CLINICAL EFFICACY OF SILK SERICIN DRESSING WITH COLLAGEN FOR SPLIT-THICKNESS SKIN GRAFT DONOR SITE TREATMENT

Miss Apirujee Punjataewakupt

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

A Dissertation Submitted in Partial Fulfillment of the Requirements

for the Degree of Doctor of Philosophy in Pharmaceutical Care

Department of Pharmacy Practice

FACULTY OF PHARMACEUTICAL SCIENCES

Chulalongkorn University

Academic Year 2021

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ประสิทธิศักย์ทางคลินิกของแผ่นปิดแผลเซริซินจากไหมที่ผสมคอลลาเจนสำหรับการรักษาบาดแผลจากการปลูกถ่ายผิวหนังที่มีหนังแท้เหลืออยู่บางส่วน

น.ส.อภิรุจี ปัญจะเทวคุปต์

จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรดุษฎีบัณฑิต
สาขาวิชาการบริบาลทางเภสัชกรรม ภาควิชาเภสัชกรรมปฏิบัติ
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2564
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

Thesis Title	CLINICAL EFFICACY OF SILK SERICIN DRESSING WITH COLLAGEN FOR SPLIT-THICKNESS SKIN GRAFT DONOR SITE TREATMENT
By	Miss Apirujee Punjataewakupt
Field of Study	Pharmaceutical Care
Thesis Advisor	Professor PORNANONG ARAMWIT, Pharm.D., Ph.D.
Thesis Co Advisor	Associate Professor Apichai Angspatt, M.D.

Accepted by the FACULTY OF PHARMACEUTICAL SCIENCES, Chulalongkorn University in Partial Fulfillment of the Requirement for the Doctor of Philosophy

..... Dean of the FACULTY OF PHARMACEUTICAL SCIENCES

(Professor PORNANONG ARAMWIT, Pharm.D., Ph.D.)

DISSERTATION COMMITTEE

..... Chairman

(Assistant Professor Thitima Wattanavijitkul, Ph.D.)

..... Thesis Advisor

(Professor PORNANONG ARAMWIT, Pharm.D., Ph.D.)

..... Thesis Co-Advisor

(Associate Professor Apichai Angspatt, M.D.)

..... Examiner

(Associate Professor CHANKIT PUTTILERPONG, Ph.D.)

..... Examiner

(Assistant Professor NUTTHADA AREEPIUM, Ph.D.)

..... External Examiner

(Associate Professor Sumate Ampawong, Ph.D.)

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อภิรุจี ปัญจะเทวคุปต์ : ประสิทธิศักย์ทางคลินิกของแผ่นปิดแผลเซริซินจากไหมที่ผสมคอลลาเจนสำหรับการรักษาบาดแผลจากการปลูกถ่ายผิวหนังที่มีหนังแท้เหลืออยู่บางส่วน. (CLINICAL EFFICACY OF SILK SERICIN DRESSING WITH COLLAGEN FOR SPLIT-THICKNESS SKIN GRAFT DONOR SITE TREATMENT) อ.ที่ปรึกษาหลัก : ศ. ภญ. ดร.พรอนงค์ อร่ามวิทย์, อ.ที่ปรึกษาร่วม : รศ. นพ.อภิชัย อังสพัทธ์

แผ่นปิดแผลเซริซินจากไหมผสมคอลลาเจนได้ถูกนำมาทดสอบในการศึกษาทางคลินิก 2 การศึกษา ในการศึกษาแรกเป็นการทดสอบความปลอดภัยทางคลินิก โดยใช้การทดสอบการเกิดผื่นแพ้สัมผัสในอาสาสมัครสุขภาพดีจำนวน 103 คน พบว่าผลที่ได้ส่วนใหญ่ (ประมาณร้อยละ 90) คือไม่มีปฏิกิริยาหรือมีปฏิกิริยาที่ไม่ชัดเจน และผลที่เหลืออีกร้อยละ 10 คือมีผลบวกอย่างอ่อน หลังจากนั้นจึงได้ทดสอบประสิทธิศักย์และความปลอดภัยของแผ่นปิดแผลต่อในคนไข้จำนวน 21 คนที่มีบาดแผลจากการปลูกถ่ายผิวหนังที่มีหนังแท้เหลืออยู่บางส่วนจำนวน 30 บาดแผล โดยแต่ละบาดแผลได้ถูกแบ่งออกเป็น 2 ส่วนที่มีขนาดเท่ากัน ก่อนที่จะปิดทับแต่ละส่วนโดยการสุ่มว่าจะปิดด้วยแผ่นปิดแผลเซริซินจากไหมผสมคอลลาเจนหรือแผ่นปิดแผลที่มีในท้องตลาด (แบคทิกราส) ผลที่ได้พบว่าแผ่นปิดแผลเซริซินจากไหมผสมคอลลาเจน (15.00 ± 7.00 วัน) สามารถลดเวลาการหายของบาดแผลได้อย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับแผ่นปิดแผลแบคทิกราส (16.00 ± 8.00 วัน) และได้มีการพิจารณาคุณภาพของแผลเป็นโดยใช้ทั้งการประเมินจากความคิดเห็นซึ่งประกอบด้วยแบบประเมินแผลเป็นแวนคูเวอร์และแบบประเมินในส่วนของคนไข้ และการประเมินโดยใช้เครื่องมือซึ่งประกอบด้วยการประเมินสีผิว เกราะป้องกันผิวหนัง ความชุ่มชื้นของผิวหนัง และความยืดหยุ่นของผิวหนัง พบว่าผลจากการประเมินหลังจากแผลหายทันทีและใน 1 3 และ 6 เดือน มีความสอดคล้องกันคือแผ่นปิดแผลเซริซินจากไหมผสมคอลลาเจนสามารถเพิ่มคุณภาพของแผลเป็นได้ โดยแผลเป็นในกลุ่มของแผ่นปิดแผลเซริซินจากไหมผสมคอลลาเจนมีความดำและความแดงที่น้อยกว่า และฝั่งที่เคยรักษาด้วยแผ่นปิดแผลเซริซินจากไหมผสมคอลลาเจนก็มีความยืดหยุ่นของผิวหนังจากแบบประเมินแผลเป็นแวนคูเวอร์ที่ดีกว่าฝั่งที่รักษาด้วยแผ่นปิดแผลแบคทิกราส นอกจากนี้แผ่นปิดแผลเซริซินจากไหมผสมคอลลาเจนยังอาจช่วยให้เกิดการฟื้นฟูการทำงานของผิวรวมถึงการเจริญเต็มที่ของแผลเป็นได้ดีขึ้น โดยยังพบอีกว่าคนไข้มีความพึงพอใจในแผลเป็นฝั่งที่เคยรักษาด้วยแผ่นปิดแผลเซริซินจากไหมผสมคอลลาเจนมากกว่าฝั่งที่รักษาด้วยแผ่นควบคุม สำหรับผลการประเมินความเจ็บปวด พบว่ามีค่าค่อนข้างต่ำและไม่แตกต่างกันระหว่างแผ่นปิดแผลทั้งสองชนิด และไม่พบการติดเชื้อรวมถึงผลข้างเคียงจากการใช้แผ่นปิดแผลทั้งสองชนิด ด้วยคุณสมบัติในการช่วยควบคุมความชื้นและผลการเสริมฤทธิ์ทางเภสัชวิทยาของเซริซินจากไหมและคอลลาเจนอาจช่วยส่งเสริมการหายของบาดแผลจากการปลูกถ่ายผิวหนังที่มีหนังแท้เหลืออยู่บางส่วนจึงทำให้มีการหายของแผลที่เร็วขึ้นและแผลเป็นที่ได้มีคุณภาพที่ดี จากผลที่ได้นั้นอาจสรุปได้ว่าแผ่นปิดแผลเซริซินจากไหมผสมคอลลาเจนสามารถนำมาใช้เป็นแผ่นปิดแผลทางเลือกสำหรับการรักษาบาดแผลจากการปลูกถ่ายผิวหนังที่มีหนังแท้เหลืออยู่บางส่วนได้

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สาขาวิชา	การบริบาลทางเภสัชกรรม	ลายมือชื่อนิสิต .....
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		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5776556233 : MAJOR PHARMACEUTICAL CARE

KEYWORD: Silk sericin, Collagen, wound dressing, clinical efficacy, clinical safety, Split-thickness skin graft donor site

Apirujee Punjataewakupt : CLINICAL EFFICACY OF SILK SERICIN DRESSING WITH COLLAGEN FOR SPLIT-THICKNESS SKIN GRAFT DONOR SITE TREATMENT. Advisor: Prof. PORNANONG ARAMWIT, Pharm.D., Ph.D. Co-advisor: Assoc. Prof. Apichai Angspatt, M.D.

Sericin dressing containing collagen hydrolysate (SDC) was evaluated in this study, consisting of two phases of clinical trials. The clinical safety of SDC was determined using a patch test in 103 healthy volunteers (phase I). Most of the results (around 90%) in the SDC group were interpreted as negative and doubtful reactions, and the remaining results (around 10%) were weak reactions. After that, the clinical efficacy and safety of SDC were investigated in 21 patients with 30 split-thickness skin graft (STSG) donor sites (phase II). Each donor site was divided into two equal sites. SDC or the commercial dressing (Bactigras®) was randomly applied as the primary dressing at each site. It was found that SDC (15.00 ± 7.00 days) significantly reduced the wound healing time when compared to Bactigras® (16.00 ± 8.00 days), p=0.015. The scar quality after complete healing were also examined by both subjective (Vancouver scar scale; VSS and patient part of Patient and Observer Scar Scale; POSAS) and objective methods (Mexameter®, Tewameter®, Corneometer®, and Cutometer®). The findings from all measurements at 0, 1, 3, and 6 months seemingly agree that SDC could improve the scar quality. The darkness and redness of the scar appear to be better in the SDC group. The scar pliability evaluated by VSS in SDC treated sites was also preferable to Bactigras® treated sites. Moreover, the recovery of epidermal functions and scar maturation might be enhanced by SDC. The patient's satisfaction in SDC treated site also seems to be superior to the control-treated site. The pain scores in both dressings were relatively low and comparable. There was no infection and adverse effects in both dressings' applications. The moisture control and the synergistic effects from sericin and collagen hydrolysate might facilitate STSG donor healing, leading to faster healing and better scar quality. Accordingly, it can be concluded that SDC is possibly used as the alternative dressing for STSG donor sites.

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Field of Study:	Pharmaceutical Care	Student's Signature .....
Academic Year:	2021	Advisor's Signature .....
		Co-advisor's Signature .....

ACKNOWLEDGEMENTS

Foremost, I would like to express my sincere gratitude to my advisor, Professor Pornanong Aramwit, Pharm.D., Ph.D., Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, and my co-advisor, Associate Professor Apichai Angspatt, M.D., the Division of Plastic and Reconstructive Surgery, Department of Surgery, Faculty of Medicine, Chulalongkorn University for the continuous support, invaluable guidance, and constant encouragement throughout my study period. Besides, I would like to extend my gratitude to the rest of my thesis committee: Assistant Professor Thitima Wattanavijitkul, Ph.D., Associate Professor Chankit Puttilerpong, Ph.D., Assistant Professor Nutthada Areepium, Ph.D., Associate Professor Sumate Ampawong, Ph.D. for their insightful comments and helpful suggestions. I would like to gratefully acknowledge the financial support from the Thailand Research Fund through Research and Researcher for Industry Program (RRi) under Grant No. PHD58I0006 to Apirujee Punjataewakupt and Pornanong Aramwit. My sincere thanks also go to the healthy volunteers and patients who participated in Phase I and Phase II clinical studies as well as the clinical staff and the plastic surgery residents at King Chulalongkorn Memorial Hospital who facilitated me during the research process. Last but not the least, I would like to thank my family and my friends for supporting and encouraging me throughout my entire life.

Apirujee Punjataewakupt

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
ALP	Aspartate aminotransferase
ALT	Alanine aminotransferase
ALT flap	Anterolateral thigh flap
AST	Aspartate aminotransferase
BMI	Body mass index
BSE	Bovine Spongiform Encephalopathy
BUN	Blood urea nitrogen
CH	Collagen hydrolysate
ECM	Extracellular matrix
FMD	Foot-and-mouth disease
FTSG	Full-thickness skin graft
ICC	Intraclass correlation coefficient
ICDRG	International Contact Dermatitis Research Group
MCID	Minimal clinically important difference
MMPs	Matrix metalloproteinases
NMF	Natural moisture factors
PHMB	Polyhexamethylene biguanide

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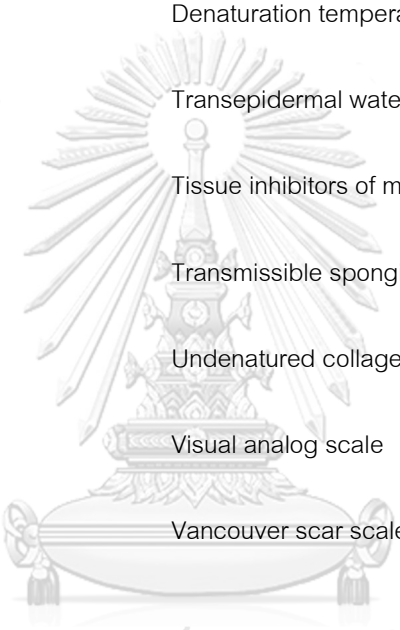
POSAS	Patient and Observer scar assessment scale
PVA	Polyvinyl alcohol
SC	Stratum corneum
SDC	Sericin dressing containing collagen hydrolysate
STSG	Split-thickness skin graft
T_d	Denaturation temperature
TEWL	Transepidermal water loss
TIMPs	Tissue inhibitors of metalloproteinases
TSE	Transmissible spongiform encephalopathy
UC	Undenatured collagen
VAS	Visual analog scale
VSS	Vancouver scar scale



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CHAPTER I
INTRODUCTION

1.1 Background and Rationale

Wounds are a public health problem found worldwide. More than the billions of dollars are spent as an annual cost of wound care in the healthcare system, especially for chronic wounds [1, 2]. Moreover, the wound complications can lead to amputation or fatality, affecting patients' quality of life and expenditure [3, 4]. The faster healing may prevent wound infection and other complications because skin functions as a barrier, protecting the human body from the outer environment and microorganisms. The large or deep wounds that cannot be closed spontaneously may require additional interventions to facilitate the healing.

