayn, *et al*., 1990). Lertchaiporn, Vayamhasuwan and Pongsamart (2002) have successfully formulated vitamin E gel and lotion using PG as a surfactant. Toxicity test of polysaccharide gel have been reported, a high oral dose (2g/kg) did not induce severe toxicity in male mice and rats (Pongsamart, Sukrong and Tawatsin, 2001). No toxic effects have been observed in subacute treatment in male mice (Pogsamart, Jesadanont and Markman, 1989) and subchronic toxicity test of durian polysaccharide gel in male and female mice has not found to induce toxic effect (Pongsamart, Tawatsin and Sukrong, 2002).

Biological properties of PG has been investigated, PG showed antibacterial activity against both gram positive and gram negative bacteria such as *Staphylococcus aureus*, *Escherichia coli, Staphylococcus epidermidis*, *Bacillus subtilis, Micrococus luteus*, *Lactobacillus pentosus* and *Proteus vulgaris* (Lipipan, *et al*., 2002 and Nantawanit, 2001). Furthermore, PG also has film-forming property that can be used to prepare a film dressing for pharmaceutical purpose. Satisfactory film dressing product has also been prepared (Girddit *et al*., 2001). The recent study of wound healing efficacy of PG dressing film was evaluated by treatment of open wound with PG products in pig skin *in vivo* (Nakchat, 2002). The results demonstrated that PG film dressing preparation has shown wound closure acceleration, smaller wound area obtained after 12 days treatment with PG film dressing when compared to the control (applying 1% povidone iodine), groups of treatment and control wounds have shown completely wound closure on days 18 and 21, respectively. In histopathologically study of tissue reaction in pig skin, the wound treated with PG dressing has shown

mild score of chronic inflammatory cells aggregated in dermal layer, mild fibroblasts and no remarkable lesion to mild dermal granuloma formation.

According to the fascinating results of the previous study, PG showed the noteworthy property for medical use. Therefore, the objectives of this study were to prepare PG dressing and evaluate the effect of PG dressing preparations on wound healing compare with conventional method and commercial moist dressing by using dog skin (*in vivo*) as a model because it is known for evaluating small animal wound healing. Moreover, this study aim to develop the wound dressing for animal wound management.

2. Literature reviews

2.1 The Skin and wound management

2.1.1 The structure of the skin (Wynsberghe, *et al*., 1995)

The skin is the largest organ of the body in surface area and weight. Structurally, the skin consists of two principal layers. The superficial, thinner portion which is composed of epithelial tissue, is the epidermis. The deeper, thicker connective tissue part, is the dermis. Deep to the dermis is the subcutaneous layer and also called the hypodermis (Figure 2a)

(1). The epidermis

The outermost layer of skin is the epidermis. It is avascular, having no blood supply. The multi-layered epidermis is the body's front line of defense, guarding against both mechanical threats (cuts, abrasions, temperature extremes) and biological invasions (bacteria, fungi, viruses). The epidermis forms the external surface of the skin and is mainly composed of keratinocytes which differentiate to form 5 layers;

- Stratum Basale. The deepest layer of the epidermis composed of a single row of cuboidal or columnar keratinocytes. It undergoes

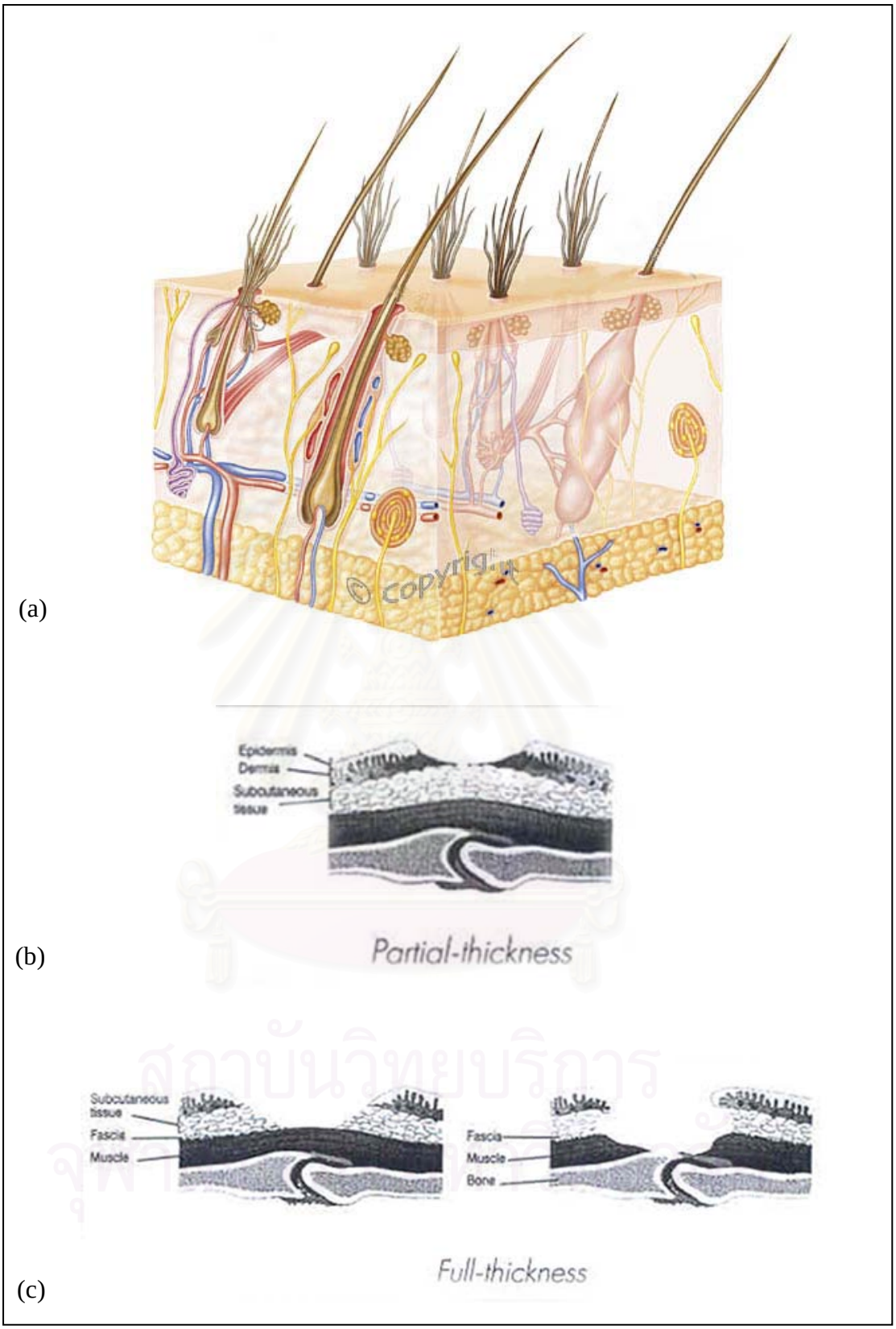

Figure 2. The structure of skin composed of 2 layers; epidermis, dermis (Wynsberghe, *et al*., 1995) (a), partial thickness wound (b), full-thickness wound (c).

continuous cell division to produce new cell to replace those being shed in the exposed superficial layer. It also contains melanocytes, cells that produce malanin (a pigment that helps protect from UV radiation).

- Stratum Spinosum. It is the superficial to the stratum basale, where 8-10 layers of keratinocytes fit closely together by spiny projections. These cells have limited capacity for mitosis.

- Stratum Granulosum. This layer consists of three to five laters of flattened keratinocytes that are undergoing apoptosis. Cells begin to die due to their accumulation of eleidin and their increasing distance from the dermal blood supply.

- Stratum Lucidum. It consists of three to five layers of clear, flat, dead keratinocytes that contain a protein called eleidin (a keratin precursor). The stratum lucidum is present only in the palms and soles, acting as a protective shield against the ultraviolet ray of the sun.

- Stratum Corneum. The upper layer consists of 25-30 layers of dead, flat keratinized cells (keratin). The keratin is a protein with waterproofing properties, preventing water loss from the deep tissue. Keratinized cells are dead, and so these cells are continuously shed and replaced by the division of deeper cells from the deeper strata.

(2). The dermis

Underlying the epidermis, the dermis, is the thickest layer of the skin. It contains mainly of connective tissue containing collagen and elastic fibers. The cell types present in the dermis include fibroblasts, some prowling white blood cells (macrophage) and the occasional fat cell. The dermis is rich in sensory nerve fibers, highly blood vessels and lymphatic vessels. The hair follicles and glands are also embedded in dermal tissue. Based on its tissue structure, the dermis can be divided into both a superficial papillary region and a deeper reticular region. The papillary layer of the dermis consists of loose connective tissue with fine bundles of elastic fibers. The reticular layer is made up of dense connective tissue containing bundles of collagen and some coarse elastic fibers that crisscross to from a strong and elastic network.

The hypodermis (subcutaneous) is beneath the dermis. This layer consists of loose, fibrous, connective tissue and adipose tissues. It serves as a storage depot for fat and contains large blood vessels that supply the skin. This region also contains nerve ending, the coiled ducts or sudoriferous (sweat) glands and the base of hair follicles.

2.1.2 Function of Skin (Wynsberghe, *et al*., 1995)

The skin helps to regulate body temperature by excreted sweat through the pores of sweat glands, serves as a water-repellent and protective barrier between the external environment and internal tissues, excretes small amounts of waste materials such as urea through the skin by perspiration; contains sensory nerve ending that respond to heat, cold, touch, pressing and pain and excretes a small amount of salts and several organic help to synthesize the active form of vitamin D.

2.1.3 Type of Skin Wounds

(1). Partial-thickness wound

Partial-thickness wound is loss of skin involving epidermis and/or dermis (Figure 2b). This superficial ulcer presents clinically as an abrasion, blister or shallow crater.

(2). Full-thickness wound

Full thickness wound is skin loss involving damage or necrosis of subcutaneous tissue that may extend down to, but not through, underlying fascia (Figure 2c).

2.1.4 Inflammation (Kumar, *et al*., 1997)

(1). Acute inflammation

The acute inflammation is referring to immediate and early response to tissue injury (physical, chemical, microbiologic, *etc.*). The acute inflammatory response to an injurious influence of brief duration is characterized principally by vascular and exudative change. The inflammatory responses consist of changes in blood flow, increased permeability of blood vessels (vasodilation) and escape of cells from the blood into the tissues (vascular leakage and edema and leukocyte emigration (mostly PMNs)). Acute inflammation is short-lasting, lasting only a few days.

(2). Chronic inflammation

The chronic inflammation is an inflammatory response of prolonged duration. Principal cells of chronic inflammation are lymphocytes, macrophages, plasma cells (mononuclear cell). Tissue destruction caused both by the causative agent and inflammatory cells. There are two types of chronic inflammation; nongranulomatous and granulomatous. The granulomata is clusters of T cell-activated macrophages, which engulf and surround indigestible foreign bodies.

2.1.5 Patterns of Inflammation (Kumar, *et al*., 1997)

The severity of reaction, its specific cause, and particular tissue and site involved all introduce morphologic variations in basic patterns of acute and chronic inflammation.

(1). Serous inflammation

Serous inflammation is marked by the outpouring of a thin fluid that, depending on the size of injury, is derived from the blood serum. The serous inflammation is mild injury, with epithelial destruction. The skin blister resulting from a burn or viral infection represents a large accumulation of serous fluid.

(2). Fibrinous inflammation

With more severe injuries and the resulting greater vascular permeability, larger molecules such as fibrin pass the vascular barrier. A fibrinous exudate develops when the vascular leaks are large enough to permit the passage of fluid rich in plasma proteins, containing fibrinogen molecules. A fibrinous exudate is characteristic of inflammation in body cavities, such as the pericardium and pleura.

(3). Suppurative or purulent inflammation

More severe inflammatory responses, particularly those caused by microbiologic agents are characterized by the production of large amounts of purulent exudates consisting of neutrophils, necrotic cells, and edema fluid. A common example of an acute suppurative inflammation is acute appendicitis. Abscesses are focal localized collections of purulent inflammatory tissue caused by suppuration buried in a tissue. Abscesses have a central region that appears as a mass of necrotic white cells and tissue cells.

(4). Ulcers

An ulcer is a local defect, or excavation, of the surface of an organ or tissue that is produced by the shedding of inflammatory necrotic tissue. Ulceration can occur only when an inflammatory necrotic area exists on or near the surface, such as the mucosa of the mouth, stomach, intestines. During the acute stage, there is intense polymorphonuclear infiltration and vascular dilatation in the margins of the defect. With chronicity, the margins and base of the ulcer develop fibroblastic proliferation, scarring, and the accumulation of lymphocytes, macrophages, and plasma cells.

2.1.6 Skin Wound Healing (Bertone, A. L. 1989.)

Two kinds of wound healing processes can occur, depending on the depth of the injury. Epidermal wound healing occurs following wounds that affect only the epidermis; deep wound healing occurs following wounds that penetrate the dermis and/or subcutaneous layer.

The wound healing process occurs in three overlapping phases: an inflammatory phase, a proliferative phase and a remodeling or maturation phase (Figure 3).

(1). Inflammatory phase

Injury disrupts the tissue and causes haemorrhage from damaged vessels and lymphatics. During the inflammatory phase, a blood clot forms gives rise to a fibrin plug to arrest blood loss and provide a protective covering in the wound. Coagulation and activation of complements (kinins and prostaglandins) produce biologically active compounds that cause local vasodilation, increase permeability of blood vessels and promote cell migration into the wound. Polymorphonuclear leucocytes (PMN) and neutrophil are the first blood leucocytes to enter the wound site. They initially appear in the wound shortly after injury and subsequently their numbers increase steadily, peaking at 24-48 hrs. Their main function appears to be phagocytosis of debris, foreign material and microorganisms. The next cellular, immune elements to enter the wound are macrophages. They have a much longer life span than the PMN and persist in the wound until healing is complete. Macrophages just like neutrophils phagocytose and digest pathological organisms and tissue debris. In addition, they also release a variety of chemotactic (cytokines) and growth factors such as fibroblast growth factor (FGF), epidermal growth factor (EGF), transforming growth factor beta (TGF-β) and interleukin-1 (IL-1) which are necessary for the initiation and propagation of granulation tissue formation. This phase occurs in 0-5 days after injury.

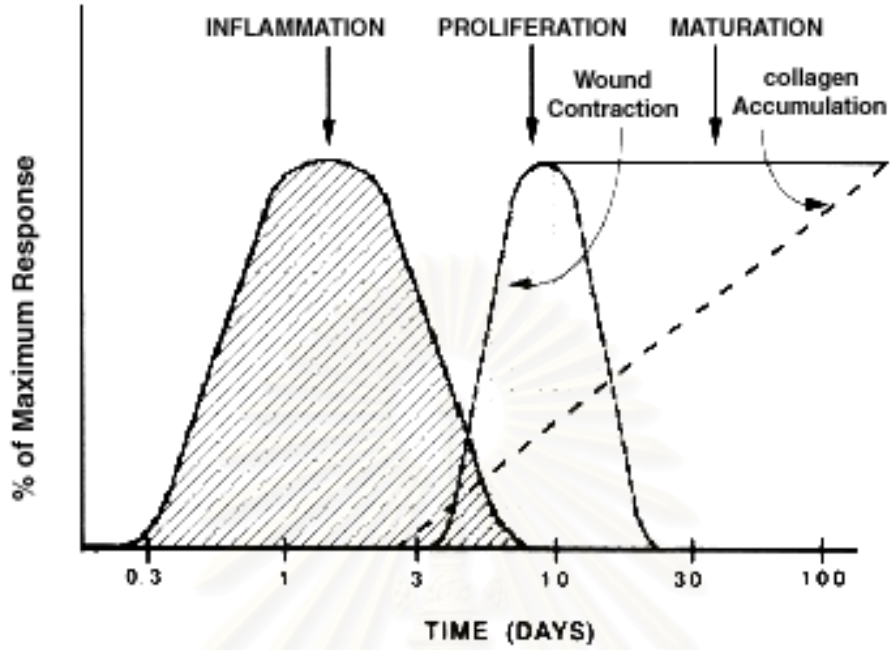

Figure 3. Three overlapping phases of wound repair: inflammation , proliferation and maturation (Regan and Barbul, 2000).

(2). Proliferative phase

After the wound has been successfully cleared of devitalized and unwanted material, it gives way to the proliferative phase of healing. The clot becomes a scab, and epithelial cells migrate beneath the scab to bridge the wound. The proliferative phase is characterized by the formation of granulation tissue in the wound. It is characterized clinically by the presence of red tissue in the wound base. Granulation tissue consists of a combination of cellular elements, including fibroblasts and inflammatory cells, along with new capillaries embedded in a loose extra cellular matrix of collagen, fibronectin and hyaluronic acid. Fibroblasts are the primary synthetic element in the repair process and are responsible for production of the majority of structural proteins used during tissue reconstruction. In particular, fibroblasts produce large amounts of extracellular matrix including collagen, fibronectin, hyaluronic acid and the glycosaminoglycans to repair the dermis. The process of fibroblast proliferation and synthetic activity is known as fibroplasias. Revascularization of the wound proceeds in parallel with fibroplasia. Capillary buds sprout from blood vessels adjacent to the wound and extend into the wound space. On the second day post-injury, endothelial cells from the side of the venule closest to the wound begin to migrate in response to angiogenic stimuli. New sprouts then extend from these loops to form a capillary plexus. The potential cytokine mediators of neovascularization are basic fibroblast growth factor (bFGF), acidic FGF (aFGF), transforming growth factors-α and β (TGF-α and -β) and epidermal growth factor (EGF)**.** Re-epithelialization of the wound begins within a couple of hours of the injury. Epithelial cells, arising from either the wound margins or residual dermal epithelial appendages within the wound bed, begin to migrate under the scab and over the underlying viable connective tissue. The cytokines stimulating the re-epithelization are EGF, TGF-β, bFGF, platelet-derived growth factor (PDGF) and insulinlike growth factor-λ (IGF-λ). The process occurs in 3-14 days after injury.

(3). Remodeling or maturation phase

The reorganization of the extracellular matrix is begun. Collagen fibers become more organized, fibroblasts decrease in number, and blood

vessels are stored to normal. Initially, the extracellular matrix is rich in fibronectin, which forms a provisional fiber network. This serves as a template for collagen deposition by fibroblasts. The initially randomly distributed collagen fibers become cross-linked and aggregated which gradually provide the healing tissue with increasing stiffness and tensile strength. Collagen remodeling during scar formation is dependent on both continued collagen synthesis and collagen catabolism. The degradation of wound collagen is controlled by a variety of collagenase enzymes. Grossly this can be observed as a reduction in erythema associated with the earlier scar and some reduction in the scar volume, resulting in a pale thin scar. The summary of three phases of wound repair is shown in Figure 4.

The final product of the healing process is a scar. This relatively avascular and acellular mass of collagen serves to restore tissue continuity, strength and function. Scar tissue differs from normal skin in that its collagen fibers are more densely arranged and also has fewer blood vessels. Delays in the healing process cause the prolonged presence of wounds, while abnormalities of the healing process may lead to abnormal scar formation. If a scar remains within the boundaries of the original wounds, it is a hypertrophic scar; if it extends beyond the boundaries of the original wound into normal surrounding tissues, it is a keloid scar. This phase continues for up to 1 year or even longer.

2.1.7 Types of wound healing (Kumar, *et al.*, 1997)

(1). Healing by first intention

The least complicated example of wound repair is the healing of a clean, uninfected surgical incision approximated by surgical sutures. Such healing is referred to as primary union or healing by first intention. The incision causes death of a limited number of epithelial cells and connective tissue as well as disruption of epithelial basement membrane continuity. The narrow incisional space immediately fills with clotted blood containing fibrin and blood cells; dehydration of the surface clot forms the well-known scab that covers the wound.

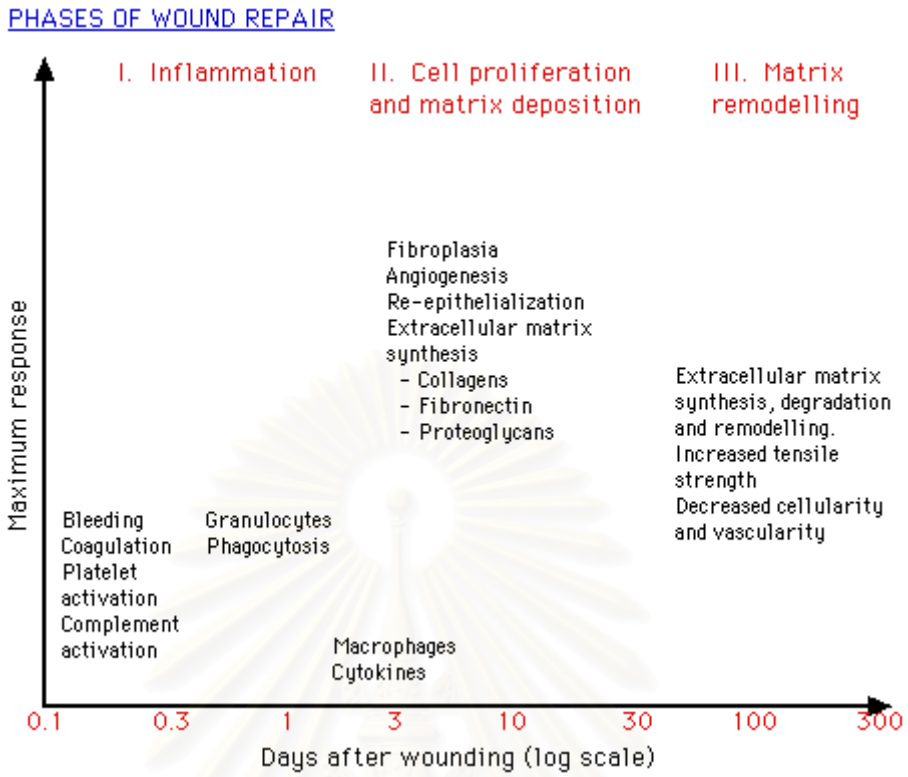

Figure 4. The summary of three phases of wound repair. The figure available from http://shef.ac.uk/uni/projects/mc/plastic2.html

Within 24 hours, neutrophil appears at the margins of the incision, moving toward the fibrin clot. The epidermis at its cut edges thickens as a result of mitotic activity of basal cells, and within 24-48 hours spurs of epithelial cells from the edges both migrates and grows along the cut margins of the dermis, depositing basement membrane components as they move. They fuse in the midline beneath the surface scab, thus producing a continuous but thin epithelial layer.