Skin grafts are the reconstructive techniques that harvest healthy tissue from the intact area, called the donor site. The taken tissue would be used for temporary or permanent covering at the defected area, called the recipient site. Split-thickness skin graft (STSG) is a standard treatment for wounds that cannot be closed primarily and those that are contraindicated for secondary closure such as full-thickness burns, wounds after excision of aggressive tumors, and chronic ulcers [5, 6]. In order to perform STSG, the entire of epidermis and portion of dermis would be collected by dermatome or knife, resulting in inevitable open wounds on donor sites. Although the STSG donor site could heal spontaneously within 2-3 weeks [7], the problematic issues caused by STSG donor sites are recently established, including pain, scar formation, wound infection, and impaired quality of life [8, 9]. Therefore, the appropriate treatment on the STSG donor site should be emphasized to prevent possible complications and improve the healing quality.

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Wound dressing plays an essential role in STSG donor site care, protecting from attack, preventing infection, reducing pain, and managing exudate [7, 10]. Several dressings are used to treat STSG donor sites; however, those dressings still have some limitations, e.g., ability to control environment, mechanical properties, ease of use and removal, biocompatibility, biodegradability, and affordability cost. Therefore, there is still no ideal wound dressing for STSG donor sites treatment [11]. Among wound dressings, the hydrogel has the potential to become an ideal wound dressing [12, 13] because of its several advantages such as healing acceleration, relieving pain, conformity,

compatible interface [14], less adherence [12], and being drug carrier [15]. The limitations of hydrogel dressing seem to be the poor mechanical properties and less absorption capacity. Moreover, there are still only a few hydrogel dressings containing bioactive substances available nowadays, and the cost of those hydrogels seems to limit the accessibility of patients. Thus, developing a new hydrogel dressing containing bioactive components, which has suitable mechanical strength and exudate management, may overcome the existing limitations.

Polyvinyl alcohol (PVA) is a biodegradable and biocompatible synthetic polymer, which is seemly one of the promising materials for biomedical devices [16]. The advantages of PVA consist of good mechanical properties, controllable properties, protein-binding properties, and versatile properties. PVA has been fabricated as wound dressing by several methods such as freeze-drying, freeze-thawing, electrospinning, chemical crosslinking, and cast drying. PVA can physically form tough and transparent hydrogel using the cast drying method without crosslinking agent addition [17]. The method is seemly economical and straightforward. The disadvantages of other methods could be diminished, which are the opaque appearance, cytotoxicity from the crosslinking agent, and requirement for the specific device. However, the prepared PVA hydrogel has insufficient elasticity [18], is relatively brittle [19], and has less bioactive properties. The addition of protein, which is a natural polymer, into the synthetic polymer could influence the mechanical properties and improve the bioactive properties of PVA hydrogel. Accordingly, the addition of bioactive protein into PVA hydrogel possibly enhances its use as a wound dressing.

Sericin is the natural glue protein contained in a silk cocoon. It acts like a glue that combines another silk protein called fibroin, forming the cocoon [20]. Sericin consists of several amino acids with sizes ranging from 20 to 200 kDa. It is a waste product in the textile industry that can lead to environmental pollution. Several studies show the benefits of sericin, including anti-inflammatory, antioxidant, anti-melanogenesis, and wound healing enhancement [21-25]. It has biocompatibility, biodegradability, and less immunogenicity. Because of those benefits, sericin has been used in several biomedicals, pharmaceuticals, cosmetics, food, and wound dressing [25-27]. However, the main limitation of sericin is poor mechanical strength [28, 29]. Sericin film has been prepared by cast drying method, but its drawback was the brittleness of film [30]. The mechanical properties of sericin might be modified by interaction and copolymerization with other polymers via

its various polar groups [31]. Several polymers were combined with sericin to improve its mechanical properties, including polyvinyl alcohol (PVA). Wound dressings consisting of sericin and polyvinyl alcohol (PVA) have been fabricated by using freeze-thawing [31], freeze-drying [32, 33], and gamma radiation [34]. Although several dressings containing sericin have been developed, only a few clinical studies have evaluated the effects of sericin dressing in human wounds. Moreover, the inconsistency of results in the reduction of the wound healing time is still suspected.

Collagen is the primary protein found in tissues, bone, cartilage, tendon, ligament, and teeth of living creatures, including humans. It is the main component of the extracellular matrix (ECM) and functions as a structural scaffold giving strength and flexibility to organs and tissues [35, 36]. Moreover, collagen has biocompatibility, biodegradability, and low immunological response [37]. It plays a crucial role in all phases of the wound healing process [36, 38]: hemostasis [37], inflammation, proliferation, and remodeling. Therefore, collagen has been extensively used in biomedical applications, including wound dressing [12]. Several studies also have established the benefits of collagen on animals [39, 40] and human wound healing [41-44]. There are several sources of collagen, such as porcine, bovine, and marine sources. Due to the religious constraints and risk of Bovine Spongiform Encephalopathy (BSE), fish collagen seems to be the potential source of collagen [45, 46]. Collagen can be divided into undenatured collagen, partially hydrolyzed collagen (gelatin), and collagen hydrolysate. Although undenatured collagen could be fabricated as a scaffold, the denaturation temperature (Td) is relatively low, especially for fish collagen, leading to unstable integrity at the temperature of the mammalian body [47, 48]. Moreover, the processes for extraction and formulation of undenatured collagen are relatively complicated. In contrast, collagen hydrolysate (CH) is a small peptide fragment (molecular weight, M.W. 1-10 kDa) of collagen that can be extracted by a straightforward method. It also provides advantages on wound healing [40]. It is a very soluble substance leading to easy formulation, but it cannot form film or scaffold by itself [46]. Accordingly, incorporating CH into other polymers may facilitate the application of collagen and preserve its bioactive properties. Moreover, based on the recent articles, there is still a lack of evidence for the effects of collagen hydrolysate applied topically in the human wound [49-51].

According to the literature, sericin and collagen seemly enhance each other benefits on wound healing [52, 53]. In addition, the interaction between sericin and collagen seems to increase

protein stability [54]. The biomedical products containing both sericin and collagen have been fabricated using the cast drying and freeze-drying method. However, glutaraldehyde is needed as the crosslinking agent in those products [55-58]. Although the safety of products was illustrated in those studies, the possibility of harming the biological tissue of the chemical crosslinking agent should still be concerned [18]. Based on current knowledge, there is scarce wound dressing containing collagen hydrolysate and sericin prepared without a crosslinking agent because of their low mechanical properties. Consequently, the dressing in the current study used PVA as a base for preparing the physically crosslinked hydrogels containing sericin and CH by the cast drying method. It might bring about the moist wound dressing with the pharmaceutical effects on wound healing. Protein release was evaluated in the in vitro study showing the sustained release of protein from dressing along 168 h. The safety of dressing was also evaluated in L929 mouse fibroblast. It was found that silk sericin dressing with collagen showed no cytotoxicity comparing with positive control. Moreover, the wound dressing has transparent appearance with good mechanical properties. Therefore, the prepared wound dressing would be further examined for its clinical safety and efficacy in this study.

The clinical safety of wound dressing would be determined by using patch test in healthy volunteers. The irritation on the attached skin would be investigated by using Mexameter and evaluation of the dermatologists. The results from the attachment of the prepared dressing would be compared with the commercial dressing. Then, the clinical efficacy would be evaluated in patients' wounds if the safety was found in healthy volunteers.

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To evaluate the clinical efficacy of wound dressing, wound healing time is considered the primary outcome in wound care. While the scar quality after complete healing is also one of the essential goals of treatment [59, 60], affecting both functional and psychological problems of patients, especially for the pathological scar [61]. The recent review states that the long-term scar outcomes of donor sites at 3 and 6 months are still the gap, especially for objective assessment outcomes [8]. Moreover, the opinion of the patient on scar outcome is a vital issue for patient-centered care. Patients' opinion is seemly less consistent with observer opinion [62, 63], and it was determined in only a few studies [64]. Scar outcome evaluated by patients was therefore suggested to be included in donor site treatment study [8, 64, 65] in addition to the evaluation by clinician and

objective device. The current study examined the effects of prepared wound dressing on the healing time, scar quality, and other morbidities of STSG donor sites, including pain score, infection, and adverse effects, compared with the commercial dressing.

1.2 Research questions

Phase I

1. Does sericin dressing with collagen change the melanin and erythema level of the healthy volunteer's skin compared with the Bactigras®?
2. How does sericin dressing with collagen result in the irritation responses evaluated by dermatologists on the healthy volunteer's skin compared with the Bactigras®?

Phase II

Primary research question

1. Does the sericin dressing with collagen reduce the complete healing time of the STSG donor site compared with Bactigras®?

Secondary research questions

1. Does the sericin dressing with collagen improve scar quality of STSG donor site compared with Bactigras®?
    a. Does the sericin dressing with collagen decrease the VSS score compared with Bactigras®?
    b. Does the sericin dressing with collagen decrease the POSAS score compared with Bactigras®?
    c. Does the sericin dressing with collagen reduce the scar darkness compared with Bactigras®?
    d. Does the sericin dressing with collagen reduce the scar redness compared with Bactigras®?
    e. Does the sericin dressing with collagen group promote the skin barrier function compared with the Bactigras® group?

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f. Does the sericin dressing with collagen promote the water holding capacity compared with Bactigras®?

g. Are there any differences in skin elasticity from normal skin in the sericin dressing with collagen and Bactigras® groups?

2. Does the sericin dressing with collagen reduce pain score on the STSG donor site compared with Bactigras®?
3. Is there any incidence of infection in the STSG donor site treated with sericin dressing with collagen and Bactigras®?
4. Is there any adverse effect on the patient treated with sericin dressing with collagen and Bactigras®?

1.3 Objectives

Phase I

1. To evaluate the safety of sericin dressing with collagen on the skin of healthy volunteers compared with Bactigras®

Phase II

Primary objective

1. To examine the complete healing time of STSG donor site treated with sericin dressing with collagen comparing with Bactigras®

Secondary objectives

1. To determine the scar quality of STSG donor site treated with sericin dressing with collagen comparing with Bactigras®
2. To evaluate the pain control in STSG donor site treated with sericin dressing with collagen comparing with Bactigras®
3. To estimate the incidence of infection in STSG donor site treated with sericin dressing with collagen comparing with Bactigras®
4. To investigate the adverse effect, including local and systemic effects obtained from the application of sericin dressing with collagen and Bactigras®

1.4 Hypotheses

Phase I

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1. The sericin dressing with collagen group has comparable safety on healthy volunteers' skin compared with the Bactigras® group.

Phase II

Primary hypothesis

1. The sericin dressing with collagen group has a lower complete healing time for treating the STSG donor site than the Bactigras® group.

Secondary hypotheses

1. The sericin dressing with collagen group has better scar quality compared with the Bactigras® group.
    a. The sericin dressing with collagen group has a lower VSS score compared with the Bactigras® group.
    b. The sericin dressing with collagen group has a lower POSAS score compared with the Bactigras® group.
    c. The sericin dressing with collagen group has a lower melanin level compared with the Bactigras® group.
    d. The sericin dressing with collagen group has a lower erythema level compared with the Bactigras® group.
    e. The sericin dressing with collagen group has lower TEWL compared with the Bactigras® group.
    f. The sericin dressing with collagen group has a higher hydration level compared with the Bactigras® group.
    g. The skin elasticities in sericin dressing with collagen and Bactigras® groups were comparable with normal skin.
2. The sericin dressing with collagen group has a lower pain score on the STSG donor site treatment than the Bactigras® group.
3. There is no difference in the incidence of infection between the STSG donor site treated with sericin dressing with collagen and Bactigras®.
4. There is no adverse effect obtained from the application of sericin dressing with collagen and Bactigras®.

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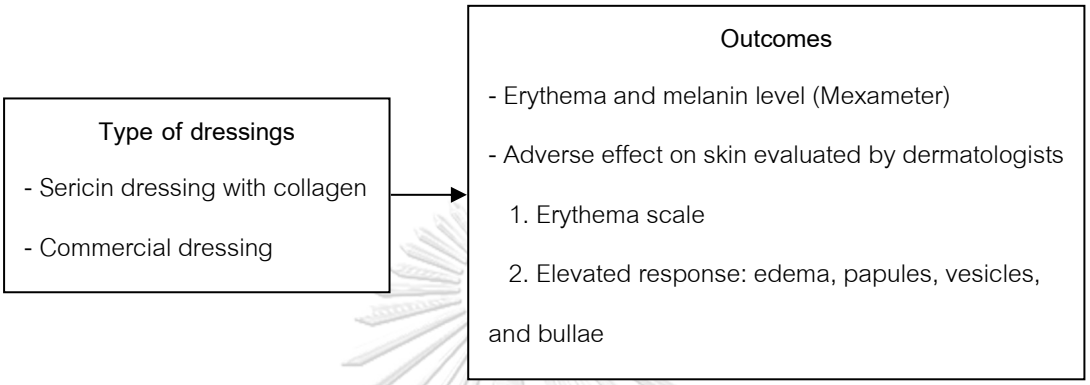
1.5 Conceptual framework

Phase I



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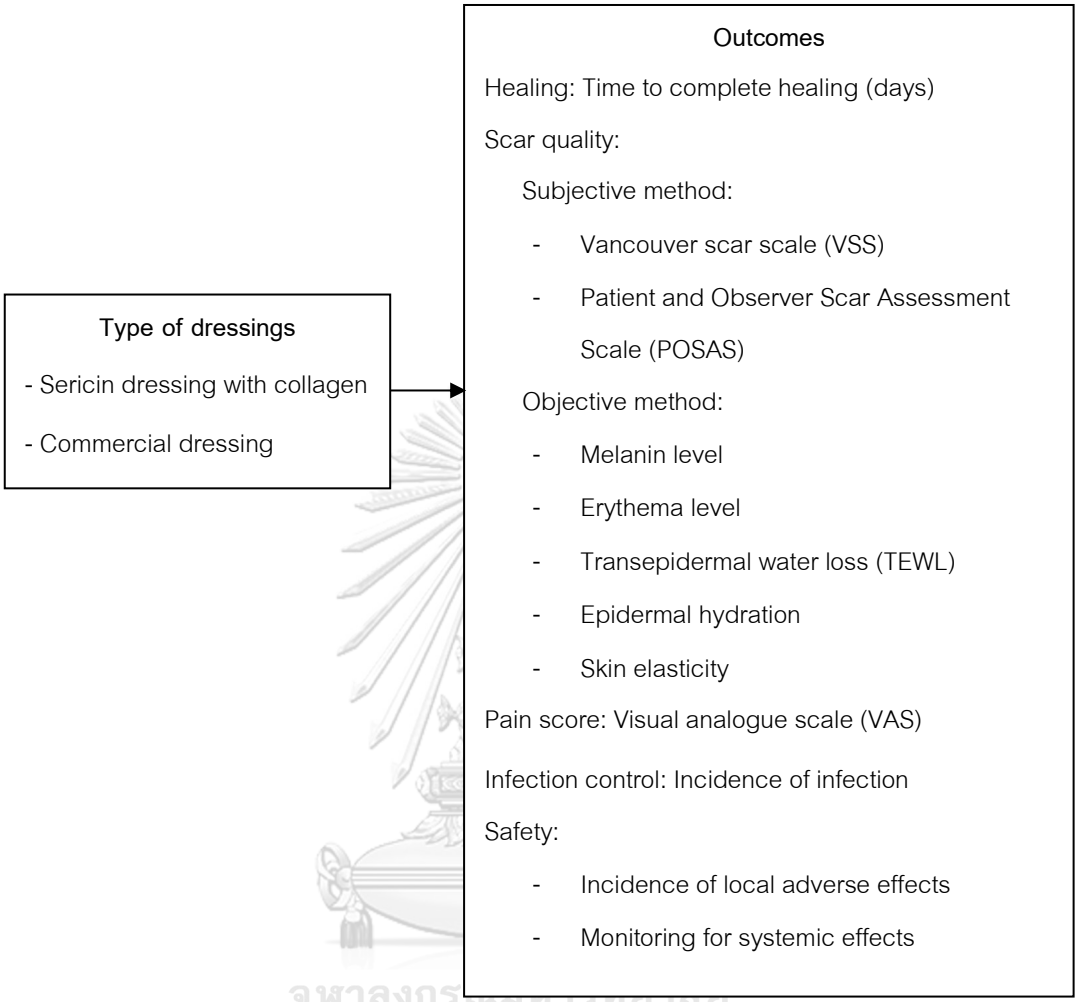
Phase II



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CHAPTER II
LITERATURE REVIEW

2.1 Skin grafts and STSG donor site management

Skin grafts

Skin grafts are the reconstructive techniques performed more than 3,000 years ago in India [5, 66]. The purpose of skin graft is to cover the extensive or deep wounds that cannot be closed by primary closure and are contraindicated for secondary closure. The defects commonly treated by skin grafts are wounds from the surgical removal of skin cancers, full-thickness burns, chronic ulcers, and hair restoration in patients with alopecia [5]. Those wounds should have healthy granulation tissue with adequate blood supply and no infection. For wounds with inadequate blood supply, exposed bone, cartilage, tendon, and fibrotic chronic granulation tissue, more complex reconstructive techniques such as skin flaps or muscle flaps are required [67].

Terminology of skin graft classified by the source of grafts [5, 6]

- *Autograft* is a graft taken from one part of an individual's body and grafted onto a different part of the same individual's body.
- *Isograft* is a graft taken from the genetically identical donor and recipient individuals, such as litter mates of inbred rats or identical human twins.
- *Allograft (Homograft)* is a graft taken from one individual and grafted onto another individual of the same species.
- *Heterograft (Xenograft)* is a graft taken from one species and placed onto a different species.

Skin grafts can be divided into 2 groups depending on the depth of grafts, as presented in Figure 1.

1. Split-thickness skin graft (STSG) contains all of the epidermis and varying thickness of dermis (0.006 to 0.024 inches in thickness) [67]. STSG can be harvested from large donor sites, including thighs, buttocks, and back [68], considered from color, texture, thickness, amount of skin required, and scar visibility [67]. The graft can be harvested from the donor site using a dermatome or Humby

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knife (Watson knife), which can adjust for the depth and width of the graft. The taken graft can be expanded by several techniques such as pinch grafts, relay transplantation, meshing, meek island grafts, microskin grafts, and the Chinese intermingling autografts and allografts (6). The advantage of STSGs is that this graft can be taken easier than FTSGs; even the wound failed to take FTSGs. Its disadvantages are more significant wound contracture, susceptibility to trauma, and pigment abnormality (6).

2. Full-thickness skin graft (FTSG) consists of the all dermis and epidermis with portions of the sweat glands, sebaceous glands, and hair follicles [67]. FTSGs are harvested by scalpel, and then all fat has to be removed with scissors before placing on recipient sites. The donor sites can be closed by primary sutures or skin grafts. Due to the greater thickness, FTSG can better prevent trauma compared with STSG. Moreover, contraction during healing on wounds treated with FTSG is less than STSG. Therefore, FTSG gives a better cosmetic outcome. However, there is limited area for FTSFs donor sites, so FTSGs should be used only for minor defects on the face, hands, and feet [68]. Moreover, FTSGs need re-vascularization from recipient sites with sufficient blood supply to be taken [6, 67].

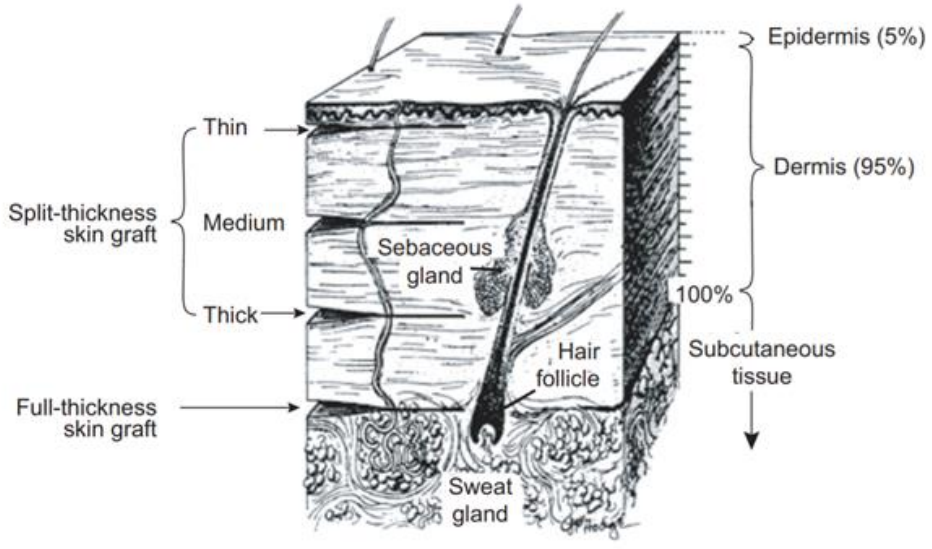

Figure 1 The anatomy of skin [69]

STSG donor site management [6]

Wounds occurred from grafts harvesting are called donor site wounds. The STSG donor site is considered a standard wound because the size and depth of STSG donor sites are controllable and matchable. The equally dividing in the same wound of the individual patient could be done. Therefore, the factors affecting wound healing, e.g., age, comorbidities, and nutrition, are diminished. STSG donor site has been used for examining the effects of treatment on wound healing in several studies [70, 71]. Moreover, the results found in STSG donor site healing might be extrapolated to other wounds with similar characteristics, such as partial-thickness burns and abrasion wounds.

Usually, STSG donor site wounds should be healed by re-epithelialization between 7 to 21 days, depending on the thickness of the collected graft [7, 68]. The standard treatment for STSG donor sites has not been established. The characteristics of STSG donor sites are sterile wounds, high exudation, equal thickness, and usually pain, and the goal for donor site management consists of rapid epithelialization, minimum pain, infection control, and well scar quality. The donor site management can be classified as follows.

1. Open wound techniques

Leaving wounds open allows for completely wound monitor; however, it is associated with delayed healing, a higher risk of infection, and increased pain.

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2. Biological dressings

Autografts

The excess harvested skin can be used for treating donor sites [72]. However, there are some questions about the expense and the careful planning for harvesting only an adequate amount of graft.

Allografts

Cadaver skin could temporarily cover wounds to reduce pain, restore function, control fluid loss, and promote wound healing. However, the limitation of these allografts and the expense are still its drawback.

Xenografts

Xenografts (collagen–elastin prostheses) have relatively low cost, ready availability, easy storage, and easy sterilization; however, there is a lack of antimicrobial activity and potential for absorption of toxic degradation products. Moreover, there is no proof for re-epithelialization promotion and pain reduction when compared with other dressings.

3. Synthetic dressings

Due to donor sites being standard wounds, various kinds of dressing can be used for donor sites [73] management, which can be classified as follows: [7, 74, 75]

1. Traditional dressing: gauze, bandages, tulles, and absorbent pads

These dressings have a lower cost and are commonly found; however, they can only protect the wound from the outer environment without moisture control properties. They are easily managed but may cause more pain to the patient [7].

2. Advance wound dressings

Several kinds of advanced dressings are developed for healing enhancement properties, such as moisture control and drug release control.

- Film dressings: Opsite™ (Smith & Nephew), Tegaderm™ (3M)

The transparent polyurethane dressings are semi-occlusive and permeable to air and water vapor but impermeable to fluid and bacteria. They can create a moist environment for granulating wounds; however, the excess fluid trapped by films may lead to maceration of wound edges, and modification is needed [76]. Because films are thin, flexible, transparent, they easily conform to the patient's body and allow for wound monitoring [77]. When treating donor sites with polyurethane dressing, the exudate may accumulate beneath the dressing which can be solved by aspiration with a needle and syringe [78].

- Hydrogel dressings: FlexiGel™ (Smith & Nephew), Nu-gel® (Systagenix), Tegagel™ (3 M)

The main components of hydrogels are water or glycerin. Hydrogel has the potential to become an ideal dressing [12, 13] due to its several advantages [14]. Most hydrogel dressings are transparent, allowing for wound visualization. The dosage form can be gel or sheet, which can maintain a moist environment for wounds. Moreover, hydrogel gives cooling effects to wound, resulting in the reduction of pain. Hydrogels also have autolytic debridement properties. They can

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use in various types of wounds such as pressure sores, surgical wounds, and burns. Due to high water content, hydrogels are suitable for wounds with mild to moderate exudate. Furthermore, hydrogels can be used as drugs carriers to provide local releasing of bioactive substances to wound beds [12, 15]. In donor site treatment, hydrogels can reduce pain and absorb some fluid excreted from wounds [12, 13]. Due to hydrogels being usually non-adherent to the wounds [12], hydrogels can be removed painlessly [60, 79]. However, excess fluid leakage may be found because of their limitation of absorption capacity [79].

- Hydrocolloid dressings: Duoderm® (ConvaTec), Cutinova® (Smith & Nephew)

Hydrocolloids contain polymers with absorption properties such as carboxymethylcellulose, pectin, and gelatin which can be in the form of pastes and powders. When contact with water, hydrocolloids absorb water and form a gel, which can adhere to wounds. Hydrocolloids can absorb mild to moderate exudate and also create autolytic debridement similar to hydrogels. Moreover, hydrocolloids have occlusive properties, so water, oxygen, and bacteria cannot penetrate wounds. Due to the opaque nature of dressings, wound monitoring is unable to perform without dressing removal. Hydrocolloids accelerated donor site healing; however, there are some drawbacks: the time required for applying the dressing, the chance of exudate leakage, and the cost of dressing [80].