By day 3, the neutrophils have been largely replaced by macrophages. Granulation progressively invades the incision space. Collagen fibers are now present in the margins of the incision, but at first these are vertically oriented and do not bridge the incision. Epithelial cell proliferation continues, thickening the epithelial covering layer.

By day 5, the incisional space is filled with granulation tissue. Neovascularization is maximal. Collagen fibrils become more abundant and begin to bridge the incision. The epidermis recovers its normal thickness, and differentiation of surface cells yields a mature epidermal architecture with surface keratinization.

During the second week, there is continued accumulation of collagen and proliferation of fibroblasts. The leukocytic infiltrate, edema, and increased vascularity have largely disappeared. At this time, the long process of blanching begins, accomplished by the increased accumulation of collagen within the incisional scar, accompanied by regression of vascular channels.

By the end of the first month, the scar comprises a cellular connective tissue devoid of inflammatory infiltrate, covered now by intact epidermis. The dermal appendages that have been destroyed in the line of the incision are permanently lost. Tensile strength of the wound increases thereafter, but it may take months for the wounded area to obtain its maximal strength.

(2). Healing by second intention

When there is more extensive loss of cells and tissues such as large defects of surface wounds, the reparative process is more complicated.

Abundant granulation tissue grows in from the margin to complete the repair. This form of healing is referred to as secondary union or healing by second intention.

Secondary healing differs from primary healing in several respects:

a. Large tissue defects initially have more fibrin and more necrotic debris and exudates that mist be removed. Consequently, the inflammatory reaction is more intense.

b. Much larger amounts of granulation tissue are formed. When a large defect occurs in deeper tissues, granulation tissue with its numerous scavenger white cells bears the full responsibility for its closure.

c. Perhaps the feature that most clearly differentiates primary from secondary healing is the phenomenon of wound contraction, which occurs in large surface wounds. Contraction has been ascribed, at least in part, to the presence of myofibroblasts which altered fibroblasts that have the ultrastuctural characteristics of smooth muscle cells.

2.1.8 Principle of Moist Wound Healing (Field, and Kerstein, 1994)

Since Winter reported in 1962 that wound is more healed faster and with better structure in healed tissue under moist wound environment by dressed with occlusive dressing material (polythene film) than those dry wound environment by left exposed to the air. In his experiment Winter described that when the wound kept moist under a polythene film, epidermis migrate and mitosis through the serous exudate on the wound surface above the fibrous tissue of the dermis. In contrast to the normal dry wound environment, epidermal migrates below the dehydrated fibrous tissue where there is sufficient moisture for the cells to live as illustrated in Figure 5. The concept of moist wound treatment supported by the results reported by Winter and Scales in 1963. They demonstrated that wound dressed with occlusive dressing showed twice the rate of wound re-epithelization compared with wounds exposed to

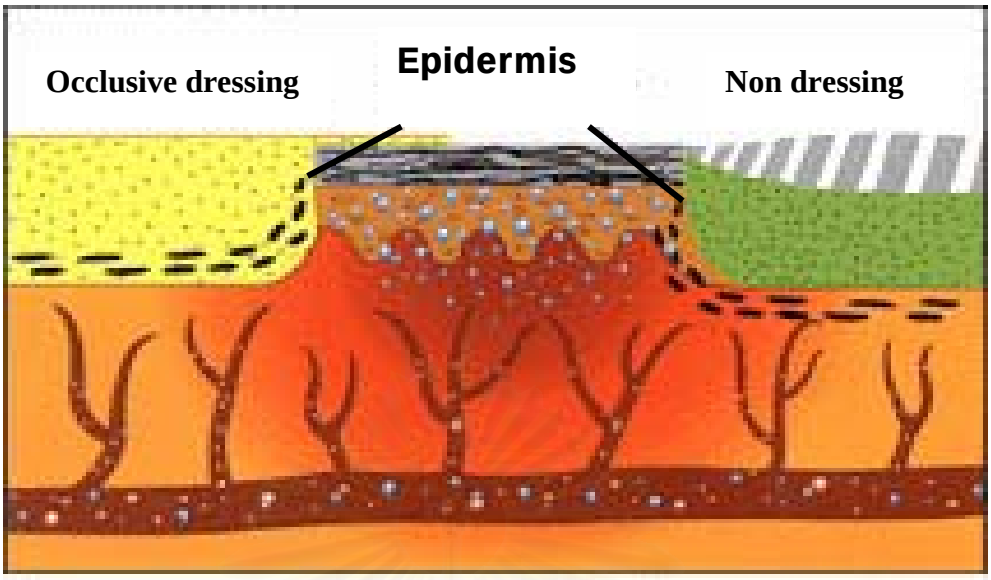

Figure 5. Winter's moist wound healing model. The wound surface moist under dressed with occlusive dressing showed migration of epithelial cells over granulation tissue. In contrast, non dressing provides dry environment, epithelial cells migrate beneath wound surface which moist than those surface that lead prolong healing. The figure available from http:// www.biopol.co.kr/ensub/skin_1.gif.

the air in pig skin. It is the same results reported by Hinman, Maibach, and Winter (1963), they demonstrated in human skin. Leipziger *et al.* (1985) reported that wound dressed with polymer dressing accelerated collagen synthesis than air expose wound in pig skin. The effects on dermal repair of moist condition and dry condition were compared by Dyson *et al.* (1988). From the results, the moist condition dressed by polyurethane dressing more rapid increase fibroblasts and endothelial cells, and more repair than that dry condition dressed (gauze dressing). These results correlated with the results of Vogt *et al*. (1995) and Ueno *et al*. (1999). From the reasons that wound rapid healed in moist condition, the wound dressing was developed from the materials that can retain moisture on wound and promote wound healing.

2.2 Properties of Wound Dressing (Biopol, 2002)

No single dressing is appropriate for all wound types and all stage of healing. The characteristics of the ideal wound dressing and a short summary of the main issues are included below:

- Promote healing
- Protect the wound
- Act as a barrier to viruses and bacteria
- Maintain a moist environment
- Allow for gaseous exchange
- Afford pain relief and be comfortable to wear
- Provide thermal insulation
- Not introduce toxins, foreign particles or fibers into wound
- Not shed fibers into the wound
- Not adhere to the wound
- Absorbent
- Haemostatic
- Debriding
- Nontoxic
- Hypoallergenic
- Be produced in a sterile form
- Easy to use
- Inexpensive

2.3 Types of wound dressing (Lawrence, 1994)

In recent years, the methods for treating wounds have considerable improved, not least because of the development of novel wound dressings. A modern wound treatment strives to keep out not only external novae from tie wound, but to support it as optimally as possible in its needs during the individual phase of healing, and to stimulate the cellular processes during each stage of the healing process. This particularly requires the phase-specific application of wound dressings with different physical characteristics (Table 1).

Lawrence (1994) classified the wound dressing into 2 main groups, such as traditional wound dressing and modern wound dressing.

2.3.1 Traditional Wound Dressing

Traditional wound dressing, such as gauze, lint and cotton dressing. Gauze dressing is a dry wound dressing which provide dried wound environment. Gauze dressing can inhibit wound contraction because these dressings are not highly absorptive. They may require frequent dressing changes and have break-through drainage. These dressings can also adhere to the wound and traumatize healthy tissue upon removal. However, gauze is still the most widely used in the wound care dressing because it is cost-effective and readily available.

2.3.2 Modern Wound Dressing

Since Winter reported in 1962 that wound healing is more efficient under moist wound environment, extensive investigations have been undertaken. Currently, wound dressing method which makes moist wound environment is rapidly replacing conventional gauze dressing method which makes dried wound environment. The modern wound dressing was developed for maintain moist environment and accelerates wound healing. Moist wound dressings include foams,

alginates, hydrocolloids, hydrogels and transparent films. The characteristic and uses of modern wound dressing are summarized in Table 1.

2.4 Mechanical Property of dressing Films (Aulton and Abdul-Razzak, 1981)

Tensile testing

The tensile test gives an indication not only of the elasticity and strength, but also of the toughness of the film. The tensile testing process is to apply increasing tensile load at a constant rate to a film strip which know dimensions in the dimension perpendicular to the cross-section of the film strip until the failure takes place. The load at film failure will be measured in term of force per unit cross-section area of the film. The method of preparing tested film is cast film method. Cast film method gives a more perfect specimen, uniform thickness and free from bubbles and defects. Cast films are reproducible because environmental factors affect the film preparation less than with sprayed films. Casting is therefore a better means of obtaining accurate data on the fundamental properties of the polymer and polymer formulation (Aulton, 1982). An ideal film – dressing patch with respect to retaining its physical continuity should be soft and tough without being brittle. Polymers are divided into five categories according to a qualitative description of their mechanical behavior and corresponding stress – strain characteristics as showed in Table 2 and Figure 6.

Hard or stiff polymers are characterized by high moduli as opposed to soft ones. Strong (as opposed to weak) polymers have high tensile strengths. Tough (as opposed to brittle) polymers have large area under their stress-strain curves and require large amounts of energy to break under stress, combining high or at least modulate tensile strength with high elongation. The desirable hard, tough film must have a high yield stress large extension before breaking and high elastic modulus.

Table 1. Characteristics and Uses of Wound Dressing Materials.

Category	Description	Applications	Examples
Alginate	This seaweed extract contains guluronic and mannuronic acids, which provide tensile strength, and calcium and sodium alginates, which confer absorptive capacity. Some of these can leave fibers in the wound if not thoroughly irrigated. Dressings are secured with secondary coverage.	Highly absorbent Useful for wounds with copious exudate (Alginate rope is particularly useful for packing exudative wound cavities or sinus tracts.)	AlgiSite Comfeel CURASORB KALTOGEL KALTOSTAT Sorbsan Tegagel
Debriding agents	This variety of products provides some degree of chemical or enzymatic debridement.	Useful for necrotic wounds as an adjunct to surgical debridement	Hypergel (hypertonic saline gel) Santyl (collagenase) Accuzyme (papain urea)
Foam	Polyurethane foam has some absorptive capacity.	Useful for clean granulating wounds with minimal exudate	LYOFOAM Spyrosorb Allevyn
Hydrocolloid	This is a microgranular suspension of natural or synthetic polymers such as gelatin or pectin in an adhesive matrix. The granules transform from a semihydrated state to a gel as wound exudate is absorbed.	Useful for dry necrotic wounds or those with minimal exudate Also useful for clean granulating wounds	AQUACEL CombiDERM Comfeel DuoDerm CGF extra thin Granuflex Tegasorb
Hydrogel	These are water- or glycerin-based semipermeable hydrophilic polymers; cooling properties may decrease wound pain. These gels can lose or absorb water depending on the state of hydration of the wound. Dressings are secured with a secondary covering.	Useful for dry, sloughy, necrotic wounds (eschar)	Aquasorb DuoDerm IntraSite gel Granugel Normlgel Nu-Gel Purilon gel K-Y jelly

Table 1. Characteristics and Uses of Wound Dressing Materials (cont.).

Category	Description	Applications	Examples
Low-adherence dressing	This is a variety of materials designed to remove easily without damaging underlying skin.	Useful for acute minor wounds such as skin tears or as a final dressing for chronic wounds that have nearly healed	Mepore Skintact Release
Transparent film	These are highly conformable acrylic adhesive films with no absorptive capacity and little hydrating ability. Dressings may be vapor permeable or perforated.	Useful for clean dry wounds with minimal exudate Also used to secure an underlying absorptive material Protection of high-friction areas and areas that are difficult to bandage, such as heels Also used to secure intravenous catheters	OpSite Skintact Release Tegaderm Bioclusive

Table 2. Qualitative description of polymer and it's stress-strain characteristics

Polymer Description	**Characteristics of stress-strain curve**			
	Young's Modulus (MPa)	Yield Stress	Tensile Strength (MPa)	Elongation to Break (%)
Soft, weak	Low	Low	Low	Low to modulate
Soft, tough	Low	Low	Moderate	Very high (20-100%)
Hard, brittle	High	None (break around yield point)	Moderate to high	Very low (<2%)
Hard, strong	High	High	High	Moderate (~5%)
Hard, tough	High	High	High	High

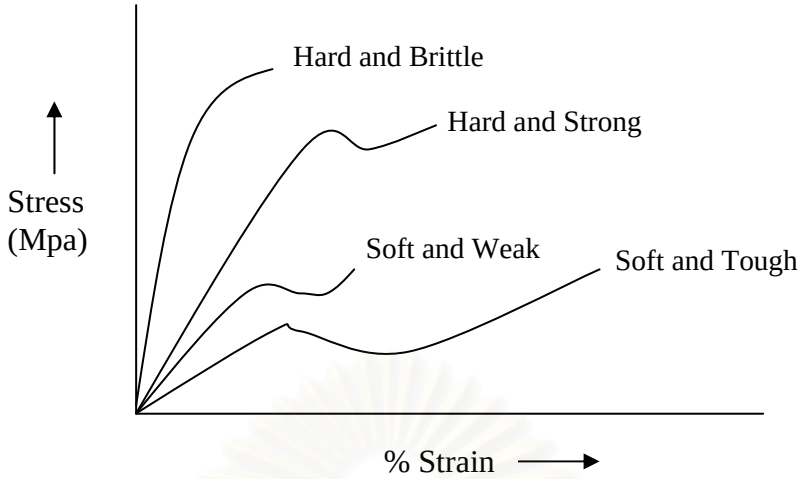

Figure 6. Characteristic of polymer properties in stress-strain curves

A typical stress-strain curve is shown in Figure 6. Along the linear portion has relation between stress and strain.

Tensile strength, ultimate strength or breaking stress is the maximum stress applied to a point at which the film specimen breaks. The determination of tensile strength alone is not very useful in predicting mechanical performance of the films, however higher values of tensile strength of the films are desirable for abrasion resistance.

$$\text{Tensile strength} = \frac{\text{load at failure}}{\text{film thickness} \times \text{film width}}$$

Strain or elongation is a measure of the ductility of the film. It is calculated by dividing the increase in length by original length. It can also be expressed as a percentage.

Young`s modulus or Elastic modulus is the most basic and structurally important of all mechanical properties and is a measure of stiffness and rigidity of the film. It is calculated as applied stress divided by the corresponding strain in the region of linear elastic deformation (slope). The greater slope of the curve the higher the elastic modulus. The high value of the elastic modulus indicates the stiffness and the strength of film.

$$\text{Elastic modulus} = \frac{\text{slope}}{\text{film thickness} \times \text{film width} \times \text{cross-head speed}}$$

Work of Failure is a function of work done in breaking the film specimen and is representative of the film toughness. It can be calculated from the area under the stress-strain curve.

$$\text{Work of faliure} = \frac{\text{area under curve} \times \text{cross-head speed}}{\text{film thickness} \times \text{film width}}$$

CHAPTER II

MATERIALS AND METHODS

Materials :

Chemicals

- Amoxicillin (Duphamox® LA) was purchased from Duphar B. V. Weesp, Holland.
- Azaperone (Stresnil®) was purchased from Janssen Pharmaceutica, Belgium.
- Buprenorphine (Temgesic®) was purchased from Reckitt & Colman Product Ltd., Britain.
- Hematoxylin crystal from Fluka, Switzerland.
- Paraffin (Paraplast®) was purchased from Sherwood medical Co., U.S.A.
- Propylene glycol of analytical reagent grade was purchased from Sigma chemical Co., Mo., U.S.A.
- Povidone iodine (Betadine®solution) was purchased from Mundipharma B.V., Netherlands.
- Sodium hexametaphosphate, citric acid, potassium phosphate monobasic, sodium phosphate diabasic (anhydrous) all of analytical reagent grade from CARLO. ERBA., Germany.
- Sodium hydrogen carbonate, absolute ethanol, hydrochloric acid, kieselguhr, calcium chloride dihydrate cryst, aluminium potassium sulfate dodecahydrate, mercuric oxide (red), and eosin Y, all of analytical reagent grade from Merck, Germany.
- Xylene was analytical reagent grade from BDH, England.
- Opsite®flexigrid from Smith & Nephew Limited.
- Hypafix® from BSN.

Equipments

- Analytical balance (Mettler Toledo, PL602-5)
- Micrometer (Thickness Gauge 0-10 mm., Code No. 17389 Inspector 3)
- pH meter (MP 230, Mettler Toledo, LE413, ME 51340 251, Switzerland)
- pH paper (pH 0-14) was obtained from Merck, Germany.
- Viscometer (Brookfield, Model LVDV-I+, Brookfield Engineering Laboratories INC., USA)
- Magnetic stirrer (Model SP 46920-26, Barnstead/Hermodyne, USA)
- Tensile tester (Universal TM, Tinius Olsen, H5KS159)
- Hot air oven (Mammert, Becthai Co.,Ltd., Thailand)
- Suction apparatus (Buchner Funnel, Aspirator, SIBATA circulating aspirator WJ-20, Japan)
- Rotary evaporator (Buchi R-200, Switzerland)
- Moisture analyzer (Presica, XM60, Becthai Co.,Ltd., Thailand)
- Freeze-dryer (LyoLab w/PC)
- Vaccuum oven (Mammert, Becthai Co.,Ltd., Thailand)
- Digital camera (Cannon Power shot S50)
- Filter paper (Whatman..No. 93, Whatman international Ltd., England)
- Vertical laminar air flow carbinet (LFV-60, MSSP, Thailand)
- Light microscope (Olympus, Japan)
- Microtome (Leica, model 820,Germany)

Methods

1. Preparation of Dried Fruit-Hulls of Durian

Fresh durian fruit-hulls waste were collected during the durian season, cleaned and ground. One kilogram of ground fresh fruit-hulls was dried by hot air oven at 50 °C until constant weight, about 200 grams weight of dried fruit-hulls were obtained. Dried fruit-hulls were kept in refrigerator (4°C) until used.

2. Isolation of Polysaccharide Gel (PG) from Dried Fruit - Hulls of Durian

Polysaccharide gel was isolated by using hot acidic water and followed by acid-ethanol precipitation. The procedure was followed the method modified by Pongsamart and Panmaung (1998).

3. Preparation of PG Aqueous Solution for PG Dressing Preparations

PG solution at 2% w/v concentration in deionized water was prepared and the viscosity and pH were measured by using viscosmeter and pH meter, respectively. Not less than 200 cps of viscosity and about 2.3 ± 0.05 of pH of PG aqueous solution were used in a casting solution.

4. Preparation of PG Dressing Films

The PG dressing films were prepared by a casting/solvent evaporating technique (Gerddit, W. 2002). The composition of casting solution composed of 2% polysaccharide gel (PG) and 15% w/w propylene glycol based on PG as a plasticizer. The casting mixture was prepared by dissolving 2 g of PG powder in 80 ml deionized water and added 0.3 g propylene glycol, deionized water was added to make 100 ml (Gerddit *et al.,* 2001). The solution was stirred until homogenous and degased by using sonicator, left to stand until trapped air bubbles were removed. The 90 ml of casting mixture was carefully poured on a casting glass plate. (casting area = 331.24 cm^2, solid content of PG was 5.4 mg/cm^2) The casting mixture was dried in

hot air oven at 45°C for 12 hrs. The dried film was peeled off from the casting glass plate and then cut into pieces of 3x3 cm^2, sealed in a plastic bag, and stored in a desiccator. The PG dressing film was sterilized under UV light, 254 nm of wavelength in the laminar air flow for 1 hr before use.

5. Preparation of PG Fiber Dressing Patches

The PG fiber dressing patches were prepared by using a freeze-dry method. The various formulation of PG fiber dressing patch composed of 0.6, 0.8, 1 and 1.5% PG. Each concentration of PG solution was prepared by dissolving 0.6, 0.8, 1 and 1.5 g, respectively of PG powder in deionized water 100 ml. The PG solutions were stirred until homogeneously mixed**.** The 90 ml of each PG solution was carefully poured onto a stainless steel tray. (tray area = 219.04 cm^2) The PG solutions were dried in a freeze-dryer. The freeze-drying process was shown in Table 3. The dried fiber dressing patches were peeled off from the tray and then cut into pieces of 3x3 cm^2 and sealed in a plastic bag and stored in a desiccator. The PG fiber dressing patches were sterilized under UV light, 254 nm of wavelength in the laminar air flow for 1 hr before use.

6. Evaluation of PG Dressing Films

6.1 Appearance and Physical Properties of PG Dressing Films

6.1.1 Appearance of PG dressing films

An appearance of PG dressing film preparations were visually evaluated including color, transparency, flexibility and ease of detachment from glass plates.

Table 3. The process of freeze-drying the PG fiber dressing patch

Process	Temperature (°C)	Time (mins)	Vacuum (mT)
Freeze	0	0	600
	-5	0	600
	-10	0	600
	-15	0	600
	-20	0	600
Extra freeze	-20	60	600
Primary drying	-20	0	600
	-20	120	600
	-10	0	600
	-10	120	600
	0	0	600
	0	120	600
	10	0	600
	10	120	600
	20	0	600
	20	120	600
	30	0	600
	30	60	600
Secondary drying	20	60	600

Where “0” in the TIME field is warm the shelves as fast as possible during any step.