- Hydrofiber dressings: Aquacel® (ConvaTec)

Hydrofibers have a high-water absorption capacity (around 25 times their weight). They consist of sodium carboxymethyl cellulose, which turns gel when contacting with wound fluid, giving a moist environment and autolytic properties to wounds. The risk of maceration is reduced because of the dressing structure allowing vertical wicking. Compared with the meshed paraffin gauze dressing, the hydrofiber dressing resulted in faster epithelialization, less pain, better scar quality, and more accessible use in the treatment of split-thickness skin graft donor site [81]. The cost of hydrofiber dressing may limit the accessibility of the patient.

- Alginate dressings: Algisite™ (Smith & Nephew), Kaltostat® (ConvaTec)

Fibrous products derived from seaweed could form a gel after contact with exudate lead to absorption up to 20 times of dressing weight. Alginates are unsuitable for dry wounds or wounds with mild exudate due to their high absorption ability. The alginates may dry when leaving on wounds

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without changing at least weekly, leading to wound adherence and pain from removal. Accordingly, secondary dressing such as foams or hydrocolloids should be considered with appropriate monitoring to prevent drying out. Moreover, calcium released from dressing gives hemostatic properties which help manage wound bleeding. Using in STSG donor sites, alginates can reduce patient discomfort, shorten healing time, and improve new skin quality compared with tulle dressing [82]. However, there were reports for dermal calcification in donor site occurred after applying calcium alginate dressing, which is unusual reactions and remains suspicious [83].

- Foam dressings: Allevyn® (Smith & Nephew), Polymem® (Ferris Corp), Aquacel® Foam (ConvaTec), Mepilex® (Molnlycke Health Care)

Typically, foam base consists of polyurethane or silicone with either hydrophilic or hydrophobic properties. They can be used in wounds with moderate to high exudate. Moreover, Foams provide thermal insulation and create a moist environment for wounds. Various shapes with adhesive or non-adhesive properties are developed along with drug-releasing ability. Although Mepilex showed no significant decrease in healing time, it showed a significant decrease in postoperative pain [84]. The main limitation of foam dressings is their cost, which is relatively high.

There are variations of wound dressings chosen for treating STSG donor sites among health care professionals [73]. Synthetic dressings, including traditional and advanced dressings, seem to be a reasonable option for treating STSG donor sites. The disadvantages of traditional wound dressings consist of wound dressing adherence, insufficient absorption, uncontrolled moist environment, and deficient healing enhancement. Therefore, advanced wound dressings have been developed to improve desirable properties, especially moisture control. The moist environment is well-known to be associated with faster healing [7, 85, 86]. Clinical trials that evaluated the effects of moist wound dressing on STSG donor sites are presented in Table 1, supporting the use of moist dressing for donor sites [64, 87]. Although there are numerous advanced wound dressings available nowadays, the limitations of each dressing seemly still exist. Those limitations include the insufficient absorption capacity, tissue incompatibility, the complicated application, the removal difficulty, fewer bioactivities, and the cost of dressing.

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Therefore, there is still no standard dressing for STSG donor site management [11]. The morbidities caused by donor sites are also recently established [8], suggesting the need for new dressing development. Hydrogels seem to have the potential to become the ideal dressing for wound care [12, 13] because of the biocompatible interface [14], moist environment control, pain reduction, less adherence [12], and ability to hold drugs [15]. However, the high-water content of hydrogels seems to limit their mechanical properties and absorption capacity. Besides, healing enhancement is typically the most crucial outcome in wound care. The dressing that could accelerate the healing process might decrease the complication in particular patients with intrinsic factors that negatively influence wound healing. Moreover, it was reported that scar formation is associated with the long-term consequences affecting the patients, emphasizing the scar outcome as the entire goal of treatment. However, the wound dressing that could enhance healing and minimize scar formation seems to be under examination. Presently, the dressings containing the bioactive substance are usually expensive, decreasing the accessibility of patients. Accordingly, the development of new medicated hydrogel dressing, which has the appropriate mechanical properties, adequate absorption capacity, and affordable cost, may fill current wound dressings' gap.

Table 1 Summary of clinical studies evaluating the effects of moist wound dressing on STSG donor sites healing

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Studies	Participants	Intervention	Outcomes	Result
Weber et al., 1995 (RCT) [88]	68 adult patients - Area: thigh - Depth: 0.015 inch	Hydrophilic polyurethane foam dressing (HPFD) VS Petrolatum gauze (PG) + heat lamp exposure for 30 min 3 times a day	- Healing scores at day 14 (1=no, 2=scattered or spotty, 3=complete epithelialization)	- Complete healing: Not sig. 37% in HPFD VS 17% in PG (p=0.06) - Mean healing scores: Not sig. 2.3±0.6 (HPFD) VS 2.2±0.6 (PG) (p=0.20)
			- Pain score (0-100)	- HPFD had lower pain score at postoperative days 1, 2, 3 (p=0.003, 0.03, 0.04)
			- Infection	- One infection (PG group)

Table 1 Summary of clinical studies evaluating the effects of moist wound dressing on STSG donor sites healing (cont.)

Studies	Participants	Intervention	Outcomes	Result
Barnea et al., 2004 (RCT) [81]	23 adult patients - Area: thigh (divided area) - Size: ≥ 8x10 cm^2 - Depth: 0.012 inches	Aquacel® VS Paraffin gauze (PG)	- Complete re-epithelialization time	- 7-10 days (Aquacel®) VS 10-14 days (PG) (p = 0.0156)
			- Percentage of re-epithelialization	- On day 15 and 30: Aquacel® sig. ↑ %epithelialization (p = 0.006 and p = 0.014)
			- Pain level: VAS (1, 3, 7, 10, and 15 days)	- Aquacel®: sig. ↓ VAS at all time points (p < 0.05)
			- Scar: VSS at 30, 60, 90 days, 1 year	- Aquacel®: sig. better scar at all time points (p < 0.05), except at 3 months
Lohsiriwat et al., 2009 (RCT) [89]	18 adult patients with 20 donor sites - Area: thigh	Ionic silver-containing Hydrofiber VS Paraffin gauze dressing	Time to complete re-epithelialization (days)	7.90±2.47 VS 11.20±3.52, p=0.031
			Pain scores at rest and during dressing removal	- At rest: 0.74 VS 0.80, p=0.894 - During removal: 3.12 VS 4.70, p=0.027.
			Infection and seroma	No infection and seroma in both groups

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Table 1 Summary of clinical studies evaluating the effects of moist wound dressing on STSG donor sites healing (cont.)

Studies	Participants	Intervention	Outcomes	Result
Brölmann et al., 2013 (RCT) [90]	288 adult patients	Six-armed: 1) Alginate 2) Film 3) Gauze 4) Hydrocolloid 5) Hydrofibre 6) Silicone	- Time to complete re-epithelialization	- Hydrocolloid (16 days) VS others (23 days) (p<0.001)
			- Pain (VAS)	- Film: sig. ↓ VAS compared to other dressings (p=0.038)
			- Adverse events	- Infection: Gauze (18%), Film (16%), Hydrofibre (15%), Silicone (4%), Hydrocolloid (2%)
			- Scar: POSAS at 12 weeks after complete healing	Patient: Film had sig. less satisfied scar (p=0.018), esp. wound relief (p=0.046) Observer: No sig.
Poh Yuen Wen et al., 2018 [91]	25 patients (divided area)	15% gamat extract in gel form VS Duoderm® gel	% Epithelialization at day 10, 14, and 21	No sig. differences - Day 10: 79.08±25.12% VS 79.32±23.35% - Day 14: 79.08±25.12% VS 79.32±23.35% - Day 21: 100.0% VS 99.9±0.40%
			Pain and pruritus (day 10, 14, 21)	No sig. difference
			Scar: mVSS at 2 months	No sig. difference: 3.76±2.13 VS 3.64±2.02

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2.2 Silk Sericin

Silk cocoon consists of two proteins which are sericin and fibroin. Sericin is a glue protein combining the fibroin in order to form the cocoon [20]. In the silk textile, sericin is a waste product that can pollute the environment, so developing sericin products increases the value of sericin and decreases the pollution for the environment [21]. The benefits of sericin, which are anti-inflammatory, antioxidant, anti-melanogenesis, moisture absorbing and releasing, and wound healing enhancement, have been established in several studies [21-25, 92]. Moreover, Sericin has biocompatibility, biodegradability, and less immunogenicity. It was proven as a safe material both in vitro and in vivo test [93]. Therefore, sericin has been widely used in pharmaceutical, cosmetic, food, and biomedical products, including wound dressing [21, 25-27]. The wound healing enhancement properties of sericin are obtained from the several amino acids contained in sericin, especially for cysteine and methionine. Sericin consists of several amino acids with sizes ranging from 20 to 200 kDa. The differences in composition and properties of sericin depend on the strains of the silk worm and the extraction method [25, 94, 95]. The findings suggested that the Chul 1/1 strain extracted by autoclave would give the highest amount of cysteine and methionine, the lowest toxicity to fibroblast cells, and the highest collagen production enhancement [95, 96]. The proliferation of fibroblast and the migration of keratinocytes could also be accelerated by sericin [97]. Moreover, sericin can promote scarless wound healing and facilitate the skin appendages recovery [98]. According to mentioned advantages, sericin seemly becomes the potential biomaterial for developing wound care products.

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In addition to the in vitro studies, sericin was added to silver-zinc sulfadiazine cream. Its clinical efficacy was examined in 29 patients with 65 burn wounds [23]. It was found that the cream containing sericin could significantly reduce healing time compared with the similar control cream containing no sericin. However, there are some limitations of the cream dosage form as it could not protect the wound. Besides, it has to be re-applied frequently around one to two times per day, resulting in suffering to patients, healing disturbance, and inconvenience for the healthcare provider. These limitations bring about the development of wound dressing that could provide the sustained release of sericin.

Fabrication of sericin as a wound dressing might overcome the mentioned problems. Pure sericin has been fabricated as wound dressing [30, 99]; however, the poor mechanical strength of sericin becomes the main limitation to developing sericin wound dressing [28-30]. Due to sericin containing various polar groups, it can interact and copolymerize with other polymers, possibly improving the mechanical properties of dressing [31]. Several polymers were combined with sericin to fabricate as the wound dressing, such as chitosan [100], gelatin/ fibroin [101], agar [102], polyacrylamide [29, 103], PVA [33, 104]. Among the polymers, PVA is seemly one of the most attractive polymers. PVA is a biodegradable and biocompatible synthetic polymer with good mechanical properties, the ability to bind with proteins, and versatile properties for different preparation [105]. Therefore, PVA has been used for the development of sericin wound dressing by various methods, which are freeze-thawing [31], freeze-drying [33, 104], gamma radiation [34], chemical crosslinking [106], and electrospinning [107].

Although many sericin dressings have been developed, there are still only a few clinical trials evaluating the efficacy of sericin in the human wound (Table 2). The clinical efficacy of sericin dressing was evaluated in 30 STSG donor sites compared with Bactigras® by Siritientong et al., 2014 [32]. Each donor site wound was divided into 2 parts, and each part was randomly treated with either sericin dressing or Bactigras®. The sericin dressing exhibited significantly lower complete healing time (12±5.0 days) compared with Bactigras® (14±5.2 days), $p = 1.99 \times 10^{-4}$. Furthermore, the pain scores in the sericin dressing group measured by VAS were significantly lower than the Bactigras® group. The findings from the study by Hasatsri et al. (2015) also supported the benefits of sericin dressing in STSG donor site treatment [108]. The healing time of the sericin dressing (11 ± 6 days) was significantly lower than those treated with Bactigras® (14 ± 6 days). Moreover, the pain score and transepidermal water loss (TEWL) of donor sites treated with sericin dressing were significantly lower than those treated with Bactigras®. However, the study of Napavichayanun et al. (2018) found no significant difference in the healing time of STSG donor site between sericin dressing and Bactigras® [109]. Although sericin dressing had comparable healing time with Bactigras® in this study, the scar quality of wound treated with sericin dressing was seemly better than Bactigras®, especially for color and TEWL. There is still disagreement on the beneficial effects of sericin on the wound healing time. Moreover, the investigation for its effect on scar quality is seemly lacking, the

further investigation for the effects of sericin dressing on the healing time as well as the scar characteristics of STSG donor site might clarify the possibility of its advantages.

Besides the wound healing application, the effects of sericin on other clinical applications have also been investigated (Table 2). The advantages of sericin on cosmetic applications seem to be apparent [110]. Skin hydration, skin surface, and skin elasticity are probably improved by sericin [111], resulting from various bioactivities. The potential effect of sericin on bone regeneration was also presented [112]. Sericin seems to be a promising biomaterial in tissue regeneration [113]. The examination for utilizing sericin in tissue engineering applications has recently increased [114, 115]. Moreover, the sericin cream could relieve the pruritus in hemodialysis patients, improving patients' s quality of life [116].