6.1.2 The Thickness of PG Dressing Films

The thickness of each PG dressing film was measured using a micrometer at five locations (center and four corners), and the mean value of thickness was calculated.

6.1.3 The Mechanical Properties of PG Dressing Films

The mechanical properties of PG dressing films were determined by the method described by Peh, and Wong, 1999 with slight modification and evaluated by using a Tensile tester (Tinius Olsen, H5KS159) equipped with 10 N load cell. The PG dressing film samples were cut into small strips (2x20 mm). The films were free from air bubbles or physical imperfections. The film strip was carefully clamped by upper and lower clips at a distance 5 mm. During measurement, the film was pulled by top clamp at rate 3 mm/min until the film was completely ruptured. The force and elongation were measured at the film breaking force. The values of tensile strength, Young's modulus, % elongation and work of failure were examined. The mean values and standard deviation of five measurements were calculated.

6.1.4 Swelling Property of PG Dressing Films

The determination of the swelling property of PG dressing film was operated by the following procedure. The PG dressing film was cut into size 2×2 cm^2, each film sample was weighed and placed in a preweighed stainless steel wire mesh with sieve opening of approximately 60 μm. The mesh containing the film sample was then submerged into 30 ml of deionized water in a petri-dish. The weight of PG dressing films were measured every 1 hr until the constant weights were recorded. The averages of five measurements were plotted vs time (hours). The degree of swelling was calculated using parameters

$$\% \text{ of swelling} = \frac{(Wt - Wo)}{Wo} \times 100$$

Where Wt is the weight of film at time t, and Wo is the weight of film at time zero.

6.2 Bioadhesive Strength Measurement

The bioadhesive strength of films was evaluated by using a Tensile tester (Universal TM, Tinius Olsen, H5KS159) (Peh, and Wong, 1999). The dressing film used in this study was a film with backing layer. The backing membrane layer was prepared by dissolving 5% w/v ethylcellulose in absolute ethanol and pouring 20 ml of the ethylcellulose casting solution onto an 8.8 cm diameter glass petri-dish. The backing membrane layers were oven dried for 8 hours at 50°C before 20 ml of the casting mixture of 2% PG solution with 15% propylene glycol was upper layered on ethylcellulose and dried in the oven at 50°C for 10 hours.

The fresh dog skin was used as the model tissue. The dog skin was removed with the depth of the entire skin layer and the surface was used for tissue model like a wound. The dog skin was affixed on the cylindrical perspex support (2 cm diameter; surface area, 3.14 cm^2) and secured with a string. The whole perspex support was then positioned at the bottom of the measuring system and held in place by a clamp. The films were cut in the size 1 × 1 cm^2 and affixed to another perspex support of similar dimension using a double sided tape. The perspex support was then screwed onto the upper probe of the instrument. The two perspex supports were aligned to ensure that the film came into direct contact with the surface of the dog skin when the upper support was lowered. During measurement, 100 µl of normal saline solution was evenly spread on the surface of the tissue. The probe was lowered at a speed of 1.0 mm/sec to contact with the tissue at a force of 1 Newton (N) for a contact time of 30 sec. It was then withdrawn at a speed rate of 1.0 mm/sec to a distance of 10 mm. Measurements were performed in triplicate.

Work of adhesion and force of bioadhesion were used to evaluate the bioadhesive strength of the dressing films. The work of adhesion was calculated from the area under the force-distance curve, and the force of bioadhesion was taken as the maximum force needed for the detachment the film from the tissue.

7. Evaluation of PG Fiber Dressing Patches

7.1 The Thickness of PG Fiber Dressing Patches

The thickness of each PG fiber dressing patch was measured using a micrometer at five locations (center and four corners), and the mean value of thickness was calculated.

7.2 Moisture Content

The moisture content of PG fiber dressing patch was estimated by using on a moisture analyzer (Presica, XM60). Five samples of each dressing patch of approximate weight 1 g were tested. The drying temperature was set at 105°C. The results were expressed as percent moisture content of the various dressing patches.

7.3 Moisture Sorption

The determination of the moisture sorption of the PG fiber dressing patch was operated by following procedure. The fiber dressing patches were cut into size 3x3 cm^2. The initial dry weight (W_o) of fiber dressing patches were measured at room temperature (about 25 °C) after keeping them in the desiccator filled with silica gel for 24 hours or until constant weight. Then they were placed inside a desiccator containing saturated sodium chloride solution in the well (75% RH), at ambient temperature. The hygrometer was used to examination the relative humidity. At appropriate time intervals, the fiber dressing patches were taken out and weighed immediately (W_t). The percentages of moisture sorption of the films were calculated by using the following equation.

$$\% \text{ moisture sorption} = \frac{(W_t - W_o)}{W_o} \times 100$$

Where W_o is the initial weight of the strip, W_t is the weight of the strip at time t. The measurement was made in triplicate.

8. Evaluation of PG dressing preparations in treatment of wound

The protocal of the following study was approved by the Ethics Committee of the Faculty of Pharmaceutical Sciences, Chulalongkorn University.

8.1 Animals

Four adult and healthy female dogs, weighing 12-15 kg were used in this experiment. The dogs were housed individually in their cages. All dogs were acclimatized for at least 7 days before the experiment started.

8.2 Creation of skin open wounds

On the experiment day (day 0), the animals were fasted for 12 hrs before wounding operation. The dogs were anesthetized with propofol (2-4 mg/kg) by intravenous injection after premedication with atropine sulphate (0.04 mg/kg), buphrenorphine (10 μg/mg) and acepromazine malate (0.2 mg/kg) by intramuscular injection. General anesthesia was maintained with endotracheal tube of halothane gas. Each dog was then placed in prone position. The dorsal skin was shaved with electric shaver, scrubbed with 1.5% chlorhexidine digluconate, 70% ethanol and 1% povidone iodine, respectively. A full-thickness circular open wound was created by using a 2 cm diameter steriled template. The wounds were operated with scalpel and removed off 5 mm. depth of the skin layer, the wounds was operated at 2 cm from the dorsal mid-line at left and right side of the dog. A total of eight wounds were created on each dog. All freshly created wounds were washed with normal saline before dressing application.

8.3 Treatment procedures with dressings

All wounds in each dog were randomly treated by 4 treatments (total 8 wounds in each treatment) as described in the following table:

Wounds	Treatment
Control group (C)	Applied 1% povidone iodine on wounds
Treatment group 1 (T_1)	Covered with PG dressing film
Treatment group 2 (T_2)	Covered with PG fiber dressing patch
Treatment group 3 (T_3)	Applied 1%povidone iodine on wounds and covered with commercial dressing film (Opsite®flexigrid)

All wounds were covered with sterile gauzes to hold the dressings in place and further occluded with hypoallergenic adhesive tape (Hypafix®) to protect the wounds and the dressings from the environmental contamination. All dressings were changed every 3 days.

8.4 Examination of wounds

Wounds were opened every 3 days after operating day 0, on day 3, 6, 9, 12, 15, 18 and 21 postoperation, all wounds were opened, old dressings were removed and cleaned with sterile normal saline, examined wounds appearance and taken picture of the wounds by digital camera. The wound margins were traced on sterile transparent film with permanent marker for wound area determination. All wounds were treated by the same treatment as day 0. A new dressing was replaced and covered with sterile gauze.

9. Evaluation of wound healing

9.1 Gross Pathology Evaluation

9.1.1 Wound lesion

Gross lesions of wounds were clinically examined every 3 days postoperation on day 3, 6, 9, 12, 15, 18 and 21. After opened and removed old dressings, the wounds were examined in terms of wound bed, color, exudates, swelling of wound surface and the consistency of surrounding wound tissue.

9.1.2 Wound area

Each transparent film tracing of wound area was scanned and the areas of wound size were calculated using computer program “SCION image software”

9.1.3 Determination of wound healing rate

The degree of wound contraction (expressed as a percentage) was calculated from the following equation:

$$\text{Degree of wound contraction} = \frac{(A_{\text{Day }0} - A_{\text{Day }X})}{A_{\text{Day }0}} \times 100$$

Where X = 3, 6, 9, 12, 15, 18 and 21 after wound creation, A = wound area

9.1.4 Statistical analysis

The results of wound area and the degree of wound contraction were analyzed by using a one-way analysis of variance (ANOVA), the statistically significant difference of mean values between groups was assumed at $p < 0.05$: equal variances assumed using LSD.

9.2 Histopathological Evaluation

On the day of experiment and at day 21, wound tissues were dissected for histopathological examination. The animals were fasted for 12 hrs. The dogs were anesthetized with propofol (2-4 mg/kg) by intravenous injection after premedication with atropine sulphate (0.04 mg/kg), buphrenorphine (10 μg/mg) and acepromazine malate (0.2 mg/kg) by intramuscular injection. General anesthesia was maintained with endotracheal tube of halothane gas. Each dog was then placed in prone position. The skin was scrubbed with 1.5% chlorhexidine digluconate, 70% ethanol and 1% povidone iodine, respectively. The skin tissues, approximate 5 mm thick, were cut through the central part of the wounds, including 2 mm of adjacent uninjured skin and underlying the depth of the entire skin layer. The tissues were kept in a plastic classette box. All wounds on dog skin were treated until they were healed.

9.2.1 **Preparation of Histologic Sections** (Luna, 1968)

9.2.1.1 Fixation

The tissues were fixed in the fresh fixative using an aqueous 10% neutral buffered formalin for at least 24 hrs.

9.2.1.2 Processing

The tissue processing procedure was operated by the followings process.

Washing: After fixation, the specimens were washed in running water for 30 mins.

Dehydrating: the specimens were dehydrated by transferring through a series of ethyl alcohol of increasing concentrations using 80% ethyl alcohol for 30 mins, 2 times; 95% ethyl alcohol for 30 mins, 2 times; and 100% ethyl alcohol for 40 mins, 2 times, respectively.

Clearing: the specimens were transferred to a clearing agent such as xylene, which is miscible with both 100% ethyl alcohol and paraffin. The specimens were infiltrated in xylene twice for 30 mins.

Infiltration: following the replacement of alcohol by clearing reagent, the tissues were immersed in melted paraffin (60°C), which infiltrates the tissues for 30 mins, 2 times.

Embedding and blocking: when infiltration was completed, the specimens were transferred to fresh melted paraffin and embedded in a cubical paraffin mold. After cooling, the melted paraffin was hardened. The paraffin block was removed and excess paraffin was trimmed away.

9.2.1.3 Sectioning

A slide of section was prepared by the following steps: the block of paraffin was secured to the microtome and oriented appropriately with respect to the knife. With each revolution of the microtome handle, the specimen moved through the blade and a section of the desired 4-6 μm thickness was produced. Each successive section adhered to the proceeding one, forming a continuous ribbon. Subsequently, one or more sections were carefully separated from the ribbon and transferred to the surface of warm water in a water bath at 40-45 °C to produce softness of paraffin and flatness of the section as well as eliminating wrinkles. The flattened section was floated onto a slide, which was left for air drying in room temperature. As the preparation dried, the section adhered to the surface of the slide (Bacha and Wood, 1990).

9.2.1.4 Staining

After the section on the slide was dried. The sections were stained with hematoxylin-eosin and Masson's trichrome reagents.

Hematoxylin and Eosin (H&E) staining procedure:

The paraffin was removed with xylene for 10 mins, the same procedure was repeated. The specimens were rehydrated by passing through a gradual series of decreasing concentrations at absolute, 95% and 70% ethyl alcohol, respectively, for 2 min with each alcohol concentration. The specimens were washed in running water for 5 mins and stained with Harris hematoxylin solution for 6 mins. The sections were a bluish-violet color and washed in running water for 5 mins. The sections were removed the excess hematoxylin in 1% acid alcohol 1 dip, and washed the excess acid in running water for 5 mins. The sections were then neutralized by dipping into saturated lithium carbonate (Li_2CO_3) for 4 dips and washed in running water for 5 mins. Counterstain the sections with eosin working solution for 1 mins to

produce a pink or red color. After stained, the specimens were dehydrated by passing through a gradual series of increasing concentrations of 95% ethyl alcohol 5 dips and absolute ethyl alcohol twice for 2 mins of each. The specimens were cleared (made transparent) with xylene for 5 mins, 3 times. Permanent mounting prepared by covered the specimens with a resinous mounting medium (DPX solution) and topped with a cover slip (Luna, 1968).

Masson's Trichome staining procedure:

The paraffin was deparaffinized through xylene for 10 mins, 2 times. The specimens were rehydrated by passing through a gradual series of decreasing concentrations at absolute, 95% and 70% ethyl alcohol, respectively, for 2 mins with each alcohol concentration. The specimens were rinsed in distilled water. The specimens were mordant in Bouin's solution in oven at 60°C for 1 hour, cool and washed in running water until yellow color disappears. The specimens were rinsed in distilled water and stained with Weigert's iron hematoxylin solution for 10 mins. The specimens were washed in running water 10 mins and rinse in distilled water. The specimens were stained with Biebrich scarlet-acid fuchsin solution for 2 mins. The specimens were rinsed in distilled water and stained in Phosphomolybdic acid-phosphotungstic acid solution for 10 mins. The sections were stained in aniline blue solution for 5 mins and rinsed in distilled water. The sections were removed the excess staining in acetic water 1% for 3 mins. After stained, the specimens were dehydrated by absolute ethanol for 2 mins, twice change. The specimens were cleared in xylene for 5 mins, twice change. Permanent mounting prepared by covered the specimens with a resinous mounting medium (DPX solution) and topped with a cover slip (Luna, 1968).

9.2.2 Histopathological Analysis

The criteria of histopathological lesions were evaluated the skin layer as the following:

Epidermis; Epidermal hyperkeratosis and epidermal hyperplasia
Epidermitis

Dermis; Dermal fibrosis

Dermatitis was classified as upper and lower layers depend on the location of lesion.

- Subacute suppurative upper dermatitis
- Subacute suppurative lower dermatitis
- Subacute suppurative lower dermatitis with pyogranuloma

The lesions were given a score ranging from 0 (no remarkable lesions), 1 (mild), 2 (moderate) and 3 (severe).

9.2.3 Statistical analysis

The values of the histopathological scores were averaged and expressed as the mean and standard error of mean. All statistical evaluations were performed by one-way analysis of variance (ANOVA). The results were considered significant at $p < 0.05$; equal variances assumed using LSD.

CHAPTER III

RESULTS

1. Isolation of Polysaccharide Gel (PG) From Dried Durian Fruit-Hulls

Polysaccharide gel (PG) of durian was isolated and purified from dried fruit-hulls (Pongsamart and Panmaung 1998). The polysaccharide gel extract was pulverized to a fine powder and passed through 60 mesh sieve, the beige to pale brown powder was obtained. The PG gel dried powder is shown in Figure 7. The PG gel swelled and dissolved in distilled water forming a viscous gel, PG at 2% by weight had pH 2.35 ± 0.04 and the viscosity was 248.88 ± 0.98 cps (Table 4). PG was used in a PG dressing film preparation by preparing a casting PG aqueous mixture composed of 2% polysaccharide gel (PG) and propylene glycol 15% w/w based on PG as a plasticizer, the mixture had pH 2.32 ± 0.02 and the viscosity was 250.74 ± 0.72 cps as shown in Table 4.

2. Preparation of PG Dressing Films

The characteristics of good film preparation should be thin, flexible and soft or not brittle. The PG dressing films were prepared by casting/solvent evaporation method, the resulting products were very thin, transparent, flexible, colorless to pale beige in color, and easy to peel off from the glass plate. The physical appearances of PG dressing film products are illustrated in Figure 8.

3. Preparation of PG Fiber Dressing Patches

The PG fiber dressing patches were prepared by dehydrating frozen aqueous PG solution in freeze-dryer. A white color of fluffy and soft fiber of PG fiber dressing patches was obtained. The physical appearance of PG fiber dressing patches are illustrated in Figure 9. An aqueous solution of 1% PG was used to produced a satisfactory dried fiber after drying in freeze dryer.

Figure 7. Polysaccharide gel (PG) powder extracted from dried fruit-hulls of durian, the purified extract of PG was a light brown to pale beige powder.

Table 4. The viscosity and pH properties of a PG aqueous mixture and a mixture of PG with propylene glycol as a plasticizer; n = 5, DW = distilled water.

Parameter	2% PG in DW (mean ± SD)	2% PG with 15% w/w propylene glycol based on PG (mean ± SD)
pH	2.35 ± 0.04	2.32 ± 0.02
Viscosity (cps)	248.88 ± 0.98	250.74 ± 0.72

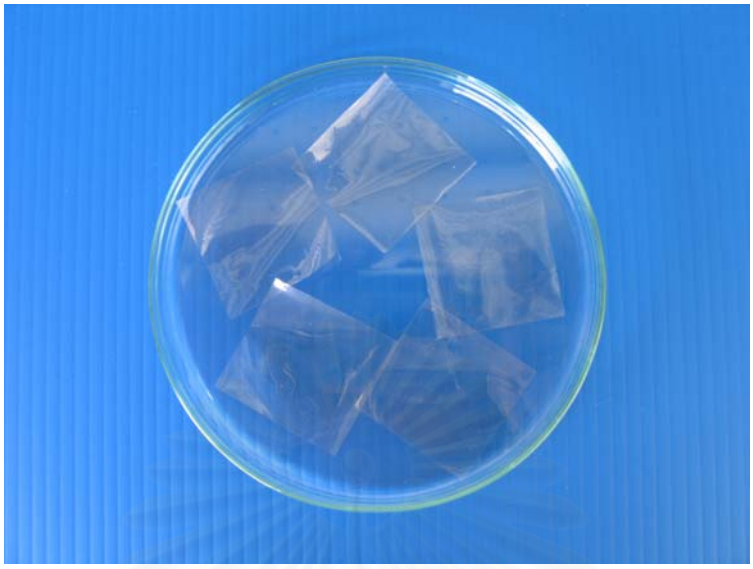

Figure 8. PG dressing films prepared from polysaccharide gel (PG) by a casting/solvent evaporating technique.

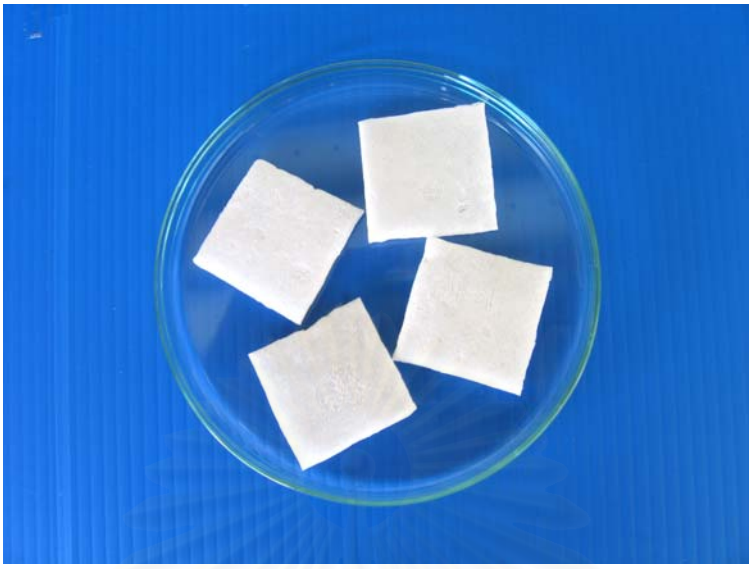

Figure 9. PG fiber dressing patches prepared from aqueous solution of polysaccharide gel (PG) by freeze- dryer.

4. Evaluation of PG Dressing Films

4.1 The Thickness of PG Dressing Films

The thickness of the PG films base and PG dressing films is shown in Table 5. The results demonstrated the mean values and SD. The thickness of PG film base and PG dressing film was 0.030 ± 0.001 and 0.031 ± 0.002 mm, respectively.

4.2 The Mechanical Properties of PG Dressing Films

The tensile testing provides an indication of the strength and elasticity of the film, which was indicated by tensile strength, % elongation at break, Young's modulus and work of failure. A dressing film that suitable for wound healing required properties of soft and flexible represented by low to medium tensile strength, low Young's modulus and high % elongation at break. The mechanical properties of PG dressing films are presented in Table 5. PG dressing films with plasticizer showed lower tensile strength and Young's modulus than that of PG film base without plasticizer. The lower values of tensile strength and Young's modulus of PG dressing film with plasticizer represented that the PG dressing film was softer and more flexible than that of PG film without plasticizer. The tensile strength of PG film base was higher than that of PG dressing film represent harder and more brittle of PG film base than PG dressing film. The results indicated that PG dressing films were softer and more flexible than that of PG film base.

In comparison, the % elongation and work of failure values of PG dressing film with plasticizer and PG film base without plasticizer demonstrated that higher values of % elongation at break and work of failure were obtained in PG dressing film with plasticizer. The result indicated that the PG dressing film with plasticizer was greater in toughness and elasticity than that of PG film base without plasticizer.

Table 5. Mechanical properties of PG films base without plasticizer and PG dressing films with 15% (w/w base on PG) propylene glycol as plasticizer; data are expressed as means (SD); n=5

Sample	Mechanical properties				Thickness (mm)
	Tensile strength (MPa)	Young's modulus (MPa)	% Elongation at break	Work of failure (mJ)	
PG film base	27.59 (0.12)	553.50 (12.06)	10.94 (3.73)	0.43 (0.18)	0.030 (0.001)
PG dressing film	21.54 (0.07)	521.64 (10.35)	22.16 (1.11)	1.33 (0.09)	0.031 (0.002)

4.3 Swelling Property of PG Dressing Films

The degree of swelling of PG dressing film in distilled water was demonstrated in Figure 10. The results showed that PG dressing film swelled in distilled water, the degree of swelling was 33.62%. Maximum swelling of PG dressing film was observed at the first hour then decreased and achieved plateau in swelling at approximately 7 hours.