Table 2 Summary of clinical studies evaluating the effects of sericin

Studies	Participants	Intervention	Outcomes	Result
Wound healing application				
Aramwit et al., 2013 (RCT) [23]	29 adult patients with 65 second-degree burn wounds	Sericin + silver sulfadiazine (AgSD) cream VS AgSD cream	Complete healing time (days)	Sig ↓ time (p=0.001): 22.42±6.33 VS 29.28±9.27
			Hospital stays (days)	No sig. differences 30.12±4.28 VS 37.48±7.57
			Infection	No infection
			Pain and adverse events	No sig. difference
Siritientong et al., 2014 (RCT) [32]	28 adult patients with 30 STSG donor sites - Area: thigh (divide area)	Silk sericin-releasing wound dressing VS Bactigras®	Time to complete healing	- Sig. ↓ time: 12 ± 5.0 VS 14 ± 5.2 days, (p = $1.99x10^{-4}$)
			Pain score (VAS) on 5 consecutive days	- Sig. ↓ VAS at all time points

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Table 2 Summary of clinical studies evaluating the effects of sericin (cont.)

Studies	Participants	Intervention	Outcomes	Result
Wound healing application				
Hasatsri et al., 2015 (RCT) [108]	23 adult patients with 30 STSG donor sites - Area: thigh (divide area)	Novel silk bilayered wound dressing VS Bactigras®	- Time to complete healing	- Sig. ↓ time: 11 ± 6 VS 14 ± 6 days ($p = 10^{-6}$)
			- Pain score (VAS)	- Sig. ↓ VAS (p <0.001)
			- Skin barrier function (transepidermal water loss; TEWL) on 0, 30, 60, 90, 120, 150 days	- Sig. ↓ TEWL median in all time points (p = <0.001)
			- Infection	No signs of infection
			- Liver & renal function	No adverse effect
Napavichayanun et al., 2018 (RCT) [109]	21 adult patients with 32 STSG donor sites - Area: thigh (divided area)	Bacterial cellulose wound dressings containing silk sericin and PHMB (BCSP) VS Bactigras®	Wound healing time	No sig. difference
			Pain scores (VAS)	Sig. ↓ VAS (p = <0.05)
			Wound quality - TEWL - Melanin and erythema levels - VSS	At 0, 1, 3, 6 months: - Sig. ↓ at 1, 3, 6 months - Sig. better at healing time and after 1 month (p = <0.05) - Sig ↓ only pigmentation item at 1 month
			- Infection and adverse effect	- No infection and adverse effect

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Table 2 Summary of clinical studies evaluating the effects of sericin (cont.)

Studies	Participants	Intervention	Outcomes	Result
Other applications				
Padamwar et al., 2005 [110]	6 healthy volunteers	Sericin gel	Hydroxyproline content	↑ hydroxyproline content in stratum corneum
			Impedance of the skin	Sig. ↓ than normal skin
			Transepidermal water loss (TEWL)	↓ TEWL compared to normal skin
			Skin surface topography (skin replica)	Increased smooth & reduced fine lines, wrinkles, and ridges
Berardesca et al., 2015 [111]	40 healthy women	Emulsion + gold silk sericin complex VS Emulsion base	Expert grading (Skin texture, skin tone, and wrinkle severity)	Sig. improved skin texture, skin tone, and wrinkle at 4 and 8 weeks
			Surface topography (Visioface® RD)	Sig. improved wrinkling, smoothness, and uniformity
			Reflectance Confocal Microscopy (Vivascope)	Promote organization of connective tissue
			Transepidermal water loss (TEWL)	Sig. ↓ TEWL
			Skin elasticity	Sig. ↑ elasticity
			Skin hydration	Sig. ↑ hydration

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Table 2 Summary of clinical studies evaluating the effects of sericin (cont.)

Studies	Participants	Intervention	Outcomes	Result
Other applications				
Kim et al., 2019 (Retrospective) [112]	Patients with impacted mandibular third molars	Silk mat VS dPTFE VS no membrane	Bone gain	Compared to no membrane - Silk mat sig. ↑ at 3 months - Silk mat and dPTFE sig. ↑ at 6 months
Aramwit et al., 2012 [116]	50 dialysis patients with ESRD	8% sericin cream VS Cream base	Skin hydration, irritation and pigmentation	- Sig. ↑ hydration - Sig. ↓ irritation - Sig. ↓ pigmentation
			Itching (VAS score)	Sig. ↓ after 6 weeks
			Kidney Disease Quality of Life Short Form (KDQOL-SF): Day 0 and after 6 weeks of treatment	↑ QoL in all domains & Sig. improved: pain scores, effect on daily life, sleep quality, and symptoms or problems related to kidney disease

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2.3 Collagen

Collagen structure consists of amino acids: glycine-proline-x or glycine-hydroxyproline-x, which x is several types of amino acids arrangement. There are at least 28 types of collagen found in the human body. The primary types of collagen (around 80-90%) are types I, II, and III [36]. Collagen can be divided into 3 groups depending on the degree of hydrolysis: 1) undenatured collagen, UC (molecular weight, MW: 300 kDa), 2) gelatin, partially hydrolyzed (MW: 20-90 kDa), and 3) collagen hydrolysate, CH (MW: 2-9 kDa) [117]. Collagen is an extensive component of the extracellular matrix (ECM). It functions as a structural scaffold giving strength and flexibility to organs and tissues [35, 36]. Moreover, collagen has biocompatibility, biodegradability, and low immunological response [37]. Thus, collagen has been widely used in biomedical applications, including wound dressing [12]. There are many commercial collagen dressing products available now, such as Biobrane™ and Permacol™ (porcine collagen), Integra™ and Matriderm™ (bovine collagen), and Helisorb® Neuskin-F®, and Kolspon® (fish collagen), Table 3.

The primary sources of collagen are parts of skin, bone, scale, and tendon from porcine, bovine, and piscine. The religious beliefs lead to the restricted use of porcine collagen in Islamic and Jewish cultures and using bovine collagen in Hinduism. Moreover, collagen from terrestrial animals is concerned about the risk of bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE), and foot-and-mouth disease (FMD), limiting their use. Those drawbacks disappear in fish collagen. It can be extracted from the skin and scale, which are waste from the industrial food process [45, 118]. Therefore, fish collagen becomes a potential source of collagen for biomedical applications [45, 46, 118].

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Fish collagen has a similar structure to mammalian collagen. The main component of fish collagen is glycine, which is 30% of all amino acids. However, the denaturation temperature (Td) of fish collagen is lower than mammalian body temperature. The instability of structure and melting of collagen might become problems for applying fish collagen in the human body [118]. The physical and chemical crosslinking seems to be the necessary method for fabricating the collagen dressing with good stability [47], leading to the difficulty of the formulation. Moreover, the extraction processes of undenatured collagen are also relatively complicated. On the other hand, collagen hydrolysate (CH) could be extracted by a straightforward method. It is a small peptide fragment of collagen,

called hydrolyzed collagen or collagen peptides. Furthermore, the higher solubility and lower molecular weight of CH may lead to better bioavailability [40, 119, 120], higher penetration [121], and easier formulation. Bioactive properties of CH also seemly improved when compared to the UC [118], including the wound healing enhancement [119]. However, CH cannot form film or scaffold by itself [46]. The incorporation of CH into other polymers might facilitate the application of collagen and preserve its bioactive properties. Some natural and synthetic polymers, including cellulose [122], chitosan [123], and PVA [119], have been combined with CH for developing wound care products.

Collagen is the crucial structure of the skin. It plays an essential role in wound healing, involving several parts of the wound healing process [36, 38]. In the hemostasis phase [37], once a wound occurs, the fracture of vascular leads to the exposure of platelet and collagen, resulting in adenosine diphosphate (ADP) release. The aggregation of platelet occurs, inducing fibroblast production and migration into the wound bed. Collagen also absorbs growth-inhibiting factors such as proteases, cytokines, and free radicals [36], reducing the inflammation in the inflammatory phase. In the proliferation phase, several cells involving wound healing, e.g., fibroblast and the re-epithelialization are activated by collagen. Moreover, the strength of the scar depends on the composition and decomposition of collagen [37] during the remodeling phase. The collagen synthesis will increase after wounding for 1 day and continuing for a few weeks. After that, the collagen synthesis will decrease, and collagen deposition will stabilize [36].

The benefits of exogeneous collagen on wound healing have been established in several studies (Table 4). Collagen showed the potential to enhance healing in several kinds of human wounds, including chronic leg and foot ulcer [124], partial thickness skin graft donor site [42, 43], burns wounds [125], diabetic wounds [126], and pressure ulcers [41]. In contrast, some studies found no advantages from collagen dressing on wound healing [127, 128]. However, collagen used in most studies was UC [129, 130] from land animals [41, 42]. Administration of the CH via both oral [131-133] and topical [39, 40] could increase water content, promote collagen synthesis, and enhance wound healing in several cell lines and animal studies [123, 134]. The oral administration of CH could also facilitate healing in a human study [44, 135]. While, the clinical study determining the efficacy of CH via topical application is scarce, especially for fish CH. It was also stated in the recent review that the clinical trial evaluating the efficacy of topical collagen hydrolysate on wound healing

is still a gap at present [49-51]. Besides, the anti-oxidant activity and moisture absorption & retention properties of collagen [118] might benefit scar quality, which seems to be insufficiently investigated. Accordingly, the clinical trial determining the clinical efficacy of fish CH with the appropriate method is still needed, including the long-term effect investigation.

Table 3 The commercial collagen dressings

Trade name	Compositions	Indications
Porcine collagen		
Biobrance™	Silicone membrane and nylon mesh bonded with peptides from a porcine dermal collagen	Clean partial-thickness burn wounds and split-thickness donor sites
Permacol™	Acellular porcine-derived collagen matrix graft cross-linked through hexamethylene diisocyanate	Surgical implant in different types of surgery such as digestive and plastic
Bovine collagen		
Integra™	Pure bovine collagen, glycosaminoglycan from shark cartilage, and a thin outer layer made of silicone	Deep partial- and full-thickness burns, other full-thickness skin defects, chronic wounds, and soft tissue defects
Matriderm™	Native (non-crosslinked) collagen matrix supplemented by an elastin hydrolysate	Full-thickness or deep dermal burn wounds and chronic wounds
Marine collagen		
Helisorb® or Kollagen®-D	Lyophilized porous collagen Type I with nylon mesh backing layer	Partial- and full-thickness burns, superficial ulcers, pressure ulcers, diabetic ulcers, traumatic wounds, and surgical wounds

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Table 3 The commercial collagen dressings (cont.)

Trade name	Compositions	Indications
Neuskin-F®	Highly purified type I fish collagen film	Donor sites or non-healing donor sites, superficial to partial-thickness burns, abrasions and skin tears, venous stasis leg ulcers
Kolspon®	Sterile type I fish collagen sponge	Cavity wounds (abscesses, pilonidal sinuses), moderate to heavily exudating wounds, control of minor bleeding wounds, and traumatic lesions

Table 4 Summary of clinical studies evaluating the effects of collagen on wound healing

Studies	Participants	Intervention	Outcomes	Result
Topical administration				
Uygur et al., 2008 (RCT) [43]	26 patients with 32 STSG donor sites - Area: thigh	Collagen spray+gauze dressing VS gauze dressing	Epithelialization time	Collagen sig. ↓ time (9.09 days VS 11.2 days), p = <0.05
			Pain score: VAS	Collagen ↓ VAS (p = <0.05)
			Scar: VSS (1, 2, 3 m)	No sig. difference
			Infection	No infection
Fernandes de Carvalho et al., 2011 (RCT) [42]	34 patients with STSG donor sites (divided into 3 groups)	1) Collagen alginate w/ transparent PU film 2) PU film 3) rayon soaked in 0.9% saline	Time to complete epithelialization	Collagen group sig ↓ time to complete epithelialization (6.3 VS 8.2 VS 11.7 days, resp)
			Pain: VAS, Brief Pain Inventory (BPI), and Index of Pain Management (IPM)	Collagen group sig. ↓ pain (p = <0.05) and require less analgesic
			Infection	No infection

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Table 4 Summary of clinical studies evaluating the effects of collagen on wound healing (cont.)

Studies	Participants	Intervention	Outcomes	Result
Kloeters et al., 2016 (RCT) [41]	33 adult patients with pressure sores Campbell score: 3-4	Oxidised regenerated cellulose (ORC)/ collagen + Tielle® (n=23) VS Tielle® alone (n=10)	Healing rate (weekly f/u for 12 weeks)	ORC/collagen sig. ↓ wound surface area (65% VS 41%), $p<0.05$
			Elastase activity	ORC/Collagen sig. ↓ activity - at day 5 and all later time points, compared to day 0 - at day 5 and 14, compared to control group
			Plasmin activity	ORC/Collagen sig. ↓ activity (day 5, 14, 28, 42, compare to day 0) - at day 5 and 14, compared to control group
Rahmanian-Schwarz et al., 2011 (RCT) [136]	34 adult patients with superficial partial-thickness burns - Area: hand or face	Biobrane® VS Suprathel®	Healing time	Median healing time: Biobrane® 23 days VS Suprathel® 24.8 days ($p>0.05$)
			Cutometer parameters: Uf, Ur, Ur/Ue, Ur/Uf, Ua/Uf, and Uv/Ue	- Both groups: all parameters lower than healthy skin - Biobrane® tends to be better (not sig.)
			Scar: VSS	No sig. difference

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Table 4 Summary of clinical studies evaluating the effects of collagen on wound healing (cont.)

Studies	Participants	Intervention	Outcomes	Result
Mostow et al., 2005 (RCT) [124]	120 patients with at least 1 chronic leg ulcer	SIS wound matrix plus compression (n=62) VS Compression alone (n=58)	Incidence of healing at 12 weeks	55% in SIS group VS 34% in control group (p=0.0196)
			Time to healing Wound size Healing (weekly up to 12 weeks)	Probability of healing: 63% in SIS group VS 40% in the control group, p=0.0226
			Recurrent at 6 months	No recurrence in SIS group
			Adverse events	No sig. difference
Park et al., 2019 (RCT) [137]	30 patients with diabetic foot ulcers	Porcine type I collagen dressing + Foam dressing (n=17) VS Foam dressing alone (n=13)	Complete healing rate within 12 weeks	Collagen group sig. ↑ 82.4% VS 38.5%, p=0.023
			Healing velocity per week	Collagen group sig. ↑ 17.85±14.61% vs 9.41±8.28%, p<0.05
			Time to 50% size reduction	Collagen group sig. ↓ 21 vs 42 days; hazard ratio = 1.94, p<0.05
			Adverse events	No sig. difference
			Infection	No infection

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Table 4 Summary of clinical studies evaluating the effects of collagen on wound healing (cont.)

Studies	Participants	Intervention	Outcomes	Result
Oral administration of hydrolyzed collagen				
Sugihara et al., 2018 (RCT) [135]	112 patients with stage II or III pressure ulcers	CH: Collagen hydrolysate 1. CH-a; low Pro-Hyp and Hyp-Gly 2. CH-b; high Pro-Hyp and Hyp-Gly 3. Placebo For 16 weeks (n=38,35,39)	At week 16	CH sig. ↓ PUSH
			Pressure Ulcer Scale for Healing (PUSH)	- CH-b (6.46±0.98) VS control (9.26±2.09), p<0.01 - CH-a (8.21±2.04) VS control (9.26±2.09), p=0.029
			Pressure Sore Status Tool (PSST) scores	- CH-b (19.71±3.08) VS control (23.38±3.85), p=0.01
			Wound area	- CH-b (3.19±2.88) VS control (5.00±3.88), p=0.027
			Serum albumin	CH-a and CH-b sig. ↑
			Adverse events	CH-a: 2 constipation, 1 diarrhea, CH-b: 2 diarrheas, Placebo: 1 headache

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Table 4 Summary of clinical studies evaluating the effects of collagen on wound healing (cont.)

Studies	Participants	Intervention	Outcomes	Result
Bagheri Miyab et al., 2020 (RCT) [44]	31 men with 20-30% TBSA burn	Hydrolyzed collagen (n=16) VS isocaloric placebo (n=15) for 4 weeks	Serum pre-albumin (mg/dL)	Collagen group sig. ↑ - Wk 2: 29.7±13.6 VS 17.8±7.5 (p=0.006) - Wk 4: 35.1±7.6 VS 28.3±8.2 (p=0.023)
			Rate of wound healing	- Hazard ratio: 3.7 times in collagen group, p=0.007 - % completely healed wound between collagen VS control at Wk 2: 50% VS 6.7%, p=0.015 Wk 4: 100% VS 40%, p<0.001
			Length of hospital stay	Collagen (9.4±4.6 days) VS control (13.5±7 days), p=0.063, but clinically sig.
			Anthropometries	No sig difference in weight, BMI, dietary energy, protein intake between groups

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2.4 Silk sericin dressing with collagen

Both sericin and CH established their benefits on wound healing via several mechanisms. Besides, it was previously found that sericin can interact with collagen via the formation of tyrosine excimers [54]. This finding suggests that sericin might improve the thermal stability of collagen. Moreover, sericin addition to collagen probably enhances desired properties for wound healing applications such as swelling, oxygen permeability, and cellular attachment. The ratio of sericin was suggested to be less than or equal to the collagen component to obtain the appropriate wound dressing [56]. The attachment and morphology of fibroblast seemly ameliorated when collagen was added into sericin [53]. Likewise, the adhesion and proliferation of human adipose-derived stem cells (hADSCs) were enhanced in the collagen scaffold containing sericin compared with the pure collagen scaffold [52]. Accordingly, the combination of sericin and collagen might result in a more extraordinary effective wound dressing.

Biomedical products containing both sericin and collagen have been fabricated by freeze-drying and cast drying methods. However, a crosslinking agent such as glutaraldehyde is needed to achieve the stability of products [52, 55-58]. Although the safety of products was illustrated in those studies, it is widely known that chemical crosslinking agents can harm biological tissue [18]. Wound dressing containing collagen hydrolysate and sericin prepared without using crosslinking agent seems impractical because both substances have low mechanical properties. Their limitations on poor mechanical properties might be improved by combining with other polymers, which have good mechanical properties. Polyvinyl alcohol (PVA) is a promising biomaterial used in biomedical device development. It is a biodegradable and biocompatible synthetic polymer, and its versatile properties allow various preparations. Moreover, PVA possibly binds with proteins, including sericin and collagen, leading to better properties of the blended polymers [105].

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Wound dressings consisting of sericin and polyvinyl alcohol (PVA) have been produced by using freeze-thawing [31], freeze-drying [33, 104], gamma radiation [34], chemical crosslinking [106], and electrospinning [107]. The findings suggested that the thermostability and mechanical properties of sericin were enhanced when combined with PVA [31, 107]. Moreover, the dressings containing sericin and PVA showed the sustained release of sericin. The dressings enhance cell

adhesion, proliferation, and migration and promote wound healing compared with the dressing containing no sericin [33, 34, 104, 106, 107].

Collagen, which is mostly UC or gelatin, was also combined with PVA by several methods [105], such as freeze-thawing [138], electrospinning [139, 140], chemical crosslinking [141], and cast drying [142]. In contrast, CH has been combined with PVA in only a few studies, commonly fabricated by electrospinning [119, 143]. Similar to sericin, the combination with PVA leads to the improved mechanical properties of collagen. The bioactive properties of PVA also increased after combining with collagen. Furthermore, blending collagen hydrolysate into PVA processed by melt blow extrusion could increase water penetration into PVA film resulting in better biodegradability and improved mechanical properties [144, 145].

Each preparation method seemly has its limitations. Firstly, the freeze-thawing method [31] needs multiple-step preparation to allow the physical crosslink of PVA hydrogel. The obtained hydrogel has a white-opaque appearance and relatively lower mechanical strength than chemical crosslinked hydrogel. For the freeze-drying method, the addition of crosslinking agent, e.g., genipin [104], is still needed. The possibility to harm biological tissues of crosslinking agents [18, 106] leads to multiple processes for eliminating them. The scaffold developed by Siritientong et al. (2014) was prepared by freeze-drying before being precipitated by ethyl alcohol, diminishing the toxicity from crosslinking agent [33]. The requirement for freeze dryer and multiple-step preparation are seemly still the limitation of this method. Moreover, gamma radiation was used for fabricating PVA hydrogel. However, specific equipment is also required for gamma radiation, and the obtained hydrogel has relatively poor mechanical strength [34]. Besides the need for an electrospinning machine, the main drawbacks of the electrospinning method were the high consumption of time and cost, and the production on a massive scale seems to be limited [146]. The preparation that could decrease these disadvantages possibly becomes the desirable method for PVA hydrogel preparation.

PVA could form a physically crosslink hydrogel without using a crosslinking agent by the cast drying method. The obtained hydrogel is rigid and transparent [17]. Compared with other methods, the cast drying method is relatively uncomplicated, low-priced, eco-friendly, and harmless [17]. However, the PVA hydrogel prepared by cast drying has insufficient elasticity [18], is relatively brittle [19], and has less bioactive properties. The addition of protein into PVA could influence its

mechanical properties and bioactive properties [105]. Therefore, incorporating sericin and collagen into PVA hydrogel prepared by the cast drying method is seemly reasonable.

There is still a lack of products containing sericin and PVA prepared by the cast drying method. In a previous study, PVA was blended with UC or gelatin to prepare film by cast drying method. The mechanically compatible blends between PVA and collagen (UC or gelatin) were found, giving the transparent film with good mechanical properties [142]. Moreover, collagen-containing particles, i.e., bone particles and demineralized bone, could be embedded in PVA by the cast drying method without using crosslinking agent [147]. Although the cast drying method has been used to prepare the biomaterial consisting of PVA and collagen, CH has been used in none of the studies.

The silk sericin dressing with collagen contains 3%w/w of sericin, 5%w/w of collagen hydrolysate, 10%w/w of PVA, and 1%w/w of glycerin, was prepared by cast drying in the current study. This silk sericin dressing with collagen was produced under sterile conditions and controlled for quality by Bioactive Resources for Innovative Clinical Applications Research Unit, Pharmaceutical Sciences, Chulalongkorn University. The study of Siritientong et al. in 2011 [148] indicated the effectiveness of gamma radiation on the sterilization of sericin dressing. Therefore, the silk sericin dressing with collagen would be sterilized by gamma radiation before application. The physical, chemical, mechanical, and biological properties of the SDC were shown in Appendix O.

The transparent hydrogel with pale-yellow color was obtained. The dressing showed a high swelling ratio (approximately 264%) and water content (approximately 87%). The dressing also has fast absorption and dehydration properties. It could absorb water and concurrently release protein. Moreover, it provided the sustained release of protein along 168 h. The mechanical properties were seemly in a suitable range: tensile strength 3.98 MPa, percentage of elongation 364%, and elasticity 2.20 N/mm^2. It can suggest that the dressing had well flexibility and durability to protect the wound from the outer environment. The safety of dressing was evaluated in the L929 mouse fibroblast. Silk sericin dressing with collagen showed no cytotoxicity. It also allowed comparable proliferation and migration compared to positive control. Accordingly, a clinical study should be conducted to evaluate further the safety and efficacy of this dressing in humans. The clinical safety would be determined from the patch test before interpretation using the International Contact Dermatitis Research Group (ICDRG) [149, 150], as shown in Table 5.

Table 5 Reading criteria of the International Contact Dermatitis Research Group (ICDRG)

Symbol	Morphology	Assessment
-	No reaction	Negative reaction
+?	Faint erythema only	Doubtful reaction
+	Erythema, infiltration, possibly papules	Weak positive reaction
+ +	Erythema, infiltration, papules, vesicles	Strong positive reaction
+ + +	Intense erythema, infiltrate, coalescing vesicles	Extreme positive reaction
IR	Various morphologies, e.g., soap effect, bulla, necrosis	Irritant reaction
NT	-	Not tested

2.5 Scar quality evaluation

The scar is the outcome after complete wound healing, divided into normal and pathological scars. Pathological scars such as hypertrophic, keloids, and atrophic scars can lead to functional and mental problems of patients [61]. Although wound healing time is considered the most crucial outcome of wound care, the scar quality, including appearances and functions of healed skin, is also the critical outcome that should be included in the entire goal of treatment. [59, 60] To evaluate the scar outcome, an invasive biopsy has been used to examine the structure of the scar and its healing progression [151]. However, this method creates a new wound, leading to concern in ethical issues. It is also a relatively expensive and complicated method for practice. Therefore, the non-invasive methods are currently emphasized in scar quality assessment [152], and the methods are divided into subjective and objective methods providing differences of advantages and disadvantages.

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Scar characteristics

Scar quality may be determined from the cosmetic outcomes such as color, irregularity, thickness, and functional outcomes, e.g., epidermal barrier, skin hydration, blood flow, sensation, appendages, and mechanical properties. Moreover, scar symptoms such as pain and pruritus are significant predictors for physical impairment and related to the patient's quality of life [153]. Each scar characteristic may represent the different effects of treatment on the quality of the wound [59].

Erythema

The redness of skin called erythema is found in an immature scar in response to inflammation, vascularization, and capillary dilation. The existence of erythema is one characteristic of pathological scars such as hypertrophic and keloid. It is assumed that it is caused by persistent inflammation [154]. However, the typical scar also has redness after the first month of healing. It seems to result from the higher number and larger size of blood vessels instead of persisting inflammation [155]. Then, vascularization tends to decrease from 3 months [156] due to the re-modeling structure of the scar. Therefore, erythema may be used as a parameter for monitoring the maturation of the scar [157].

Pigmentation

Dyspigmentation of scar, i.e., hyperpigmentation and hypopigmentation, seem to be the common complication found in STSG donor site scar, especially in Asian skin [158, 159]. The abnormality of pigmentation can be affected by wound management [160]. It can lead to aesthetic and psychological problems in patients [161]. Dyspigmentation can cause by stimulants or inflammatory [162] during the early phase of wound healing before the scar formation [163]. Therefore, pigmentation that can be measured from melanin level is considered an important parameter for scar assessment in wound care studies [164].

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Height/ thickness

Scar thickness might indirectly represent the remodeling of extracellular matrix (ECM) [61] involving the accumulation of collagen, elastin, fibronectin, and hyaluronic acid in scar [154]. It can be used as an indicator for pathological scars because atrophic, hypertrophic, and keloids have a height difference compared with normal skin [165]. Time to evaluate the thickness of the scar should be within 4-12 months because the improvement of thickness typically begins from 6 months. The thickness might be overestimated at the earlier assessment before 6 months [166]. Both subjective and objective methods can be used for height evaluation.