4.4 Bioadhesive Strength of PG dressing films

The bioadhesive strength measurement of 0.016 mm thickness backing layer (5% ethylcellulose in ethanol as a casting solution), PG dressing film with backing layer and PG film base with backing layer was evaluated. The bioadhesive strength is demonstrated in Table 6. Work of adhesion and force of bioadhesion were measured. The results showed that work of adhesion and force of bioadhesion were highest for PG dressing film, followed by PG film base, and the lowest for backing layer, respectively.

5. Evaluation of PG Fiber Dressing Patches

5.1 The physical properties of PG Fiber Dressing Patches

The physical properties of the PG fiber dressing patches are shown in Table 7. The results demonstrated the mean values and SD. The thickness of PG fiber dressing patch prepared by freeze dryer using 90 ml of 0.6, 0.8, 1 and 1.5% PG aqueous solution on a 219.04 cm^2 tray were 0.284 ± 0.019, 0.306 ± 0.016, 0.366 ± 0.012 and 0.414 ± 0.023 mm, respectively. The values of density of PG fiber dressing patches prepared by the same procedure using 0.6, 0.8, 1 and 1.5% PG aqueous solution were 0.0112 ± 0.012, 0.0123 ± 0.011, 0.0133 ± 0.016 and 0.0160 ± 0.020 g/cm^3, respectively.

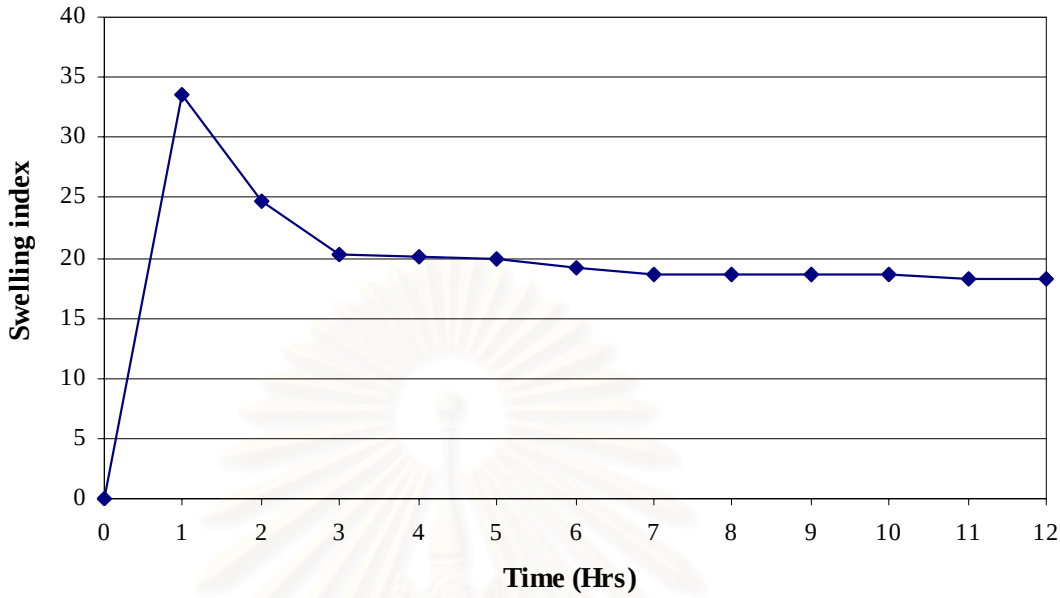

Figure 10. Swelling index versus time profile of PG dressing film in distilled water

Table 6. The bioadhesive strength of backing layer, PG dressing film with backing layer and PG film base with backing layer; data are expressed as means (SD); n = 5

Sample	Bioadhesive strength, mean (SD)	
	Work of adhesion (mJ)	Force of bioadhesion (N)
PG dressing film with backing layer	0.79 (0.24)	7.83 (0.56)
PG film base with backing layer	0.62 (0.18)	5.44 (0.61)
Backing layer	0.21 (0.03)	2.03 (0.46)

Table 7. The physical properties of PG fiber dressing patches prepared by freeze dryer; data are expressed as means ± SD; n = 5

% PG solution of 90 ml used in PG fiber dressing patch (tray area 219.04 cm^2)	Physical properties	
	Thickness (mm)	Density (g/cm^3)
0.6	0.284 ± 0.019	0.0112 ± 0.012
0.8	0.306 ± 0.016	0.0123 ± 0.011
1.0	0.366 ± 0.012	0.0133 ± 0.016
1.5	0.414 ± 0.023	0.0160 ± 0.020

5.2 Moisture Content

Percent moisture content of PG fiber dressing patch was evaluated. The results showed that PG content at 0.0112, 0.0123, 0.0133 and 0.0160 g/cm^3 contained % moisture of 14.77 ± 0.76, 15.56 ± 0.76, 15.30 ± 0.72 and 15.20 ± 0.87 %, respectively as shown in Figure 11.

5.3 Moisture Sorption

The moisture sorption of PG fiber patches are illustrated in Figure 12. The moisture sorption of PG fiber dressing patches were at equilibrium within 7 days after moisture exposure at 75% relative humidity. Percent moisture sorption of PG fiber dressing patches were increased with respect to increasing of PG content in the fiber dressing patches. Percent moisture sorption of PG content at 0.0112, 0.0123, 0.0133 and 0.0160 g/cm^3 was 16.22 ± 0.53, 17.96 ± 0.86, 21.32 ± 0.62 and 22.81 ± 0.67 percent, respectively.

6. Evaluation of PG dressing for wound healing

6.1 Gross Pathology Evaluation

6.1.1 Wound lesion

Gross lesion of wounds were clinically examined every 3 POD on day 3, 6, 9, 12, 15, 18 and 21.

On POD 3, superficial surface of wounds in each treatment was moist, red and peripheral or central swell. Some wounds were hemorrhage. Residues of dressing were cover on wound. Each wound was cleaned with normal saline and residues were removed. Only wounds in T_3 showed severe serous exudate. The wound appearances were illustrated in Figure 13.

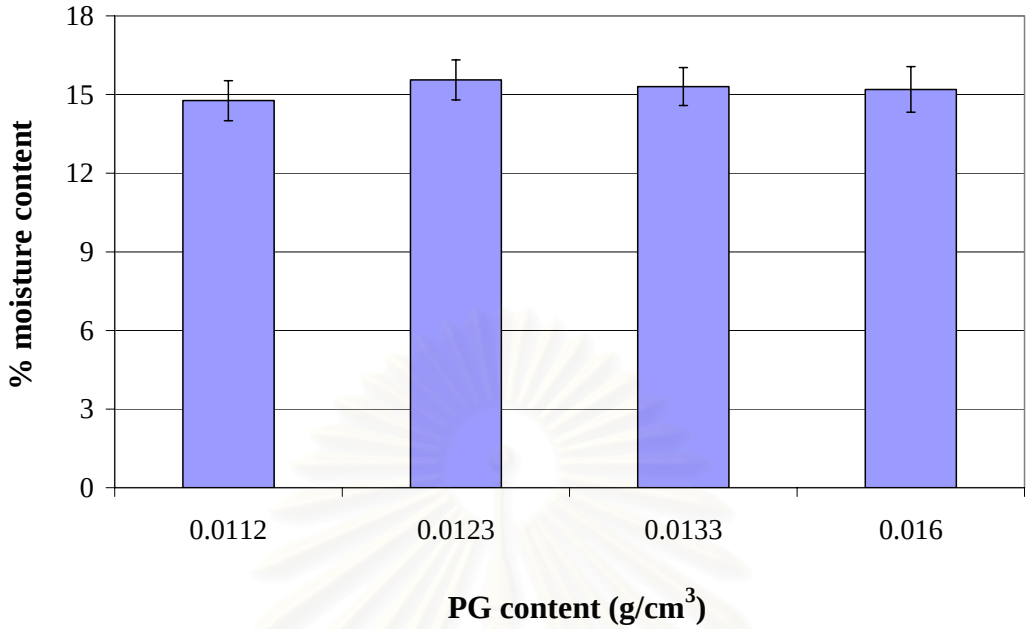

Figure 11. The moisture content of PG fiber dressing patches in various dry fiber of PG content (g/cm^3). Data are mean ± SD, n=5, mean values are not significant difference between groups ($p \geq 0.05$).

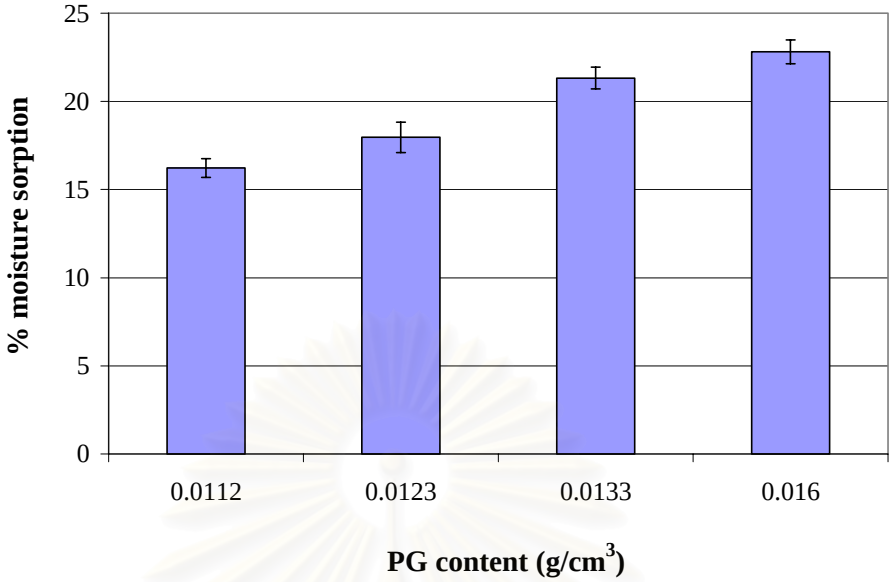

Figure 12. The moisture sorption of PG fiber dressing patches in various dry fiber of PG content (g/cm^3). Data are mean ± SD, n=5, mean values are not significant difference between groups ($p \geq 0.05$).

On POD 6, all wounds were still red, but swelling was not observed. New tissues and cells fulfilled the wound space, the wound bed was shallow which was a consequence of proliferative phase of healing, the synthesis of granulation tissue. However, wounds in control group showed less granulation tissue than that of other treatments, resulted in deep of wound bed. Scabs were covered on wound surface in control, T_1 and T_2 while the scab formations were still not observed on wounds in T_3. Wounds in T_3 showed more severe exudate than the others. The wound appearances were illustrated in Figure 14.

On POD 9, all wounds in each treatment showed red color. The wound was shallow and epithelial cells were newly synthesized around the wound margin. The wounds in T_1, T_2 and T_3 were clearly more increased in the granulation tissue, epithelial formation and the apparent wound contraction than that of control. All wounds were cleaned and scabs were removed. All wounds showed moderate exudates. The wound appearances were illustrated in Figure 15.

On POD 12, all wounds in each treatment showed red to pink color. Contraction and epithelialization of the wounds were increased, scabs were still appeared and it was removed during wound cleaning. The wounds in T_1 and T_2 were smaller in area than that of control and T_3. The wounds treated with 1% povidone iodine and covered with Opsite® Flexigrid produced more exudate than other groups. The wound appearances were illustrated in Figure 16.

On POD 15, all wounds in each treatment showed pink to red color, wounds were continuously contraction. Although, the wound areas in all treatments were decreased and became small, wounds treated with PG dressing film and PG fiber dressing patch were more decreased in wound size than the other groups. All wounds showed mild exudates. The wound appearance illustrated in Figure 17.

On POD 18, some of wounds in T_1, T_2 and T_3 treatment showed complete wound closure, pink color, the wound surface was smooth and scarring was mildly appeared. Whereas, non of wounds in control group were completely closed, wounds were pink to red color. The wound appearances were illustrated in Figure 18.

On POD 21, the day of experiment ended, all wounds treated with PG fiber dressing patch (T_2) showed complete wound closure, the wound surface was smooth, some wounds were shrinked and the scarring was mild. While, some wounds in control, T_1 and T_3 were not completely closed, wounds areas were very small and pink color. Wounds which completed closure in control were red to pink, some wounds shrinked, swell and moderate scar formation. Wounds which completed closure in T_1 were pink to red, smooth surface and mild scarring. Wounds which completed closure in T_3 were pink, smooth and mild scarring. The wound appearances were illustrated in Figure 19.

At the end of experiment, on day 21, all 8 wounds treated with PG fiber dressing patch showed 100% complete wound healing. While wounds treated with 1% povidone iodine, PG dressing film, and 1% povidone iodine and covered with Opsite® Flexigrid were complete healing at 50% (4 of 8 wounds), 87.50% (7 of 8 wounds) and 37.50% (3 of 8 wounds), respectively. The results showed in Table 8.

6.1.2 Wound area

To determine the closure rate of wound healing, wound areas were measured every 3 POD on day 3, 6, 9, 12, 15, 18 and 21 by tracing the wound boundaries using sterile transparent films with permanent marker. The wound boundaries marked transparent films or “tracing” films were scanned and the area of each wound was calculated by using computer program of image analysis by using “ SCION image software”. Data are presented in Table 9 and Figure 20.

On POD 3, the results demonstrated that the mean values of the wounds areas in each group were increased in comparison with its value on day 0. The increasing of wound size was the result of wound swelling in the inflammatory phase of wound healing process. A comparison of the wound areas of each wound treatment was found no significant difference ($p \geq 0.05$). Data are showed in Table 9 and Figure 21.

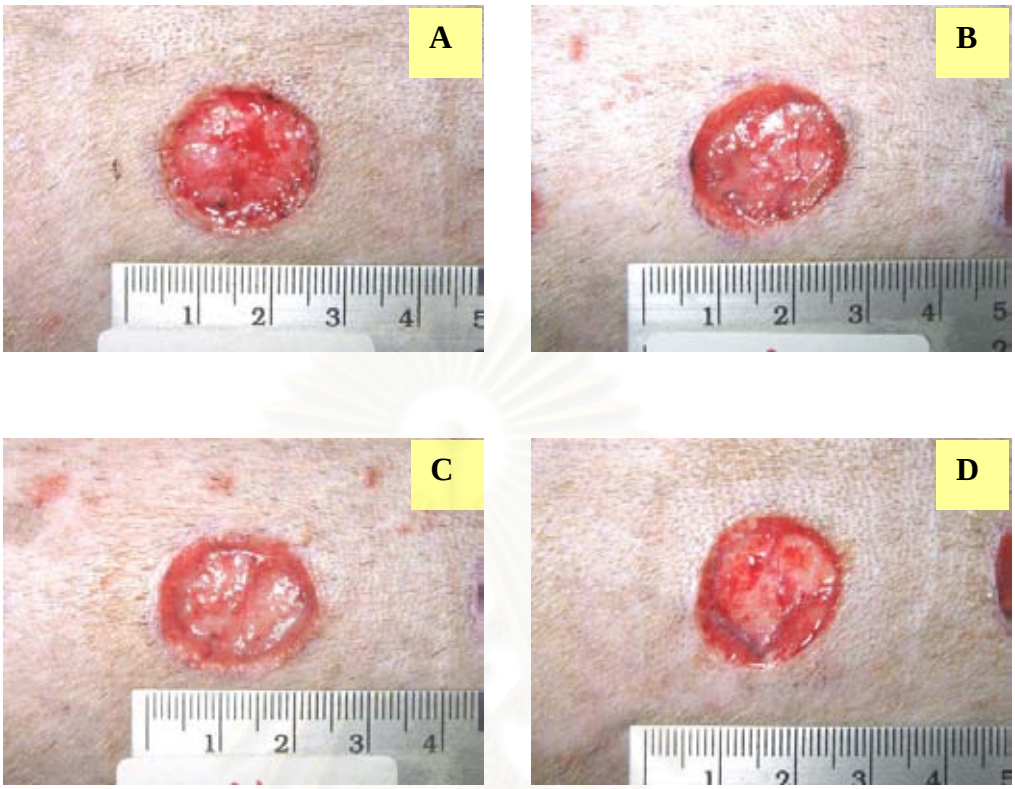

Figure 13. Gross lesion of wounds in dog skin on day 3. A = wound treated with 1% povidone iodine (control), B = wound treated with PG dressing film (T_1), C = wound treated with PG fiber dressing patch (T_2) and D = wound treated with 1% povidone iodine and covered with commercial dressing film; Opsite® Flexigrid (T_3).

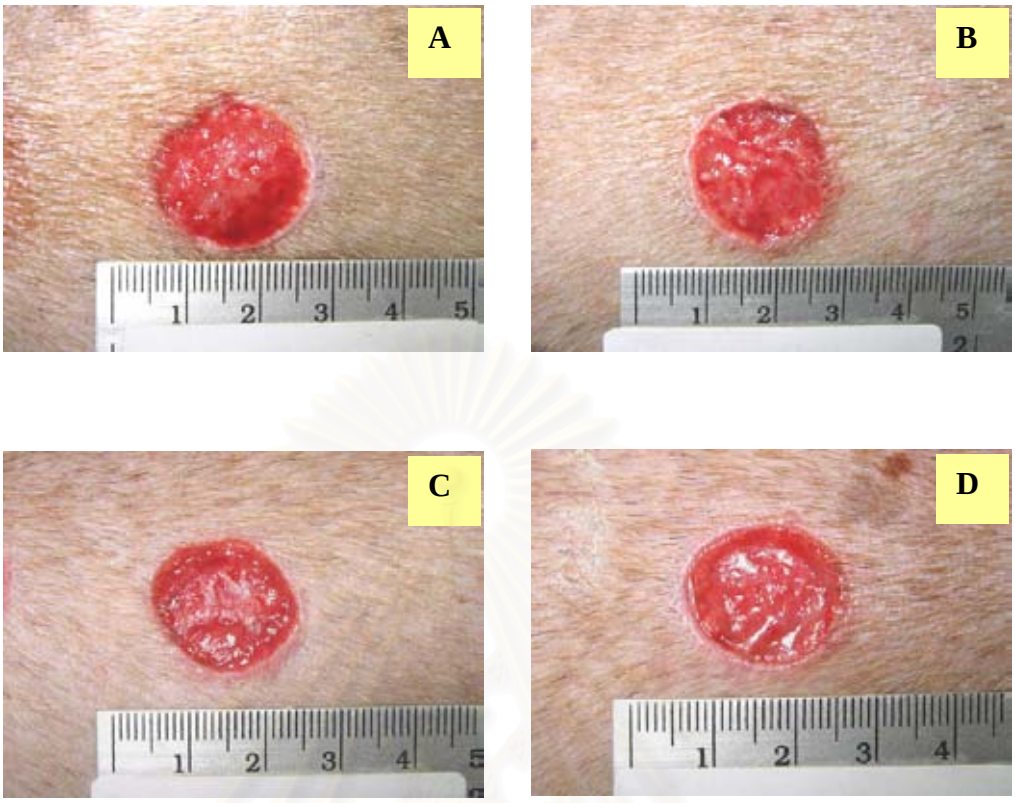

Figure 14. Gross lesion of wounds in dog skin on day 6. A = wound treated with 1% povidone iodine (control), B = wound treated with PG dressing film (T_1), C = wound treated with PG fiber dressing patch (T_2) and D = wound treated with 1% povidone iodine and covered with commercial dressing film; Opsite® Flexigrid (T_3).

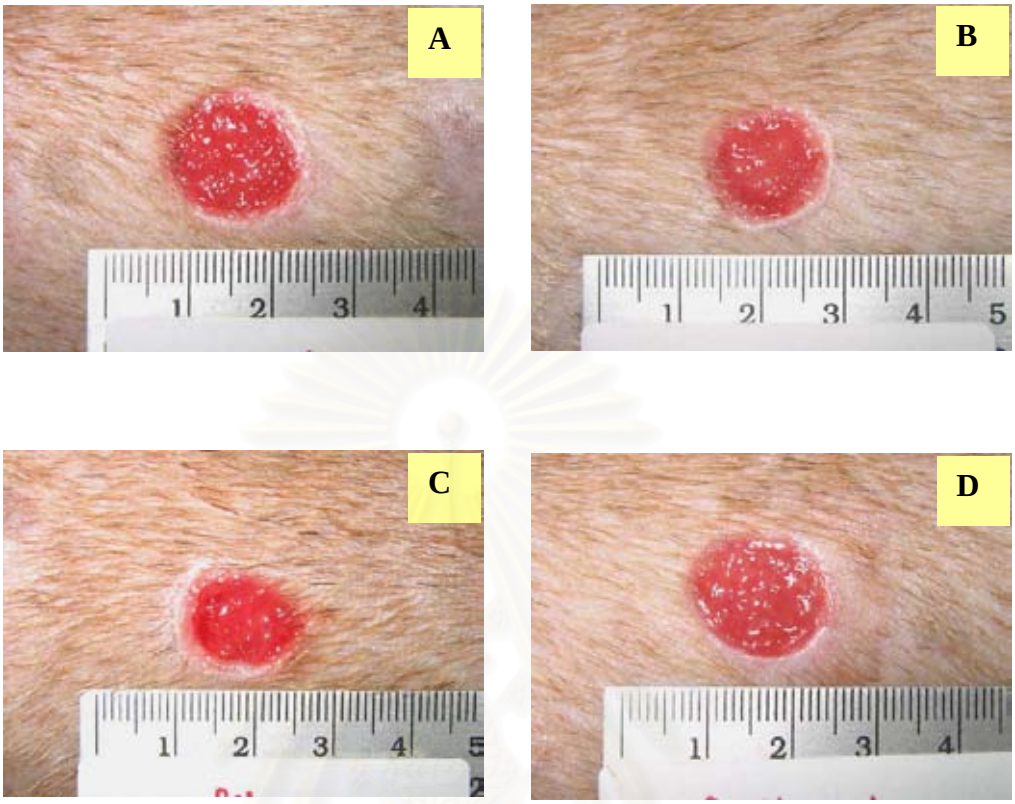

Figure 15. Gross lesion of wounds in dog skin on day 9. A = wound treated with 1% povidone iodine (control), B = wound treated with PG dressing film (T_1), C = wound treated with PG fiber dressing patch (T_2) and D = wound treated with 1% povidone iodine and covered with commercial dressing film; Opsite® Flexigrid (T_3).

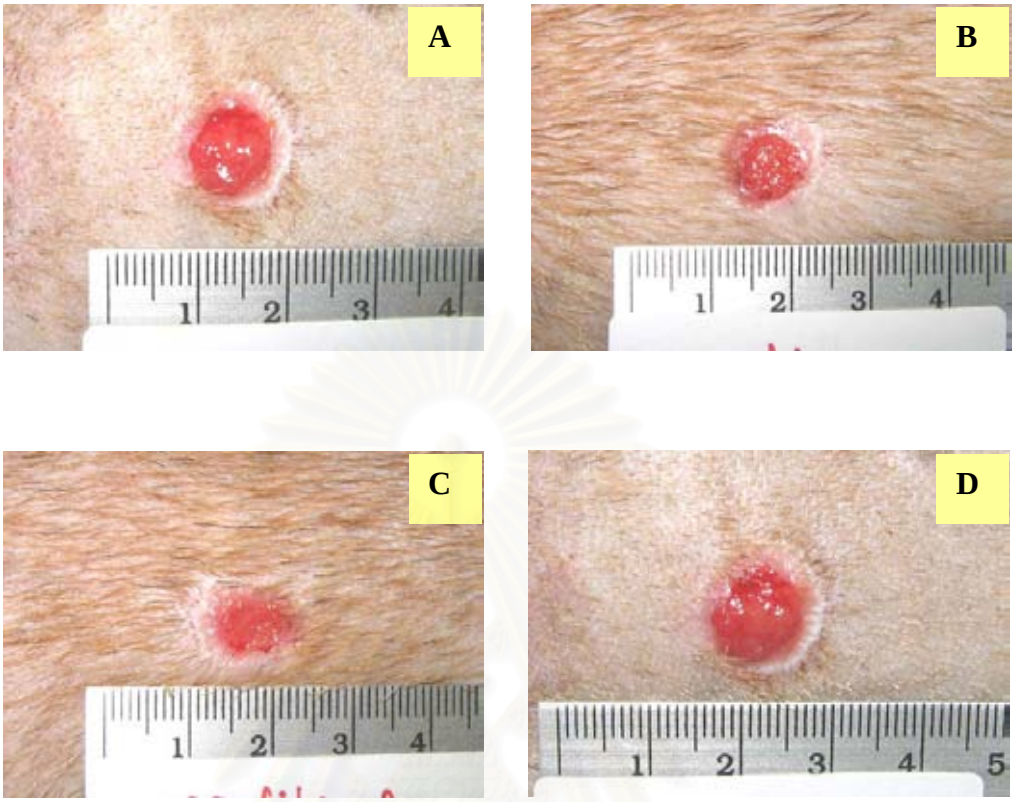

Figure 16. Gross lesion of wounds in dog skin on day 12. A = wound treated with 1% povidone iodine (control), B = wound treated with PG dressing film (T_1), C = wound treated with PG fiber dressing patch (T_2) and D = wound treated with 1% povidone iodine and covered with commercial dressing film; Opsite® Flexigrid (T_3).

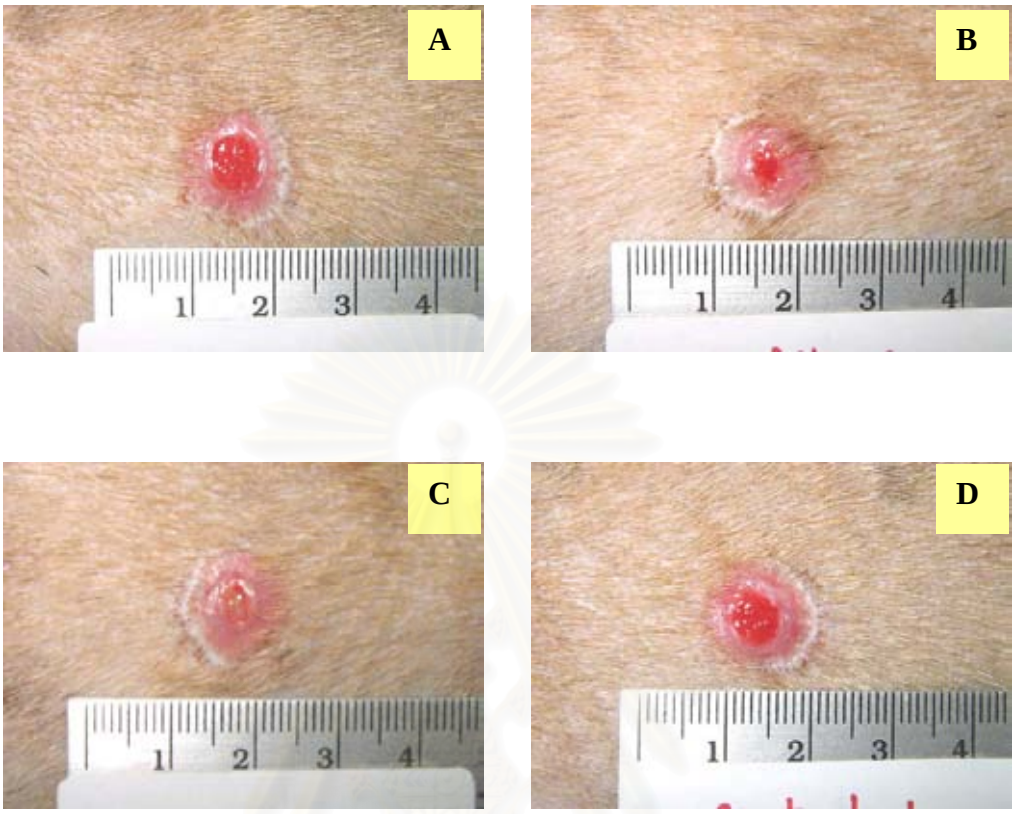

Figure 17. Gross lesion of wounds in dog skin on day 15. A = wound treated with 1% povidone iodine (control), B = wound treated with PG dressing film (T_1), C = wound treated with PG fiber dressing patch (T_2) and D = wound treated with 1% povidone iodine and covered with commercial dressing film; Opsite® Flexigrid (T_3).

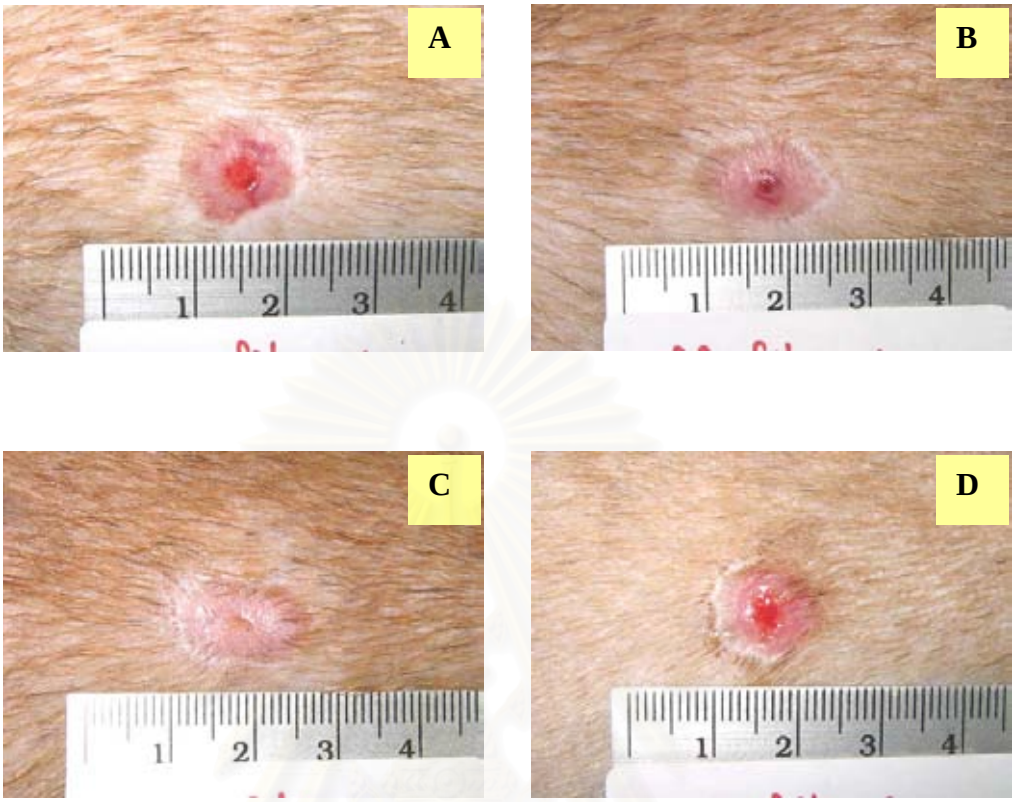

Figure 18. Gross lesion of wounds in dog skin on day 18. A = wound treated with 1% povidone iodine (control), B = wound treated with PG dressing film (T_1), C = wound treated with PG fiber dressing patch (T_2) and D = wound treated with 1% povidone iodine and covered with commercial dressing film; Opsite® Flexigrid (T_3).

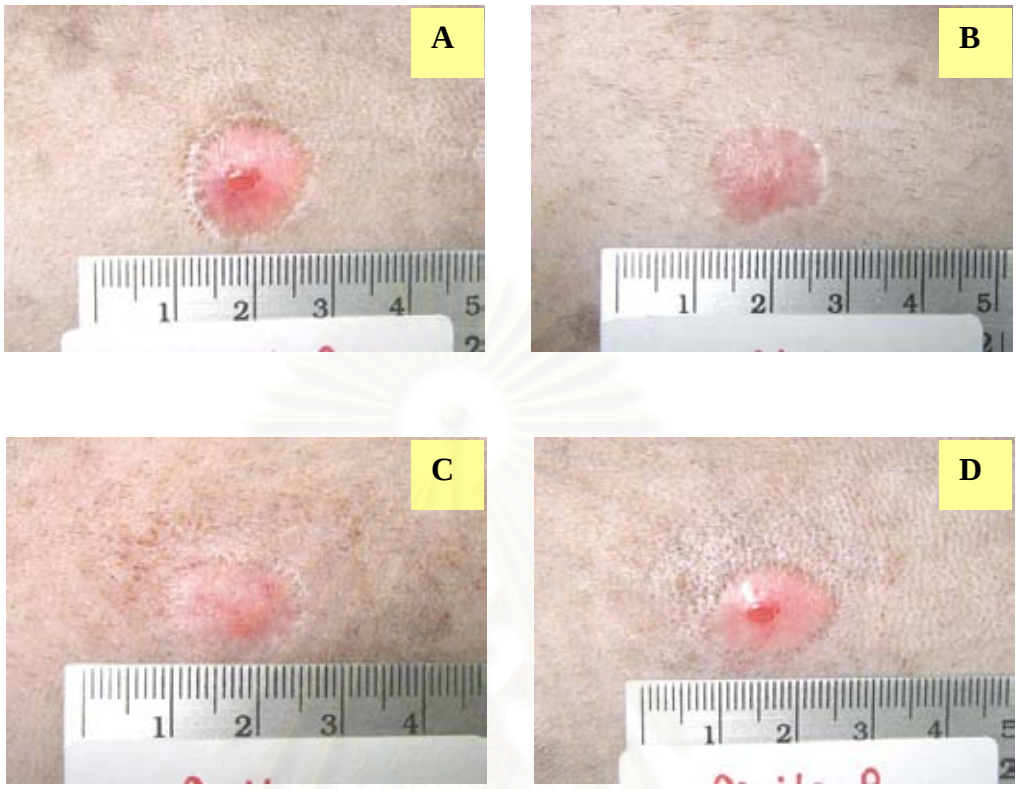

Figure 19. Gross lesion of wounds in dog skin on day 21. A = wound treated with 1% povidone iodine (control), B = wound treated with PG dressing film (T_1), C = wound treated with PG fiber dressing patch (T_2) and D = wound treated with 1% povidone iodine and covered with commercial dressing film; Opsite® Flexigrid (T_3).

Table 8. Complete wound closure rate of full-thickness wounds in dog skins after treatment with PG preparations for 21 days. Control = treated with 1% povidone iodine, T_1= treated with PG dressing film, T_2 = treated with PG fiber dressing patch, T_3 = treated with 1% povidone iodine and covered with Opsite® Flexigrid.

Treatment group	% of complete wound closure (No. of complete wound closure /n)
Control	50.00 % (4/8)
T_1	87.50 % (7/8)
T_2	100.00 % (8/8)
T_3	37.50 % (3/8)

Table 9. The rate of wound closure of full-thickness excisional wounds in dog skin determined by measuring wound area after treatment with PG dressing film (T_1) and PG fiber dressing patch (T_2) compared with povidone iodine (control) and 1% povidone iodine covered with commercial film dressing; Opsite® Flexigrid (T_3); data expressed as mean ± SD; n=8; a, b = significant difference between groups ($p < 0.05$).

Days	Wound area (cm^2) mean ± SD			
	Control	T_1	T_2	T_3
0	3.140 ± 0.000	3.140 ± 0.000	3.140 ± 0.000	3.140 ± 0.000
3	3.280 ± 0.207	3.250 ± 0.331	3.220 ± 0.299	3.230 ± 0.231
6	2.756 ± 0.232	2.665 ± 0.358	2.469 ± 0.327	2.614 ± 0.219
9	1.670 ± 0.273	1.414 ± 0.413	1.384 ± 0.290	1.606 ± 0.205
12	0.801 ± 0.214^{a}	0.631 ± 0.216^{ab}	0.608 ± 0.151^{b}	0.666 ± 0.312^{ab}
15	0.341 ± 0.179^{a}	0.215 ± 0.113^{ab}	0.175 ± 0.079^{b}	0.301 ± 0.233^{ab}
18	0.121 ± 0.102^{a}	0.043 ± 0.043^{b}	0.029 ± 0.024^{b}	0.110 ± 0.095^{a}
21	0.054 ± 0.073^{a}	0.003 ± 0.007^{b}	0.000 ± 0.000^{b}	0.054 ± 0.060^{a}

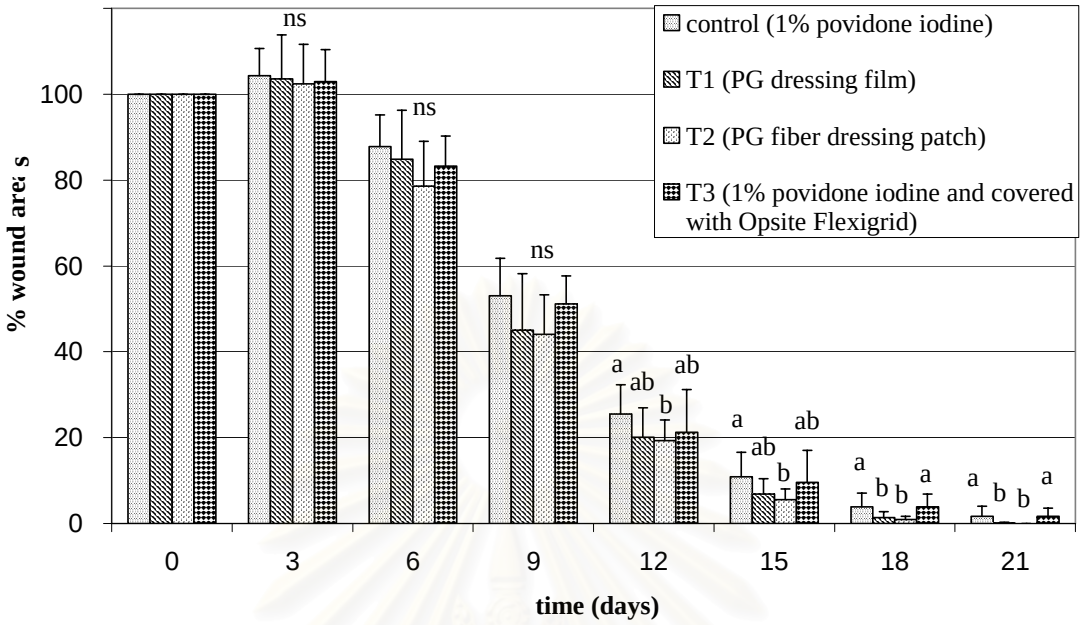

Figure 20. A comparison of wound areas (mean ± SD) of full-thickness excisional wounds in the skin of dog treated with PG dressing preparation. Control = treated with 1% povidone iodine, T_1 = treated with PG dressing film, T_2 = treated with PG fiber dressing patch and T_3 = treated with 1% povidone iodine and covered with commercial film dressing; Opsite® Flexigrid. ns = no significant difference between groups ($p \geq 0.05$). a, b = significant difference between groups ($p < 0.05$).

On POD 6, the mean values of wound areas in all treatments were decreased as illustrated in Table 9 and Figure 20. A comparison of the wound areas of each treatment was found no significant difference ($p \geq 0.05$). The results implicated that the inflammatory phase of wound healing process was ended and the swelling of wound was stopped.

On POD 9, mean values of wound areas in each treatment were more decreased than on POD 6. The mean value of each wound area was reduced to almost half of their initial wound area on day 0 as showed in Table 9 and Figure 20. It could be explained that all wounds were in the proliferative phase of wound healing process. Wounds treated with PG fiber dressing patch seemed to show the smallest of mean value of wound area. However, the values were no statistically significant difference in the wound areas ($p \geq 0.05$) between groups of each treatment.

On POD 12, wounds treated with PG fiber dressing patch (T_2) showed significantly smaller wound area ($p < 0.05$) than that of the control group (Table 9 and Figure 20). However, the area of wounds in T_2 was not significantly different from the wounds in T_1 and T_3 ($p \geq 0.05$). The area of wounds in control, T_1, T_2 and T_3 on day 12 were 0.801 ± 0.214, 0.631 ± 0.216, 0.608 ± 0.151 and 0.666 ± 0.312 % of its initial wound area, respectively.

On POD 15, the mean values of wound areas in control, T_1, T_2 and T_3 were 0.341 ± 0.179, 0.215 ± 0.113, 0.175 ± 0.079 and 0.301 ± 0.233, respectively (Table 9 and Figure 20). There was a statistical significantly smaller wound area ($p < 0.05$) of wound treated with PG fiber dressing patch (T_2) than that of control. A comparison of the wound areas in T_2 was found no significant difference compared to the wounds in T_1 and T_3 ($p \geq 0.05$).

On POD 18, the wound areas of wounds treated with PG dressing film (T_1) and wounds treated with PG fiber dressing patch (T_2) were significantly smaller ($p < 0.05$) than that of the wounds in control and T_3 as shown in Table 9 and Figure 20. However, the mean area of wounds treated with PG fiber dressing patch was the smallest compared to other groups. The mean area of wounds in control, T_1,

T_2 and T_3 on day 18 were 0.121 ± 0.102, 0.043 ± 0.043, 0.029 ± 0.024 and 0.110 ± 0.095 % of its initial wound area, respectively.

On POD 21, the day of experiment ened, all of wounds treated with PG fiber dressing patch showed complete wound closure, mean values of wound area was zero, whereas the values in control, T_1 and T_3 were 0.054 ± 0.07, 0.003 ± 0.007 and 0.054 ± 0.060 % of its initial wound area, respectively. Moreover, there was a statistical significantly smaller wound area ($p < 0.05$) of wound in T_2 and wound in T_1 than that of control and T_3. The results demonstrated in Table 9 and Figure 20.

A comparison of wound closure rates, calculated as percentages of wound area, showed in Figure 20. The results illustrated that wound closure in T_2 had significantly smaller wound areas ($p < 0.05$) than that of the wounds in control on day 12 and 15 postoperation, however, the wounds in T_1 and T_3 were healed at the same rate and not significantly different from wounds in T_2 and in control as well, represented by the wound areas of those two groups in T_1 and T_3 were not significantly different from groups of T_2 and control ($p \geq 0.05$). On day 18 and 21 postoperation, wounds treated with PG dressing film (T_1) and PG fiber dressing patch (T_2) showed a statistical significantly of smaller percentage of wound area ($p < 0.05$) than those of wounds treated with 1% povidone iodine (control) and 1% povidone iodine and covered with commercial film dressing -Opsite® Flexigrid- (T_3). The results indicated that wounds in T_2 showed the fastest complete wound closure and followed by wounds in T_1, T_3 and control, respectively.

6.1.3 Wound contraction determination

The results of wound contraction after applications with PG dressing film (T_1), PG fiber dressing patch (T_2), 1% povidone iodine and covered with commercial film dressing; Opsite® Flexigrid (T_3) and control are shown in Figure 21.