Skin barrier function

Trans-epidermal water loss (TEWL) is the amount of water permeating the stratum corneum to the outer environment. The recovery of skin barrier function may be determined from this parameter. The organization of the epidermis, especially the stratum corneum (SC), is similar to the brick and mortar. This structure can prevent water loss from the human body, and the excellent arrangement of SC leads to low TEWL. In scar, TEWL is usually higher than the normal skin, and the lowering of TEWL represents the better recovery of skin barrier function. Skin hydration may be indirectly measured from TEWL as lower TEWL should lead to higher skin hydration. However, the functions of other appendages and their ability to produce natural moisture factors (NMF) cannot be evaluated from only TEWL. Therefore, the results of TEWL should be considered together with the results from directly measuring water content [167].

Skin hydration

Skin hydration is the water content in the epidermis and dermis, indicating the recovery of water holding capacity. It was found that scars had lower hydration after complete healing for several months, leading to skin dryness and hypertrophic scar development [167]. Hydration of skin affects skin appearances such as smoothness and softness and influences skin function such as flexibility and desquamation [168, 169]. Transepidermal water loss (TEWL) is used as an objective parameter for determining complete healing and the water barrier function of the skin. However, TEWL may not directly represent the hydration state of the skin [170] which seems to have an essential role during the re-modeling process of wounds [171]. Moreover, measuring for skin hydration might elucidate the restoration of skin appendages, including sweat glands, and characterize the scar's quality compared with the normal skin [59, 172].

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Skin elasticity

The skin's elasticity is vital for movement and the ability to protect the body from outer mechanical forces, so it is the vital parameter for comparing scar recovery with normal skin.[173] The structure and organization of collagen, the viscous ground substance, and elastic fibers can affect the mechanical properties of the scar. [59] The scar is usually harder than normal skin [174] and requires 3 months up to several years to improve skin elasticity.

Pain

Pain is the symptom of a scar disturbing a patient's comfort. Inflammatory response according to the injury can cause acute pain. In contrast, chronic pain is persistent for three or more months without relieving even giving treatment [153]. Neuropathic pain, which involves peripheral and central sensitization leading to chronic pain, is frequently found in scars, especially in hypertrophic scar and keloid [175].

Pruritus/ itching

Pruritus or itch is the sensation that leads to the desire to scratch. The acute itch can be found up to 6 months after injury, corresponding to the early remodeling phase. Histamine release due to inflammatory response and collagen production can lead to itch in healing wounds. The presence of mast cells results in itching in hypertrophic scars. Moreover, chronic itch found after 6 months post-injury may represent the severity and the psychological stress [153].

Evaluation time

The individual scar may have an individual maturation pattern leading to the different progression of scar characteristics [156]. After complete healing, immature scars appear pink with slightly raised due to the inflammatory and the accumulation of blood vessels, collagen, and cells [157]. The persistence of immature scars may contribute to the development of hypertrophic scars [176]. The early evaluation may indicate the signs of pathologic scar development.[177]

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Monthly evaluation is seemly suitable for collecting more information about scar progression. First scar assessment is recommended to conduct after complete healing for 1 month [177]. At this time, the color, vascularity, and elevation of the scar can be seen. These parameters may provide signs of hypertrophic scar formation. In the third month, the hypertrophy and the pliability of the scar are apparent for investigation. Moreover, most signs can be detected in 3 months. Moreover, the elastic fibers may not be found in the first 3 months so that the strength progression might be monitored over 3 months [59]. Approximately at 6 months, the strength of the scar is maximum [157] depending on collagen production, degradation, and organization [178]. Furthermore, hypertrophic scars usually develop in 6-8 weeks, and the plateau is reached in 6

months before regression, whereas the keloids form in around 2-3 months, and the progression is continually over 6 months [177-179]. For scar symptoms, i.e., itching, the acute itch may exist for up to 6 months as it is the early remodeling phase. The persistence itch after 6 months is a chronic itch that can signify injury severity and psychological stress. Therefore, the measurement for elasticity, pain, itching, and pathological scar development should be conducted in 6 months.

Scar outcome measurement

The methods for evaluating scar outcomes could be divided into subjective and objective methods. Each method has its advantages and disadvantages.

Subjective method

The patient, clinician, and researcher could subjectively evaluate individual scar characteristics to determine the outcomes from treatment. Scar assessments scales have been developed for subjective assessment to make the assessment more objective and provide quantitative data [180]. Using the subjective method, i.e., scar scale questionnaires are relatively cheap and convenient, and multiple scar characteristics can be quickly and easily evaluated without requiring the additional device [181, 182]. The reliabilities of these questionnaires have been established, and several studies on wound care used them in their research, giving the ability for outcomes comparison. However, the evaluation for some parameters is limited for subjective methods, including the recovery of skin functions such as trans-epidermal water loss (TEWL) and skin hydration, which seem to be hardly evaluated by humans [173]. Several scar assessment scales have been developed containing different constructs and items. Among various scales, Vancouver Scar Scales (VSS) and Patient and Observer Scar Assessment Scale (POSAS) seem to remain the most frequently used scales [156].

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Vancouver Scar Scales (VSS)

Vancouver Scar Scales (VSS) is seemly one of the most popular scales that has been commonly used in numerous studies [136, 164, 182]. Several researchers modified VSS, and the most widely used is the modified VSS of Baryza et al. that showed high inter-rater reliability (ICC 0.81) [180, 183]. Scar quality is determined from four items, which are the vascularity (0-3 points),

thickness (0-4 points), pliability (0-4 points), and pigmentation of scar (0-3). The 0 point means normal skin. The higher score represents the worse scar. VSS is also recommended for scar assessment in the longitudinal study [182]. Although this scale is frequently used to provide comparability with other studies, the patient's opinion, which is also an important outcome, is not included in this scale.

Patient and Observer Scar Assessment Scale (POSAS)

POSAS is the tool for scar quality measurement, consisting of the patient and observer parts separated for measurement in different perspectives. Both of patient scale ($r = 0.94$, $p < 0.001$) and observer scale ($r = 0.96$, $p < 0.001$) shows the good reliability of total score [184]. In addition to the overall opinion item, there are 6 items for the patient part (painful, itching, color, stiffness, thickness, and irregular) and observer part (vascularity, pigmentation, thickness, relief, pliability, and surface area). Each item is rated on the 10-point score (total score 6-60), 1-normal skin, and 10-largest difference from normal skin. The pain and itching item could be directly evaluated only by patients. Patients seem to pay more attention to these parameters than the scar appearances [185]. The patient's perspective is an important issue for patient-centered care. Patient and observer's opinions are seemly less consistent [62], resulting from different perspectives and emphasis on scar characteristics [63]. However, the patient's opinion was determined as the outcome for scar quality in only a few studies [64] compared with the clinician's evaluation. The patient part of POSAS that is the validated questionnaire is recommended for examining the patient's opinion on their scar [182]. Besides, POSAS has been used for assessing the scar quality of STSG donor sites in recent studies [64, 65].

Objective method

The bioengineering method has been used for the objective scar assessment. The device could detect the subtle difference in skin, indicating a better outcome from different practices [62]. The objective methods could reduce the bias from the observer, and the outcomes are usually reported in the ratio scale, which can be used in the complex statistical analysis. Moreover, the assessment can be done by one observer with high reliability [152]. However, the cost and time-consuming seem to be drawbacks of objective methods. The results from the device cannot

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represent the scar quality in the patient's perspective [181]. Some parameters of scar, such as erythema and pigmentation, may be visually inspected. Similarly, the elasticity of the skin is also the crucial property affecting the function of the organ [173] could also be evaluated by the subjective method. However, the evaluation by the device might specify the more apparent difference between the treatments. Besides, some parameters, especially physiological parameters representing the recovery of skin functions, seem difficult to evaluate by subjective methods due to minor differences or invisibility [167]. Therefore, the devices should be used to facilitate the evaluation of these parameters. Several devices have been developed to objectively determine scar quality. The Multi Probe Adapter (MPA) device (Courage and Khazaka electronic GmbH, Köln, Germany) seemly give the superiority on scar assessment as several scar characteristics could be measured by this device using the additional probes, including Mexameter, Tewameter, Corneometer, and Cutometer.

Mexameter®

Mexameter® MX 18 is a narrow band spectrophotometer. The measurement principle of Mexameter® is based on the absorption and reflection of the three wavelengths: green at 568 nm, red at 660 nm, and near-infrared at 880 nm. The erythema index is calculated from green and red wavelengths, corresponding to the spectral absorption peak of hemoglobin. The different absorption rates of melanin pigments indicate the melanin index determined by the red and infrared wavelengths. The result presents arbitrary Mexameter units (0-999) for both melanin and erythema levels [186]. Mexameter® is one of the devices used to measure erythema and pigmentation in several studies. It shows the intraclass correlation coefficient (ICC) between 0.82 and 0.97 [161, 186, 187].

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Tewameter®

Tewameter® TM 300 is the open-chamber device measuring the difference of vapor pressure between two sensors. Due to it being an open chamber, it should be used in the closed room controlling for temperature and humidity. Several studies used Tewameter to determine the epidermis's recovery function [59, 188], and it has excellent inter-and intra-reliability, 0.96 and 0.95, respectively [167].

Corneometer®

Corneometer® CM 825 measures the skin hydration based on the capacitance method, and the measurement unit is arbitrary Corneometer units. According to the study of Anthonissen et al. [189], they suggest that Corneometer® can be used for measuring water content in the clinical trial as it showed excellent ICC values (0.985 for intra-observer reliability and 0.984 for inter-observer reliability). However, it should be done under very strict conditions with standardized test protocol to avoid the influence of environmental factors. Moreover, skin hydration is recommended to be reported as the differences or percentage change to reduce the influencing factors [167]. The results from Corneometer® should be considered together with other physiological parameters such as TEWL [167, 189].

Cutometer®

Cutometer® provides negative pressure onto the scar leading to the deformation before switching to normal pressure allowing the retraction. Then, a non-contact optical measuring system would determine the change of skin. Cutometer® has several measurement modes, and the most used is mode 1 (measurement with constant negative pressure). Several parameters, which could be divided into R-parameters, F-parameters, and Q-parameters, obtained from Cutometer®, can represent different biomechanical properties of scar (Figure 2). R-parameters, which are seemly frequently used in the literature, contain absolute parameters and relative parameters. The absolute parameters, measured in millimeters, are influenced by skin thickness varying with age, sex, and body region. Among parameters, maximal skin extension (R0 or Uf) or elasticity (Ue), which are least affected by variations in force, are recommended for use as a reliable parameter in scar elasticity assessment [186]. High ICCs of R0 (>0.78) were found for the measurement in the donor site [190]. Moreover, the relative parameters are the ratio of two absolute parameters. These parameters are seemly less influenced by the size of the aperture, force, thickness of skin, and other factors. There are variations of Cutometer parameters that were recommended by various researchers [191]. Considering all R-parameters together is also recommended for determining the overall mechanical properties of skin because the increase of numerator and the decrease of denominator could result

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in the same value of relative parameters [192]. Because of good reliability [193], this device has been used for determining the effects of the treatment after wound healing [136, 172].

R-parameters

- R0 or Uf is the maximum amplitude of the first curve that is the final deformation of skin representing the stretchability or firmness of the skin in mm.
- R1 (Uf – Ua) is the remaining deformation in mm after relaxation at the end of the first cycle that can represent the ability to return to the original state of skin (the minimum amplitude of the first curve).
- R2 (Ua/Uf) is the ratio of total retraction to total deformation. It includes viscous deformation, so it is called gross elasticity or overall elasticity.
- R3 is the maximum amplitude of the last curve that indicates the tiring effect (fatigue) from repeating suction and relaxation.
- R4 is the minimum amplitude of the last curve after relaxation showing the ability to return to the original state.
- R5 (Ur/Ue) is the net elasticity calculated from the ratio of elastic portion of suction and relaxation without viscous deformation.
- R6 (Uv/Ue) is the ratio between delayed and immediate deformation. It is the viscoelastic ratio or the ratio of viscoelastic to elastic distention.
- R7 (Ur/Uf) is the ratio of immediate retraction to the total deformation, called biological elasticity.
- R8 or Ua is the final retraction, total recovery, or complete relaxation after stop suction.
- R9 (R3 – R0) is the difference between the total skin deformation of the last curve to the first curve, which is hysteresis (H).

F-parameters (Area parameters)

- F0 is the area of the rectangle above the curve (Uf x suction time).
- F1 is the area of the rectangle underneath the curve (Uf x relaxation time)
- F2 is the area above the upper enveloped curve (area A). It is the surface between the real curve and the value corresponding to the maximal deformation.

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The less area of F 0, F1, and F2 closing to the value “0” indicates the more elastic of the skin. The completely elastic material will show no area at all.

- F3 is the area within the enveloped curve (area B), representing skin fatigue.
- F4 is the area beneath the envelope curve (area B + area C), representing the skin's firmness that resists suction.

Q-parameters

- Q0 is the maximum recovery area, decreases with increasing firmness of the skin.
- Q1 (QE/Q0) is the elastic recovery, which increases with higher elasticity.
- Q2 (QV/Q0) is the viscous recovery.
- Q3, (QE + QV)/Q0, is the viscoelastic recovery (overall elasticity), higher with more elasticity.