The results on POD 3 demonstrated that the contraction of wounds in each treatment was not observed due to the inflammation in wound healing process

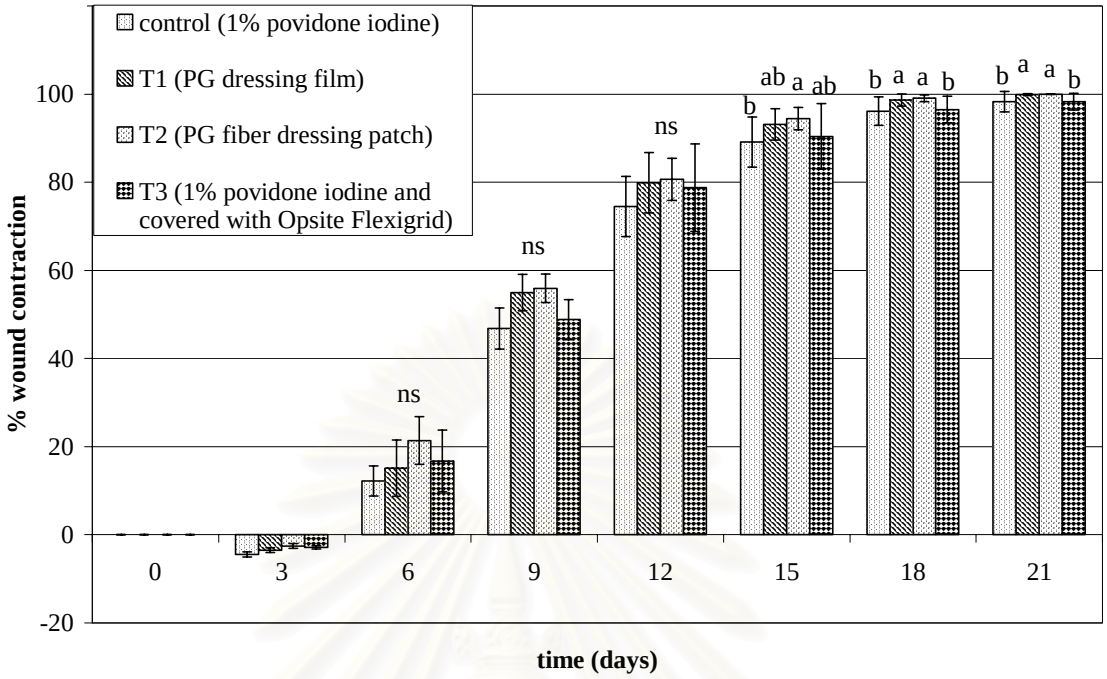

Figure 21. Percentage of wound contraction (mean ± SD) of full-thickness excisional wounds in dog skin treated with PG dressing film (T_1) and fiber dressing patch (T_2) compared with 1% povidone iodine (control) and 1% povidone iodine and covered with commercial film dressing; Opsite® Flexigrid. (T_3). ns = no significant difference between groups ($p > 0.05$). a, b = significant difference between groups ($p \leq 0.05$).

and wounds were swelled. The wounds contraction was gradually progress during the treatment period and clearly observed on day 6 postoperation. However, a comparison of the wound contraction in each treatment was found no significant difference ($p \geq 0.05$) on day 6, 9 and 12 postoperation. On POD 15, wounds treated with PG fiber dressing patch (T_2) showed significantly more contracted ($p < 0.05$) than that of wounds in control, however, wound contraction in T_2 was no statistically different from wounds in T_1 and T_3 ($p \geq 0.05$). Percent of wound contraction in control, T_1, T_2 and T_3 on day 15 were 89.132 ± 5.685, 93.153 ± 3.583, 94.427 ± 2.531 and 90.406 ± 7.429, respectively. On POD 18 and 21, the significantly highest percentage of wound contraction ($p < 0.05$) in T_1 and T_2 was obtained compared to that of control and T_3. On day 18 postoperation, wounds in T_2 showed higher percentage of wound contraction than those of the other treatments. Percentage of wound contraction of wounds in control, T_1, T_2 and T_3 were 96.139 ± 3.236, 98.646 ± 1.380, 99.084 ± 0.751 and 96.497 ± 3.019, respectively. On POD 21, wounds in T_2 showed 100% wound contraction, whereas wounds in control, T_1, and T_3 were 98.288 ± 2.334, 99.920 ± 0.225 and 98.288 ± 1.895 percent, respectively (Figure 21).

6.2 Histopathological Evaluation

The criteria of histopathological lesions were evaluated as followings: epidermal hyperplasia and hyperkeratosis, epidermitis, upper dermatitis (suppurative dermatitis), lower dermatitis (suppurative dermatitis and suppurative dermatitis with pyogranuloma) and dermal fibrosis. The lesions were presented as a score ranging from 0 (no remarkable lesions), 1 (mild), 2 (moderate) and 3 (severe).

Epidermal hyperplasia and hyperkeratosis were determined by proliferation of multilayer epithelium with increased keratin production in stratum corneum layer. The results demonstrated in Figure 22 and 24 that wounds treated with 1% povidone iodine (control) and 1% povidone iodine and covered with commercial film (Opsite® Flexigrid) showed mild to moderate lesion while wounds treated with PG dressing film and PG fiber dressing patch were mild lesion.

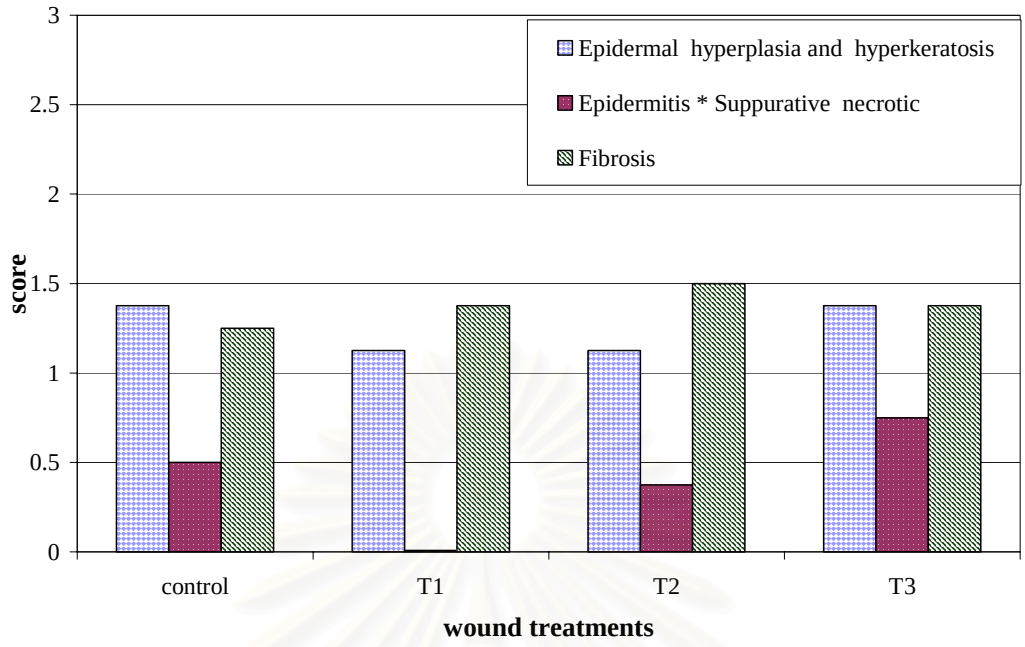

Figure 22. Histopathological evaluation of epidermal hyperplasia and hyperkeratosis lesion, epidermitis lesion and dermal fibrosis lesion of wounds in dog skin treated with PG dressing film (T_1), fiber dressing patch (T_2), and 1% povidone iodine and covered with commercial film dressing; Opsite® Flexigrid (T_3) compared with povidone iodine (control). Data are mean values, mean values of treated group are not significantly different from control ($p \geq 0.05$).

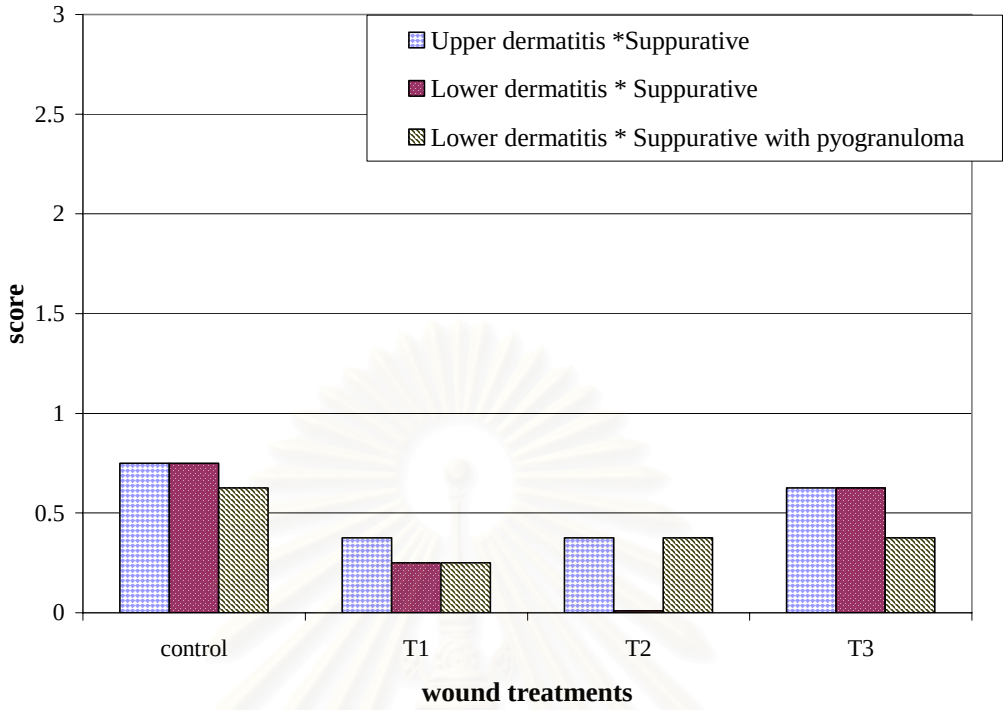

Figure 23. Histopathological evaluation of subacute suppurative upper dermatitis lesion, subacute suppurative lower dermatitis lesion and subacute suppurative lower dermatitis with pyogranuloma lesion of wounds in dog skin treated with PG dressing film (T_1), fiber dressing patch (T_2), and 1% povidone iodine and covered with commercial film dressing; Opsite® Flexigrid (T_3) compared with povidone iodine (control). Data are mean values, mean values of treated group are not significantly different from control ($p \geq 0.05$).

Epidermitis was characterized by a number of inflammatory cells such as PMNs, macrophages, and lymphocytes aggregated in epidermal layer. Epidermis is usually superficial necrosis and peripheral hemorrhage. The results illustrated in Figure 22 and 25 showed no remarkable lesion of wounds treated with PG dressing film. Wounds treated with PG fiber dressing patch (T_2) showed no remarkable to mild lesion, however, one wound in T_2 showed mild epidermal necrosis. Wounds treated with 1% povidone iodine (control) and 1% povidone iodine and covered with commercial film (Opsite® Flexigrid) showed no remarkable to mild lesion and moderate epidermal necrosis.

Dermal fibrosis was determined by an increasing a number of reactive fibroblasts producing collagen fibers which increased in the dermal layer. Including the irregular arrangement of reactive fibroblast and increased of capillary nests. The results demonstrated in Figure 22, 26 and 27 that all treatments showed mild fibrosis. However, the irregular arrangement of reactive fibroblast was observed in wounds in T_1.

Subacute suppurative upper dermatitis was characterized by a number of PMNs, lymphocytes and some macrophages diffusely aggregated in upper dermis and interface dermis. The results demonstrated that wounds treated with 1% povidone iodine (control) showed the higher score of dermatitis while wounds in T_1, T_2 and T_3 were no remarkable to mild lesion. However, some wounds in control showed severe suppurative dermatitis. The results showed in Figure 23 and 28.

Subacute suppurative lower dermatitis was characterized by a number of PMNs, lymphocytes and some macrophages diffusely aggregated in deep dermis adjacent to subcutaneous fat and muscular layer. Wounds treated with 1% povidone iodine (control) showed the highest score of upper dermatitis than T_3, T_1 and T_2, respectively. Wounds treated with 1% povidone iodine (control) and commercial film (Opsite® Felexigrid) (T_3) showed no remarkable to mild lesion while wounds treated with PG dressing film (T_1) and PG fiber dressing patch (T_2)

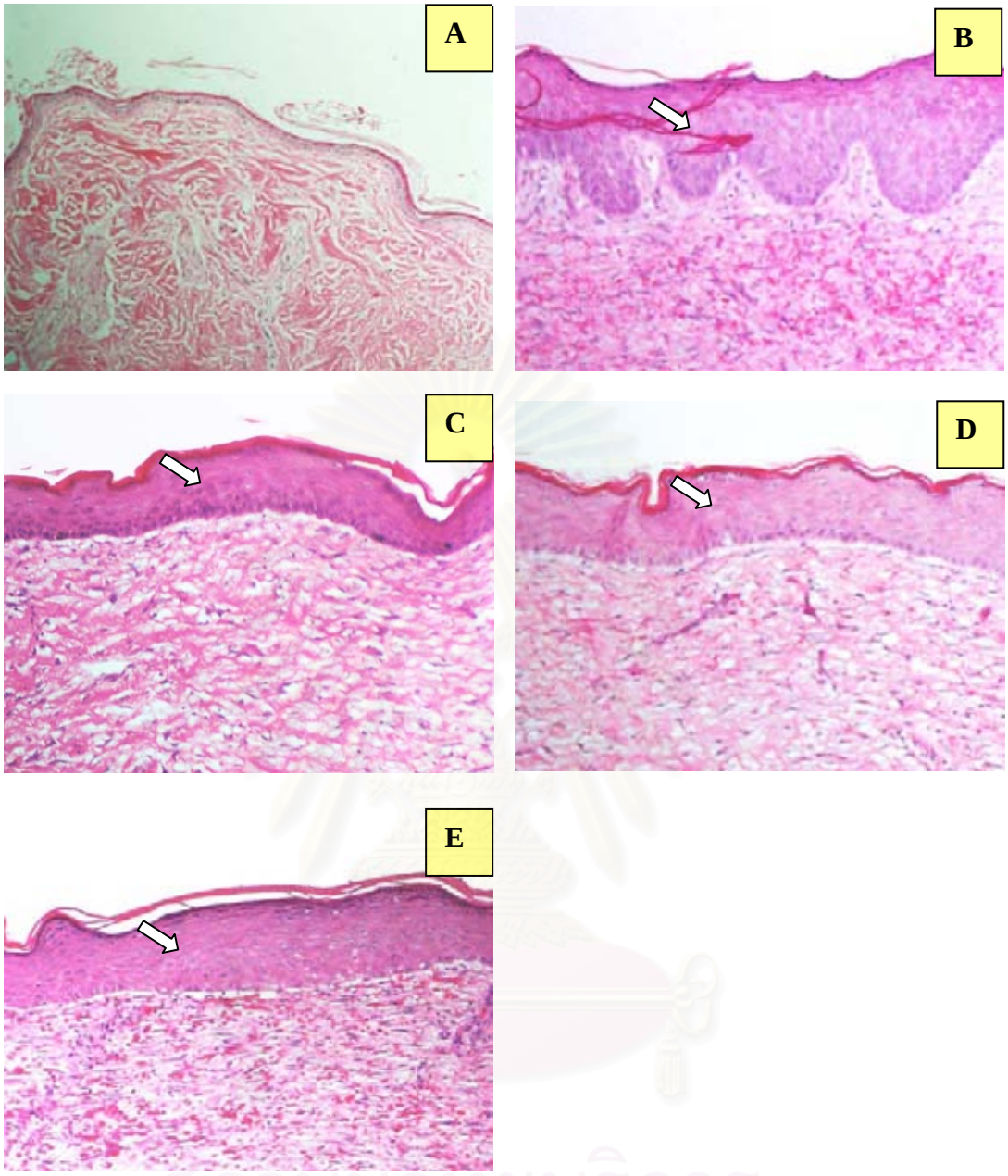

Figure 24. Photomicrographs demonstrated epidermal hyperkeratosis and hyperplasia of the wounds (Hematoxylin and eosin (HE), × 25 µm). A) normal skin. B) control : a wound treated with 1% povidone iodine. C) T_1 : a wound treated with PG dressing film. D) T_2 : a wound treated with PG fiber dressing patch. E) T_3 : a wound treated with 1% povidone iodine and covered with commercial dressing film; Opsite ®Flexigrid. Arrows pointed the epidermal hyperplasia. B and E showed mild to moderate epidermal hyperplasia while C and D showed mild epidermal hyperplasia.

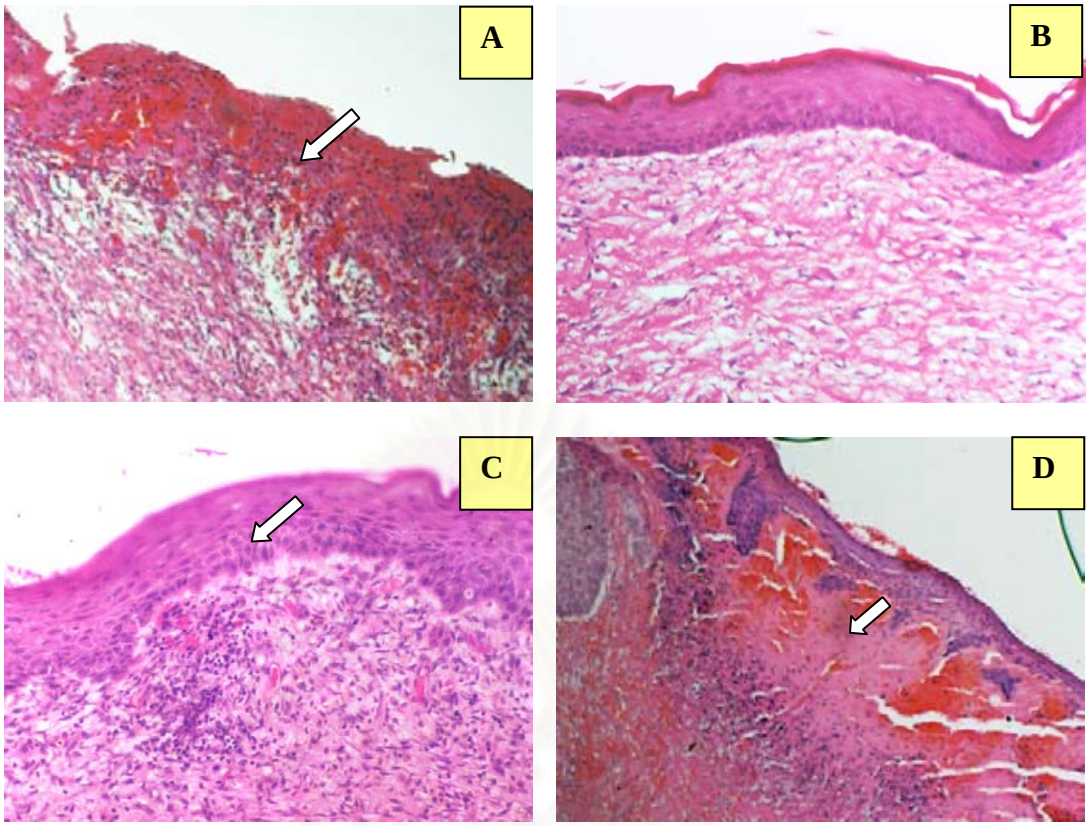

Figure 25. Photomicrographs showed epidermitis of the wounds (Hematoxylin and eosin (HE), × 25 µm). A) control : a wound treated with 1% povidone iodine. B) T_1 : a wound treated with PG dressing film. C) T_2 : a wound treated with PG fiber dressing patch. D) T_3 : a wound treated with 1% povidone iodine and covered with commercial dressing film; Opsite ®Flexigrid.; Arrows in A, C and D showed the superficial necrosis. B showed no remarkable lesion of epidermitis.

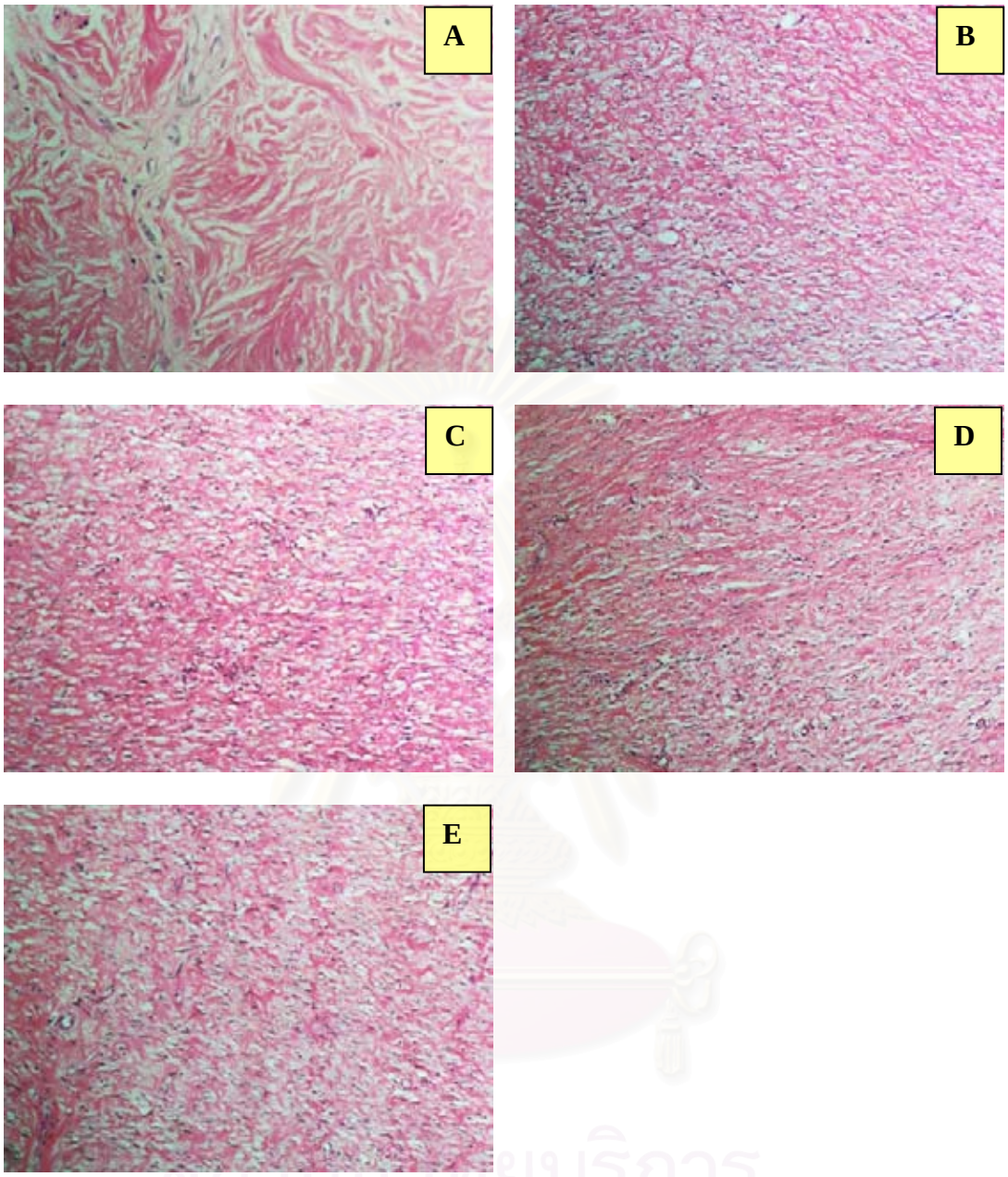

Figure 26. Photomicrographs demonstrated dermal fibrosis of the wounds (Hematoxylin and eosin (HE), × 25 µm). A) normal skin. B) control : a wound treated with 1% povidone iodine. C) T_1 : a wound treated with PG dressing film. D) T_2 : a wound treated with PG fiber dressing patch. E) T_3 : a wound treated with 1% povidone iodine and covered with commercial dressing film; Opsite ®Flexigrid. All treatments showed mild dermal fibrosis.

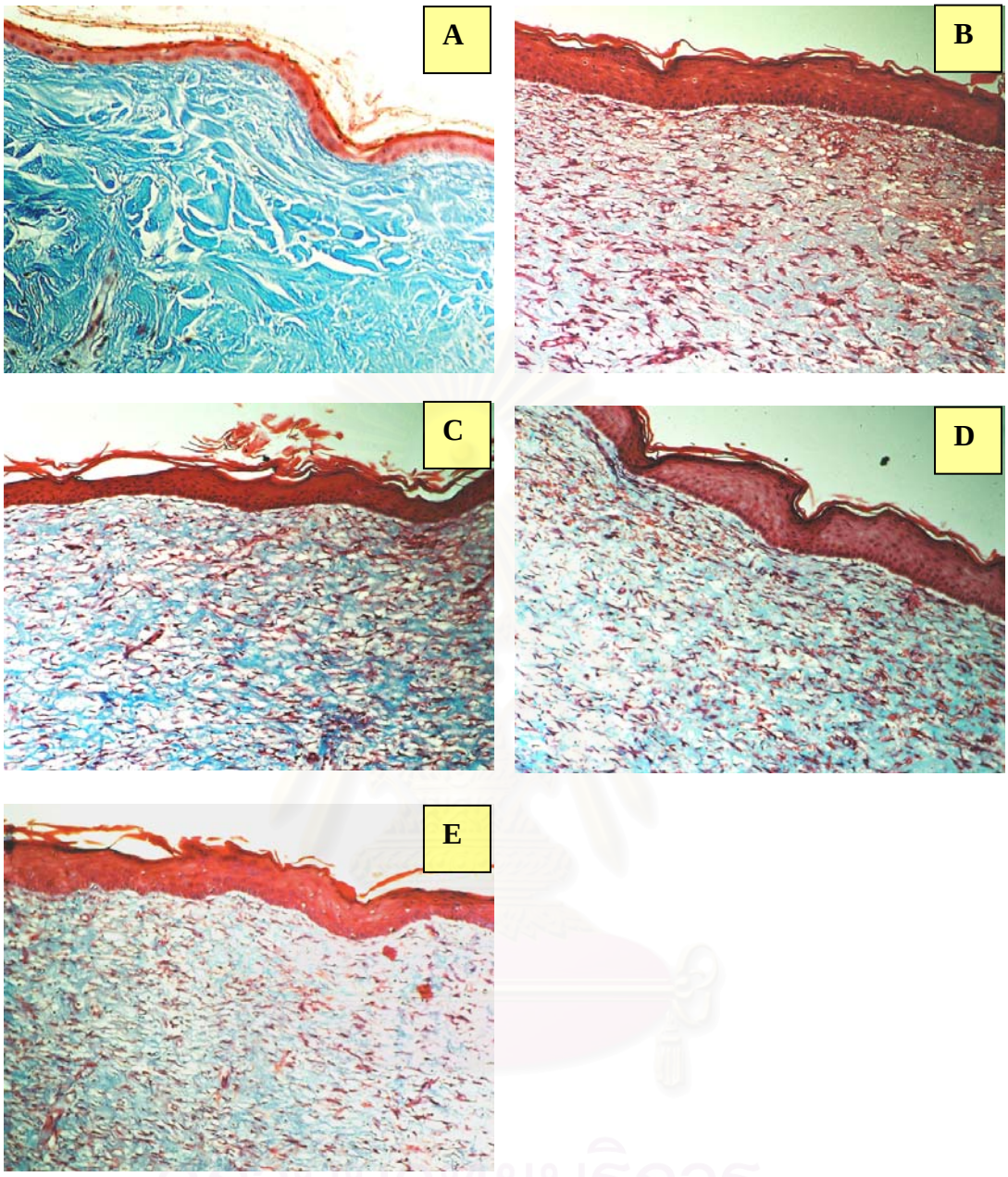

Figure 27. Photomicrographs demonstrated dermal fibrosis of the wounds (Trichrome stain, × 25 µm). A) normal skin. B) control : a wound treated with 1% povidone iodine. C) T_1 : a wound treated with PG dressing film. D) T_2 : a wound treated with PG fiber dressing patch. E) T_3 : a wound treated with 1% povidone iodine and covered with commercial dressing film; Opsite ®Flexigrid.

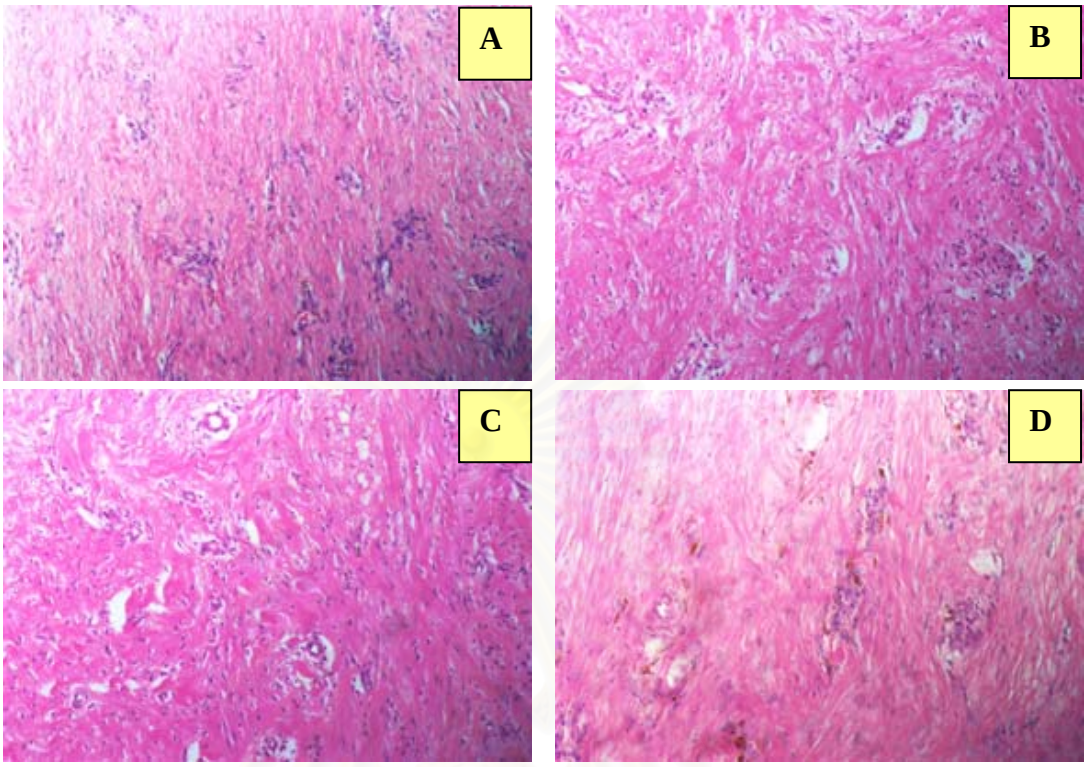

Figure 28. Photomicrographs demonstrated subacute suppurative upper dermatitis of the wounds (Hematoxylin and eosin (HE), × 25 µm). A) control : a wound treated with 1% povidone iodine. B) T_1 : a wound treated with PG dressing film. C) T_2 : a wound treated with PG fiber dressing patch. D) T_3 : a wound treated with 1% povidone iodine and covered with commercial dressing film; Opsite ®Flexigrid. Arrow showed suppurative dermatitis in upper dermis.

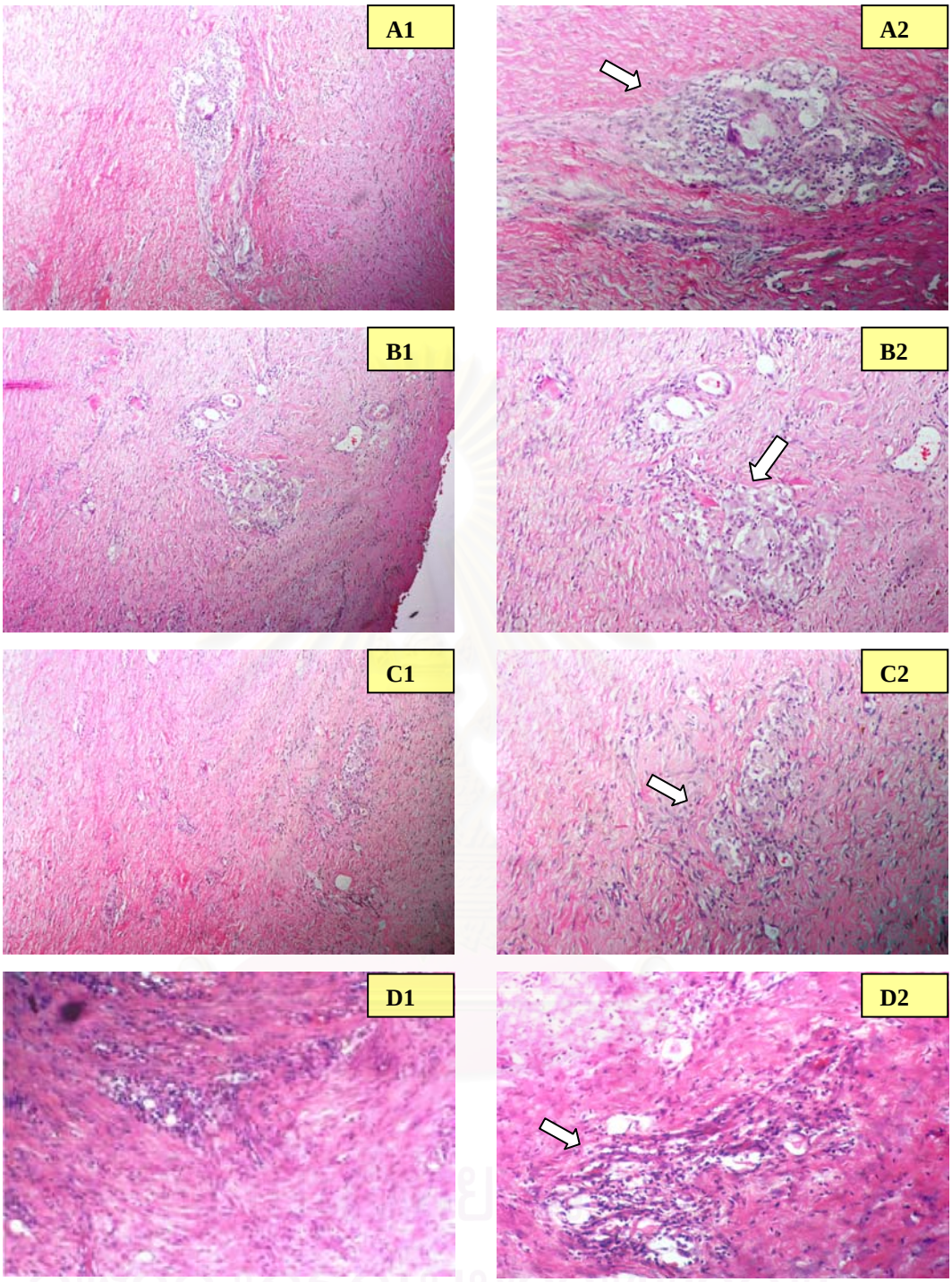

Figure 29. Photomicrographs showed subacute suppurative lower dermatitis with pyogranuloma of the wounds (Hematoxylin and eosin (HE), A1-D1× 25 µm, A2-D2 × 50 µm). A1,A2) control : a wound treated with 1% povidone iodine. B1,B2) T_1 : a wound treated with PG dressing film. C1,C2) T_2 : a wound treated with PG fiber dressing patch. D1,D2) T_3 : a wound treated with 1% povidone iodine and covered with commercial dressing film; Opsite ®Flexigrid. Arrows represented the dermal pyogranuloma in lower dermis.

were no remarkable lesion. Furthermore, some wounds in control showed moderate to severe lesion of dermatitis. The results demonstrated in Figure 23.

Subacute suppurative lower dermatitis with pyogranuloma 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 illustrated in Figure 23 and 29 that wounds treated with 1% povidone iodine (control) showed the highest tissue reaction score. Wounds in T_1, T_2 and T_3 showed no remarkable to mild lesion.

CHAPTER IV

DISCUSSION AND CONCLUSION

Discussion

1. Preparation of Polysaccharide Gel (PG) From Dried Durian Fruit-Hulls

Polysaccharide gel (PG) extracted from dried fruit-hulls of durian was prepared. The viscosity of 2% PG aqueous solution was 248.88 ± 0.98 cps and pH was 2.35 ± 0.04. Whereas, the mixture of 2% PG with 15% propylene glycol had 250.74 ± 0.72 cps viscosity and pH 2.32 ± 0.02. The results indicated that addition of propylene glycol was not much effect to the viscosity and pH of PG dispension. The results were agreed with the previous study by Gerddit, W. 2002 and Nakchat, O. 2002. The properties of PG dispersion were suitable for using in preparation of PG dressing films.

2. Preparation and Evaluation of PG dressing films

The physical characteristics of good film preparation should be thin, smooth and free of any air bubble, uniform thickness, easy to peel from glass plate after drying, flexible and not brittle. In this study, the PG film base and PG dressing film were prepared by casting/solvent evaporation method. The PG film base was a transparent thin film, colorless to pale beige in color, but brittle and not freely flexible. However, the PG dressing film which was adding plasticizer using propylene glycol at 15% w/w based on PG showed a film product with increasing in flexibility and reducing in brittleness. In consideration of the mechanical properties of the film, it is suggested that suitable films for wound dressing should be soft, flexible and tough represented by low to moderate tensile strength, Young's modulus and high % elongation at break and work of failure. However, too low value of % elongation and too high tensile strength made the unsatisfactory film due to the hardness and brittleness of film. The mechanical properties of PG dressing film product showed the higher % elongation, work of failure and lower tensile strength

and Young's modulus than that of PG film base (Table 5). The addition of a plasticizer generally protected the compaction of the polymer macromolecules by reducing the polymeric intermolecular attractions and increasing the free volume of the polymer. This allows the polymeric molecules to move more easily, thereby increasing flexibility (Gutierrez-Rocca and McGinity, 1994). The results were similar to the previous reported by Peh and Wong (1999), Khan, Peh and Ching (2000), Gerddit (2002), Nakchat (2002) and Tachatawepisarn (2003). In this study, the preparation of PG dressing film provided a product that having a suitable mechanical properties for application as a wound dressing film.

The PG dressing film showed the swelling property. PG dressing film swelled in distilled water, the degree of swelling was 33.62%. Maximum swelling of PG dressing film was observed in 1 hour then decrease and achieved plateau in swelling at approximately 7 hours. It can be explained that PG dressing film swelling and absorbing liquid to make an increasing its volume. Solvent penetrates the gel matrix. Intermolecular interactions are replaced by molecule-solvent interactions. Swelling was the initial phase of dissolution. Limited swelling is usually the result of some degree of crosslinking in the gel matrix that prevents total dissolution. The results indicated that PG dressing film swelled and absorbed water to provide the moist wound environment which suitable to promote epithelial cell proliferation and wound healing (Winter, 1962; Dyson, 1988; Vogt, 1995; Ueno, 1999). In addition, PG dressing film was also exhibited bioadhesive property. It was found that PG dressing film presented the higher value of force of adhesion and work of bioadhesion than that of PG film base (Table 6). Addition of propylene glycol as plasticizer has produced a softer and more elastic film as described earlier than PG film base without plasticizer. The increasing in flexibility of the PG dressing film have improved the contact between the film and the tissue, by promoting penetration of the polymeric chains into the tissue to form a strong bonding and leading to increase in the bioadhesion strength. Several mechanisms have been proposed to explain the *in vitro* bioadhesion phenomena including electrical double layers, electrostatic attractions, hydrogen bonding, Van der Waals force, hydrophobic bonding, wetting, diffusion-interpenetration and physical entanglements (Gupta *et al*., 1992). PG is a polyanionic polymer of long chain acidic sugar, galacturonic acid, containing polyanionic number of carboxylic groups that providing the ability to form electric interaction with soft

tissues. From the study, PG dressing film presented not only swelling property but also bioadhesive property which the swelling state of the polymer was reported to be crucial for its bioadhesive behavior. The results indicated that PG dressing film being appropriated for using as a moist wound dressing.

3. Preparation and Evaluation of PG fiber dressing patches

The physical properties of PG fiber dressing patches prepared from 0.6, 0.8, 1 and 1.5% PG aqueous solution were compared. All PG fiber dressing patches were a white and fluffy fiber. PG fiber dressing patches prepared from 0.6 and 0.8% PG aqueous solution were too soft and easily tear off whereas PG fiber dressing patch prepared from 1.5% PG aqueous solution was too stiff and not flexible. From this study, the PG fiber dressing patch prepared from 1% PG aqueous solution was a soft, flexible and not easily broken fiber product. A comparison the density of PG fiber dressing patches prepared from 0.6, 0.8, 1 and 1.5% PG aqueous solution,the density of the PG f iber was increased with the increasing of PG concentration used in freeze-drying. It was noticed that the increasing in density was depending on the concentration of PG in aqueous solution prepared for freeze-drying. The density was used to measure the compactness of the fiber material (the ratio of mass to volume). The higher concentration of PG contained more PG content therefore the higher ratio of mass to volume (density) was obtained. From these results, the PG fiber dressing patch prepared from 1% PG aqueous solution was found suitable for using as a wound dressing. The moisture content of PG fiber dressing patches prepared from 0.6, 0.8, 1 and 1.5% PG aqueous solution were 14.77 ± 0.76, 15.56 ± 0.76, 15.30 ± 0.72 and 15.20 ± 0.87 %, respectively. The results of moisture content determination showed that the moisture content of PG fiber dressing patches prepared from 0.6, 0.8, 1 and 1.5% PG aqueous solution were not significantly different. It may be due to the range of PG concentration was too narrow. The moisture sorption of PG fiber dressing patch was investigated by exposing the PG fiber dressing patches to moisture at 75% relative humidity. The results demonstrated that the moisture sorption of PG fiber dressing patches were increased with respect to increasing of PG content in the fiber dressing patches. These findings indicated that PG fiber dressing patch contain moisture itself (moisture may be present as free water) and can also absorb moisture

to provide the moist wound environment which is beneficial for promoting wound healing (Winter, 1962; Dyson, 1988; Vogt, 1995; Ueno, 1999).

4. Evaluation of PG dressing for wound healing

4.1 Gross Pathology Evaluation

The process of wound healing is composed of 3 phase; inflammatory phase, proliferative phase and remodeling phase. This involving a number of mechanisms, including induction of an acute inflammation, epithelialization, cell migration and proliferation, collagenization and wound strength (Adam, and Richard, 1999). From the previous mention, the process of wound healing may be described as follows: The inflammatory phase process within the third postoperative day, the wound is filled with blood clot, inflammatory cells appear in the wound such as neutrophil and macrophage. The gross lesion of wound is red and swelled and the exudate may observed. Followed by the proliferative phase process within the second week, the granulation tissue progressively invades the wound space. Granulation tissue consists of a combination of cellular elements, including fibroblast and inflammatory cells, along with new capillaries embedded in loose extra cellular matrix of collagen. The end of inflammation process and beginning of fibrosis process which involving in fibroblast proliferation and collagen synthesis, the wound becomes red to pink, not swelling, decrease in size, new tissues and cells fulfill the wound space, the wound bed is shallow and the scab appears. The remodeling phase within the end of the first month, wound has been closed by connective tissue and epithelialization. Fibroblasts shrink, collagen fibers become maturation and rearrangement. The gross lesion of wound is pink, complete wound closure and the scar formation appears (Regan and Barbul, 2000).

In this study, the wound healing process was evaluated every 3 POD. The results demonstrated that the lesions of wound were not apparently different between groups however the wound size in each treatment was different. Wounds treated with 1% povidone iodine were performed slower wound healing than PG dressing treated groups while wounds treated with PG fiber dressing patch showed the fastest of completed wound closure (Table 8). On day 3, all wounds became red, swelling and

the serous exudates were observed (Figure 13). The results indicated that the first phase of wound healing, all wounds were in the inflammatory phase. The swelling of wounds were gradually reduced and observed on day 6. New tissues and cells fulfilled the wound space, the wound bed was shallow (Figure 14). However, wounds in control group showed the less granulation tissue than that of the other treatments. On day 9, 12, 15, 18 and 21 all wounds were red to pink, increased wound contraction and epithelialization, the wound size of all wounds in each group was progressively decreased. These results due to the wounds were in the second phase of wound healing in the proliferative phase. Nakchat (2002) evaluated the effect of PG dressing film compared to conventional treatment (applying 1% povidone iodine and covered with gauze) on wound healing in pig skin, those results are agreeable with the results in this experiment. The results showed that all wounds in dog skin became red and swelling on days 3. The swelling was continuously reduced and observed on day 3 to day 6. The wounds in each group showed progressive decrease in size, increase wound contraction and epithelialization on day 6, 9, 12, 15 and 18 postoperation. A comparison of wound closure rate indicated that wounds treated with PG fiber dressing patch showed the fastest complete wound closure. On day 21 postoperation, all wounds treated with PG fiber dressing patch showed 100% complete wound closure, whereas 1% povidone iodine, PG dressing film, and 1% povidone iodine and covered with Opsite® Flexigrid were complete healing at 50%, 87.50% and 37.50% wound healing, respectively (Table 8 and Figure 20). A significant difference smaller of wound area ($p < 0.05$) of wounds treated with PG dressing film (T_1) and PG fiber dressing patch (T_2) than those of wounds in control and T_3. These results correlated with the data of wound contraction. Wounds in T_1 and T_2 were a significantly more contract ($p < 0.05$) than that of wounds in control and T_3. PG dressing preparations demonstrated the rapid wound closure in this study. The previous study reported that PG dressing film has swelling and moisture sorption properties (Girddit, 2002, Nakchat, 2002 and Tachatawepisarn, 2003) furthermore PG fiber dressing patch showed moisture sorption property in this study. In addition, biological properties of PG has been investigated Lipipun, *et al.*, 2002 and Nantawanit, 2001 that PG showed antibacterial activity against both gram positive and gram negative bacteria. Winter has previously reported in 1962 that wound is healed faster under moist wound environment. According to the fascinating results in this study, PG dressing

preparations has potential to provide moist wound environment as well as inhibit the wound infection. Therefore, wounds treated with PG preparations show faster wound healing and prevent infection eventhough no antiseptic was not applied to those wounds. It was also found in this study that wounds in T_3 were infected if 1% povidone iodine was not used. This study prepared two PG dressing preparations; PG dressing film and PG fiber dressing patch. It was found that PG fiber dressing patch showed the slightly faster wound closure than PG dressing film. A comparison to the study of Nakchat (2002), the two experiment used different animal species, pig and dog, the skin structure is alike. The characteristic of wound is also different so the effect of different dressing preparations on wounds are varied. However, the results indicated that PG dressing preparations both PG dressing film and PG fiber dressing patch were satisfactory used for healing wound better than the traditional treatment (1% povidone iodine) and the commercial dressing film.

4.2 Histopathological Evaluation

As previously mentioned, the wound healing process consists of the 3 overlapping phase of the inflammatory, proliferative and remodeling phase. In this study, the histopathological lesion was evaluated by the day of experiment ended on day 21 postoperation which was the late of proliferative phase, in this phase usually characterized by proliferative fibroblast proliferated and irregular collagen fiber arranged and the cellular response of inflammation was declined. If the presence of inflammatory cells prolonged, the process of wound healing will delay (Sawyer, 2002). The results showed that wounds treated with 1% povidone iodine were the slowest in wound healing. The histopathological lesion of wounds treated with 1% povidone iodine showed epidermitis and epidermal necrosis (Figure 25) which prolonged the inflammatory phase of wound healing, therefore the epidermal regeneration will delay which is result in retardation of wound closure. The results are similar to wounds treated with 1% povidone iodine and covered with commercial film (Opsite® Flexigrid). Whereas, wounds treated with PG dressing film and PG fiber dressing patch presented the least histopathological lesion compared to other groups because the histopathological lesion of wounds treated with PG dressing film and PG fiber dressing patch were mild epidermal regeneration and no remarkable to mild epidermitis (Figure 24 and 25) that providing rapid wound closure with less

inflammation. Bertone *et al.* (1985), Young *et al*. (1991), and Foster *et al*., (1995) have reported that increasing of epidermal regeneration efforted in promoting rapid wound healing. The results correlated with the previous study by Nakchat, 2002, the study on evaluating the PG dressing film on wound healing in pig skin. The results have demonstrated that PG dressing film showed mild to moderate epidermal regeneration and mild inflammatory lesion.

In addition, the results have also demonstrated that wounds treated with PG dressing preparations showed mild dermal fibrosis (Figure 22 and 26). This result was correlated with the previous studied of Nakchat, 2002 that wounds treated with PG dressing film showed mild fibroblast in dermal layer. Furthermore, Young *et al.* (1991), Ueno *et al*. (1999), and Sugihara *et al*. (2000) have reported that the fibroblasts were decreased in remodeling phase, if fibroblast still high, it will promote scar formation. Otherwise, too low fibrosis caused wound closure retardation due to slight wound contraction.

The presence of dermatitis is a cause of wound healing retardation as seen in control group. Wounds treated with 1% povidone iodine showed the highest score of suppurative upper dermatitis while wounds in T_1, T_2 and T_3 were no remarkable to mild lesion (Figure 23). Moreover, wounds treated with PG dressing preparations showed no remarkable lesion of subacute suppurative, lower dermatitis and less of pyogranuloma formation than those wounds treated with 1% povidone iodine and 1% povidone iodine and covered with commercial film (Opsite® Flexigrid) (Figure 23). The formation of pyogranuloma represents the chronic inflammation which is resulted in delay of wound healing. The no remarkable tissue reactions of PG dressing preparation in pig skin wounds have been reported by Nakchat, 2002. PG dressing film was less cytotoxicity, it produced the least tissues reaction by showing no remarkable lesion granuloma formation in dermis layer. The present study was slightly different. The pyogranulomas were occasionally observed in wounds treated with PG dressing preparations less than wounds in control and treatment 3. However, PG dressing preparations treated wounds showed less tissue reaction than that of control (1% povidone iodine). These results indicated that PG dressing preparations showed satisfactory healing wound with minimal inflammation.

Conclusion

Polysaccharide gel (PG) extracted from durian fruit-hulls was prepared as wound dressing preparations; PG dressing film and PG fiber dressing patch. The PG dressing film was produced by casting/solvent evaporation method, the resulting product was transparent thin film, colorless to pale beige in color, soft, though and flexible. The PG dressing film also presented swelling and bioadhesion properties. The PG dressing film can swell and absorb the water to provide the moist wound environment which is beneficial in wound healing. While, the PG fiber dressing patch was prepared by freeze-dryer. The PG fiber dressing patch prepared by freeze-drying of 1% PG aqueous solution provided the most satisfactory dried fiber product. The resulting product was a soft, fluffy, tough, thick fiber and white in color. Study of the property of PG fiber dressing patch was found that PG fiber dressing patch contain moisture (moisture may be present as free water) and also absorbed moisture from environment providing moist environment to the wound which is helpful for healing of wound. In addition, PG dressing can be dissolve in water, it can be remove from wound without trauma. The results indicated that PG dressing film and PG fiber dressing patch being appropriated for using as a moist wound dressing.

Evaluation the efficacy of PG dressing preparations on wound healing in dog skin was investigated. PG dressing preparations presented the satisfactory results. It was found that wounds treated with PG dressing preparations showed rapid wound healing rate, promoted epithelial proliferation and produced minimal tissue reaction. PG preparations showed more rapid wound healing rate than 1% povidone-iodine and 1% povidone-iodine and covered with commercial film (Opsite® Flexigrid). Although, PG fiber dressing patch were slightly faster healing rate than PG dressing film. These data demonstrated that PG preparations showed the properties of ideal wound dressing (Linda, 1998; Biopol, 2002) as following; maintain a moist environment, promote wound healing by reduce the inflammation, promote epithelalization and dermal fibrosis, and less toxicity. Furthermore, they do not adhere to the wound and allow removal without trauma and reduce pain because PG dressing film and PG fiber dressing patch are water soluble dressing that can be remove by washing with saline solution (Nemeth *et al*, 1991; Field and Kerstein, 1994). This study indicated that PG dressing preparations; PG dressing film and PG

fiber dressing patch were effectively used as wound dressing for healing open excisional wounds better than the traditional treatment.

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APPENDICES

APPENDIX A

Reagents

1. 10% neutral buffer formalin solution. In 1 L of solution composed of 100 ml formalin (approximately 40% formaldehyde gas in water called *formalin*), distilled water 900 ml, sodium phosphate 4 g and sodium phosphate diabasic (anhydrous) 6.5 g (Luna, 1968).

2. Eosin stain solution

   2.1 Eosin stock solution was prepared by dissolving 1 g of eosin Y in 20 ml distilled water, and adding 80 ml 95% alcohol.

   2.2 Eosin working solution was prepared by mixing eosin stock solution 1 part and 80% alcohol 3 parts. To 100 ml of the stain solution added glacial acetic acid 0.5 ml and stirred.

3. Harris hematoxylin stain solution

   Harris hematoxylin solution was prepared by dissolving 5 g of hematoxylin crystal in absolute alcohol 50 ml. Aluminium potassium sulfate dodecahydrate 100 g was added in distilled water 100 ml and heated to dissolve. The two solutions were mixed and boiled as rapidly as possible less than 1 min boiled with continuously stirred, removed from heat and added 2.5 g of mercuric oxide (red) slowly, reheated to a simmer and removed from heat immediately after the color of solution became dark purple and the solution was cool in a basin of cold water. In 100 ml of the solution 2-4 ml of glacial acetic acid was added to increase the precision of the nuclear stain. The solution was filtered before use.

APPENDIX B

The procedure for preparation of the tissue processing.

Objective	Reagent	Time (mins.)
Dehydration	80% ethyl alcohol	30
	80% ethyl alcohol	30
	95% ethyl alcohol	30
	95% ethyl alcohol	30
	100% ethyl alcohol	40
	100% ethyl alcohol	40
Clearing	xylene	30
	xylene	30
Infiltration	Melted paraffin	30
	Melted paraffin	30

APPENDIX C

The physical properties of PG fiber dressing patches prepared by freeze dryer

% PG solution of 90 ml used in PG fiber dressing patch (tray area 219.04 cm^2)	Physical properties			
	Thickness (mm)	Volume (cm^3)	Weight (g)	Density (g/cm^3)
0.6	0.284 (0.019)	62.207 (0.316)	0.698 (0.022)	0.0112 (0.012)
0.8	0.306 (0.016)	67.026 (0.426)	0.827 (0.026)	0.0123 (0.011)
1.0	0.366 (0.012)	80.169 (0.673)	1.066 (0.023)	0.0133 (0.016)
1.5	0.414 (0.023)	90.689 (0.497)	1.455 (0.029)	0.0160 (0.020)

APPENDIX D

Statistical analysis of wound area on POD 3.

Oneway

Descriptives

area

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	4	3.27500	.142478	.071239	3.04829	3.50171	3.100	3.410
PG film	4	3.25250	.249182	.124591	2.85600	3.64900	2.960	3.510
PG fiber	4	3.21500	.220530	.110265	2.86409	3.56591	3.010	3.510
Opsite	3	3.23333	.030551	.017638	3.15744	3.30922	3.200	3.260
Total	15	3.24467	.169616	.043795	3.15074	3.33860	2.960	3.510

ANOVA

area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.008	3	.003	.073	.973
Within Groups	.395	11	.036		
Total	.403	14			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: area
LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	PG film	.022500	.133985	.870	-.27240	.31740
	PG fiber	.060000	.133985	.663	-.23490	.35490
	Opsite	.041667	.144720	.779	-.27686	.36019
PG film	control	-.022500	.133985	.870	-.31740	.27240
	PG fiber	.037500	.133985	.785	-.25740	.33240
	Opsite	.019167	.144720	.897	-.29936	.33769
PG fiber	control	-.060000	.133985	.663	-.35490	.23490
	PG film	-.037500	.133985	.785	-.33240	.25740
	Opsite	-.018333	.144720	.901	-.33686	.30019
Opsite	control	-.041667	.144720	.779	-.36019	.27686
	PG film	-.019167	.144720	.897	-.33769	.29936
	PG fiber	.018333	.144720	.901	-.30019	.33686

Statistical analysis of wound area on POD 6.

Oneway

Descriptives

area

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	8	2.7562	.23231	.08214	2.5620	2.9505	2.47	3.18
PG film	8	2.6650	.35781	.12651	2.3659	2.9641	2.21	3.12
PG fiber	8	2.4688	.32678	.11553	2.1956	2.7419	2.05	2.90
Opsite	8	2.6138	.21922	.07750	2.4305	2.7970	2.32	3.03
Total	32	2.6259	.29538	.05222	2.5194	2.7324	2.05	3.18

ANOVA

area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.347	3	.116	1.373	.271
Within Groups	2.358	28	.084		
Total	2.705	31			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: area
LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	PG film	.09125	.14509	.535	-.2060	.3885
	PG fiber	.28750	.14509	.057	-.0097	.5847
	Opsite	.14250	.14509	.334	-.1547	.4397
PG film	control	-.09125	.14509	.535	-.3885	.2060
	PG fiber	.19625	.14509	.187	-.1010	.4935
	Opsite	.05125	.14509	.727	-.2460	.3485
PG fiber	control	-.28750	.14509	.057	-.5847	.0097
	PG film	-.19625	.14509	.187	-.4935	.1010
	Opsite	-.14500	.14509	.326	-.4422	.1522
Opsite	control	-.14250	.14509	.334	-.4397	.1547
	PG film	-.05125	.14509	.727	-.3485	.2460
	PG fiber	.14500	.14509	.326	-.1522	.4422

Statistical analysis of wound area on POD 9.

Oneway

Descriptives

area

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	8	1.66750	.273378	.096654	1.43895	1.89605	1.320	2.140
PG film	8	1.41375	.413416	.146165	1.06813	1.75937	.910	2.140
PG fiber	8	1.38375	.289923	.102503	1.14137	1.62613	1.050	1.900
Opsite	8	1.60625	.205492	.072652	1.43445	1.77805	1.270	1.900
Total	32	1.51781	.314988	.055683	1.40425	1.63138	.910	2.140

ANOVA

area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.472	3	.157	1.693	.191
Within Groups	2.604	28	.093		
Total	3.076	31			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: area
LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	PG film	.253750	.152465	.107	-.05856	.56606
	PG fiber	.283750	.152465	.073	-.02856	.59606
	Opsite	.061250	.152465	.691	-.25106	.37356
PG film	control	-.253750	.152465	.107	-.56606	.05856
	PG fiber	.030000	.152465	.845	-.28231	.34231
	Opsite	-.192500	.152465	.217	-.50481	.11981
PG fiber	control	-.283750	.152465	.073	-.59606	.02856
	PG film	-.030000	.152465	.845	-.34231	.28231
	Opsite	-.222500	.152465	.156	-.53481	.08981
Opsite	control	-.061250	.152465	.691	-.37356	.25106
	PG film	.192500	.152465	.217	-.11981	.50481
	PG fiber	.222500	.152465	.156	-.08981	.53481

Statistical analysis of wound area on POD 12.

Oneway

Descriptives

area

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	8	.80125	.213705	.075556	.62259	.97991	.590	1.250
PG film	8	.63125	.215701	.076262	.45092	.81158	.360	.930
PG fiber	8	.60750	.150784	.053310	.48144	.73356	.420	.800
Opsite	8	.61750	.158272	.055958	.48518	.74982	.430	.800
Total	32	.66438	.195266	.034518	.59397	.73478	.360	1.250

ANOVA

area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.202	3	.067	1.925	.148
Within Groups	.980	28	.035		
Total	1.182	31			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: area
LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	PG film	.170000	.093535	.080	-.02160	.36160
	PG fiber	.193750*	.093535	.048	.00215	.38535
	Opsite	.183750	.093535	.059	-.00785	.37535
PG film	control	-.170000	.093535	.080	-.36160	.02160
	PG fiber	.023750	.093535	.801	-.16785	.21535
	Opsite	.013750	.093535	.884	-.17785	.20535
PG fiber	control	-.193750*	.093535	.048	-.38535	-.00215
	PG film	-.023750	.093535	.801	-.21535	.16785
	Opsite	-.010000	.093535	.916	-.20160	.18160
Opsite	control	-.183750	.093535	.059	-.37535	.00785
	PG film	-.013750	.093535	.884	-.20535	.17785
	PG fiber	.010000	.093535	.916	-.18160	.20160

* The mean difference is significant at the .05 level.

Statistical analysis of wound area on POD 15.

Oneway

Descriptives

area

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	8	.34125	.178521	.063117	.19200	.49050	.130	.620
PG film	8	.21500	.112504	.039776	.12094	.30906	.100	.370
PG fiber	8	.17500	.079462	.028094	.10857	.24143	.090	.320
Opsite	8	.30125	.233265	.082472	.10624	.49626	.030	.760
Total	32	.25813	.168206	.029735	.19748	.31877	.030	.760

ANOVA

area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.140	3	.047	1.777	.174
Within Groups	.737	28	.026		
Total	.877	31			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: area
LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	PG film	.126250	.081107	.131	-.03989	.29239
	PG fiber	.166250*	.081107	.050	.00011	.33239
	Opsite	.040000	.081107	.626	-.12614	.20614
PG film	control	-.126250	.081107	.131	-.29239	.03989
	PG fiber	.040000	.081107	.626	-.12614	.20614
	Opsite	-.086250	.081107	.297	-.25239	.07989
PG fiber	control	-.166250*	.081107	.050	-.33239	-.00011
	PG film	-.040000	.081107	.626	-.20614	.12614
	Opsite	-.126250	.081107	.131	-.29239	.03989
Opsite	control	-.040000	.081107	.626	-.20614	.12614
	PG film	.086250	.081107	.297	-.07989	.25239
	PG fiber	.126250	.081107	.131	-.03989	.29239

* The mean difference is significant at the .05 level.

Statistical analysis of wound area on POD 18.

Oneway

Descriptives

area

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	8	.12125	.101621	.035928	.03629	.20621	.030	.310
PG film	8	.04250	.043342	.015324	.00626	.07874	.000	.120
PG fiber	8	.02875	.023566	.008332	.00905	.04845	.000	.070
Opsite	8	.11000	.103923	.036742	.02312	.19688	.000	.280
Total	32	.07563	.083741	.014803	.04543	.10582	.000	.310

ANOVA

area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.052	3	.017	2.969	.049
Within Groups	.165	28	.006		
Total	.217	31			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: area
LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	PG film	.078750*	.038374	.050	.00014	.15736
	PG fiber	.092500*	.038374	.023	.01389	.17111
	Opsite	.011250	.038374	.772	-.06736	.08986
PG film	control	-.078750*	.038374	.050	-.15736	-.00014
	PG fiber	.013750	.038374	.723	-.06486	.09236
	Opsite	-.067500	.038374	.090	-.14611	.01111
PG fiber	control	-.092500*	.038374	.023	-.17111	-.01389
	PG film	-.013750	.038374	.723	-.09236	.06486
	Opsite	-.081250*	.038374	.043	-.15986	-.00264
Opsite	control	-.011250	.038374	.772	-.08986	.06736
	PG film	.067500	.038374	.090	-.01111	.14611
	PG fiber	.081250*	.038374	.043	.00264	.15986

* The mean difference is significant at the .05 level.

Statistical analysis of wound area on POD 21.

Oneway

Descriptives

area

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
control	8	.05375	.073473	.025976	-.00767	.11517	.000	.200
PG film	8	.00250	.007071	.002500	-.00341	.00841	.000	.020
PG fiber	8	.00000	.000000	.000000	.00000	.00000	.000	.000
Opsite	8	.05375	.059507	.021039	.00400	.10350	.000	.170
Total	32	.02750	.052363	.009257	.00862	.04638	.000	.200

ANOVA

area

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.022	3	.007	3.274	.036
Within Groups	.063	28	.002		
Total	.085	31			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: area
LSD

(I) group	(J) group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
control	PG film	.051250*	.023703	.039	.00270	.09980
	PG fiber	.053750*	.023703	.031	.00520	.10230
	Opsite	.000000	.023703	1.000	-.04855	.04855
PG film	control	-.051250*	.023703	.039	-.09980	-.00270
	PG fiber	.002500	.023703	.917	-.04605	.05105
	Opsite	-.051250*	.023703	.039	-.09980	-.00270
PG fiber	control	-.053750*	.023703	.031	-.10230	-.00520
	PG film	-.002500	.023703	.917	-.05105	.04605
	Opsite	-.053750*	.023703	.031	-.10230	-.00520
Opsite	control	.000000	.023703	1.000	-.04855	.04855
	PG film	.051250*	.023703	.039	.00270	.09980
	PG fiber	.053750*	.023703	.031	.00520	.10230

* The mean difference is significant at the .05 level.

APPENDIX E

Blind analysis of histopathological section in skin of dog after 21 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, T1= treated with PG dressing film, T2 = treated with PG fiber dressing patch and T3 = treated with 1% povidone iodine and covered with commercial dressing film (Opsite® Flexigrid)

Group	Epidermis		Fibrosis	Dermatitis		
	Epidermal hyperplasia and hyperkeratosis	Epidermitis		upper	lower	
						pyogranuloma
control	1.375 ± 0.744	0.5 ± 1.061	1.25 ± 0.463	0.75 ± 1.165	0.75 ± 1.165	0.625 ± 0.916
T1	1.125 ± 0.354	0.000 ± 0.000	1.375 ± 0.516	0.375 ± 0.744	0.25 ± 0.707	0.25 ± 0.354
T2	1.125 ± 0.354	0.375 ± 0.744	1.5 ± 0.535	0.375 ± 0.463	0.000 ± 0.00	0.375 ± 0.463
T3	1.375 ± 0.518	0.75 ± 1.165	1.375 ± 0.518	0.625 ± 0.744	0.625 ± 0.744	0.375 ± 0.535

VITA

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