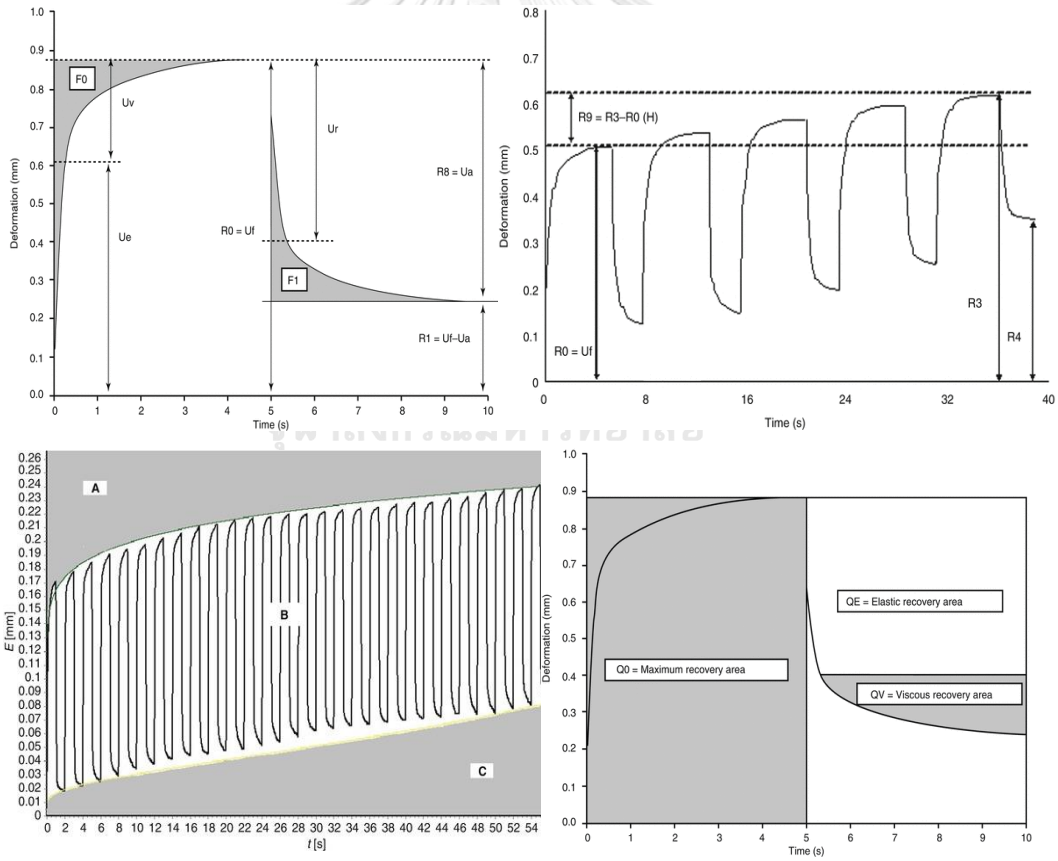

Figure 2 Cutometer parameters

The prevalence of pathological scar and abnormal appearances seems to be higher in Asians [194], including in STSG donor sites [159] that are expected to heal with minimal scar. Although the evaluation for scar outcomes in STSG donor sites is recently increasing (Table 6), the studies in the scar progression in Asians seem to be limited [158]. Moreover, some studies recalled patients back after several years, so many loss follow-ups were found, which may influence the results and interpretation. Therefore, the studies determining the effects of dressing on scar outcomes after the RCT trial are suggested [195]. Although the efficacy of sericin dressing was explored in many studies, the long-term outcomes from sericin dressing application are still less reported in those studies [23, 32, 108, 109], especially for the outcomes from multiple objective assessments and patient's perspectives. According to all mentioned above, the long-term examination for the scar characteristic of STSG donor sites by using multiple parameters may better reveal the effects of sericin dressing with collagen on wound healing in addition to overall healing time. Moreover, the consideration from both subjective and objective methods on scar outcomes might provide a better understanding of the overall outcomes of treatment [196].

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Table 6 Summary of clinical studies evaluating the scar outcomes in STSG donor sites healing

Studies	Participants	Intervention	Outcomes	Result
Rennekampff et al., 2006 [173]	81 patients for healing outcome and 33 patients for scar outcomes	1. Occlusive dressing (OD) 2. Biobrane® (BD) 3. Equine collagen dressing (CD) 4. Vaseline gauze (VD):	Duration of wound healing	OD: sig. faster healing 3 days than mean of all groups Mean (days): OD=14, BD=19, CD=19, VD=19
			Pain scores: VAS & FPRS	No sig. difference (day 2, 10)
			Scar: Cutometer parameters at 6 m	- All parameters sig. diff: pooled scar VS normal skin - Uf and Ue in VD and BD sig. ↑ than CD, other items: no sig. between groups - No sig. correlation: parameters VS healing time, parameters VS pliability VSS
			Scar: VSS at 6 m	Pooled scar VS normal skin - Sig. diff: Total VSS - No sig: Pliability Between 4 groups: No sig. for total VSS and pliability

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Table 6 Summary of clinical studies evaluating the scar outcomes in STSG donor sites healing (cont.)

Studies	Participants	Intervention	Outcomes	Result
Werdin et al., 2018 [70]	219 patients with 439 STSG donor sites	Betulin gel + moist dressing VS only moist dressing	Time to wound closure	Betulin group sig. ↓ time 15.3 VS 16.3 days, $p<0.05$
			Validated scar score: height, surface, and color At 3 and 12 months	- Betulin group sig. ↓ score At 3 m: 6.7±1.7 VS 7.1±1.7 At 12 m: 4.6±1.4 VS 4.71±1.4 - Scar: sig. improve 3 VS 12 m
			Correlation between healing time and scar quality	Pearson correlation coefficient, $p<0.000001$ - Healing time/ scar quality At 3 m: +0.46, at 12 m: +0.51 - Healing time/ all items

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Table 6 Summary of clinical studies evaluating the scar outcomes in STSG donor sites healing (cont.)

Studies	Participants	Intervention	Outcomes	Result
Karlsson et al., 2018 [197]	27 patients with 29 donor sites	Follow-up at 8 years after treatment with 1. Hydrofiber (n=11) 2. Polyurethane (PU) foam (n=9) 3. Porcine xenograft (n=9)	Scar: POSAS (patient scale)	Sig. diff btw 3 groups, p=0.03 - Hydrofiber better than PU and xenograft, p=0.03, 0.02 - PU VS xenograft: no sig.
			Correlation btw healing time and POSAS	No sig. diff btw groups - 14 VS 21 VS >21 days - ≤21 VS >21 days
			Association btw POSAS overall opinion & dressing	- Xenograft associated with ↑ overall opinion, p=0.03 - Only color item associated with overall opinion, p=0.02
			Hypertrophic scar 1st model: score > median 2nd model: thickness > 1	1st model: 3, 6, and 5 in group 1-3, resp. 2nd model: 3, 2, and 3 in group 1-3, resp.

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Table 6 Summary of clinical studies evaluating the scar outcomes in STSG donor sites healing (cont.)

Studies	Participants	Intervention	Outcomes	Result
Kim et al., 2019 [158]	18 patients with healed STSG donor site	Follow-up at 1, 2, 7, and 12 months after treated with silicone-faced polyurethane foam (Mepilex)	Hydration (TEWL)	No sig. change between time At 12 m: TEWL ≠ normal skin
			Color; chromameter *L**: 0=black to 100=white, *a**= green (-) to red (+), *b**= blue (-) to yellow (+)	- *L**: sig. diff up to 7 months and return at 12 m - *a** and *b**: tends to decrease after 2 m but not return to normal at 12 m
			Pressure threshold (monofilament)	At center: No sig. change At the edge: Sig. ↑ at 2 m
			Two-point discrimination center VS edge VS normal	No sig. between sites
			VSS between times	Pliability & height: no change Vascularity & pigmentation: sig. improved at 12 m

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Table 6 Summary of clinical studies evaluating the scar outcomes in STSG donor sites healing (cont.)

Studies	Participants	Intervention	Outcomes	Result
Karlsson et al., 2020 [195]	17 patients	Follow-up at 10 years after treatment with 1. Hydrofiber (n=5) 2. Polyurethane (PU) foam (n=6) 3. Porcine xenograft (n=6)	Observer part of POSAS	Total POSAS and all items: no sig. btw groups, p=0.968 - Vascularization, thickness, relief, pliability: median = 1 - (Hypo) pigmentation and surface: median > 1
			Healing time and POSAS	No diff between groups: ≤14 vs 15-21 vs >21 days
			Cutometer	- Only xenograft: scar ↓ F1 than normal skin, p=0.022 - No diff btw groups
			Healing time and Cutometer parameters	No diff between groups: ≤14 vs 15-21 vs >21 days
			Hypertrophic scar	No hypertrophic scar

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Table 6 Summary of clinical studies evaluating the scar outcomes in STSG donor sites healing (cont.)

Studies	Participants	Intervention	Outcomes	Result
Legemate et al., 2020 [65]	115 donor-site scars of 72 patients	Follow-up at 12 months after treatment with an alginate dressing	Patient part of POSAS at 12 months (6 items: color, pain, itch, thickness, relief, pliability) Cut-off in this study 1. low score, no diff with normal skin: POSAS = 1 2. intermediate score, minor diff: POSAS = 2, 3 3. high score, major diff: POSAS ≥ 4	Mean total POSAS = 1.9±1.2 (15.7% no diff and 84.3% at least minor diff) - Color: least appreciate: 41% major diff, 43% minor diff - Pain: 97% no diff - Other items: 8-12% major diff, 73-88% no diff Mean overall opinion: 3.2±2.2 (19.1% best scar and 80.9% at least minor dissatisfaction) - Younger age, female, darker skin type, and healing time: associated with poor scar

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According to the literature, STSGs are standard reconstructive techniques that are frequently performed in plastic surgery. Harvesting the graft brings about the wound at the donor site, which further causes morbidities to the patients. STSG donor sites need wound dressing to fasten healing, control infection, decrease pain, and manage exudate. Paraffin gauze seems to be the standard dressing used for donor site treatment in many clinical settings because of the ease of use and low cost. However, the drawbacks of this dressing are postoperative pain and adherence to the wound. Nowadays, the dressing that is considered the best dressing for STSG donor sites is still controversial [11]. There have been several studies investigating the most suitable dressing for this kind of wound. The essential outcomes that should be monitored in the donor site management are healing time, pain control, exudate absorption, infection rate, scar quality, ease of use, and cost of dressing [64].

The effects of either sericin or collagen on donor sites management have been studied. The results showed that sericin dressing could enhance the STSG donor healing in two trials [32, 108]; whereas, there was no advantage in healing time in one trial [109]. A further study exploring the effects of sericin and the reasons for the controversial results would be helpful. Some studies showed that collagen accelerated STSG donor sites healing [42, 43]; however, some studies reported no benefit of collagen on healing [10, 128]. Moreover, it seems that there is still no clinical trial investigating the effects of topical application of collagen hydrolysate on human wound healing. The combination of sericin and collagen possibly enhances their beneficial effects, and examining the effects of sericin and collagen hydrolysate in human wounds is still required. The complete healing time is the most critical outcome in wound healing studies. Besides, the evaluation for the scar outcomes is also necessary as the scar characteristics can influence patients' physical and psychological problems. However, there is still inadequate clinical trial determining the long-term outcomes from dressing application on STSG donor sites, especially in Asian patients.

Accordingly, the current study would investigate the clinical safety and efficacy of sericin dressing with collagen in the human wound, including the short- and long-term outcomes. The findings might indicate whether sericin dressing with collagen could be used as the alternative dressing for STSG donor sites. The data may illustrate the possible long-term outcomes on STSG donor sites, using for informing the patients.

CHAPTER III
RESEARCH METHODOLOGY

3.1 Study design

Phase I

Prospective, randomized, controlled matched-pair study

Phase II

Prospective, single-blinded, randomized, controlled matched-pair study

3.2 Sample and population

Phase I

3.2.1 Population

Healthy volunteers

3.2.2 Samples

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Healthy volunteers who passed all inclusion and exclusion criteria during June 2018 to November 2018

3.2.3 Inclusion and exclusion criteria

Inclusion criteria

1. Healthy volunteers aged 18 – 65 years
2. Able to communicate with the Thai language
3. Willing to attend the study and sign the informed consent

Exclusion criteria

1. Chronic skin diseases such as psoriasis and eczema

2. Immunocompromised diseases or using the medicine, e.g., immunosuppressants, antihistamine, steroids within 2 weeks before attending the study
3. Using an analgesic patch or topical application at the back within 2 weeks before attending the study
4. Known sensitivity/ allergy to sericin, chlorhexidine, collagen, fish, or Fixomull® (or any similar patch)
5. Pregnancy or lactation
6. Active or chronic scar at the back, which has no sufficient area for attaching two dressings (2x2 cm^2)

Termination criteria

1. Having any adverse skin effect during attending the study that disturbs the patient's daily life considering from the dermatologists.
2. Using immunosuppressants, antihistamines, or steroids during attending the study
3. Loss follow-up at least one time

3.2.4 Sample size calculation

The evaluation and interpretation of the patch test were reviewed by McNamee et al., 2008 [198]. At least 100 volunteers should attend until the end of the study, so there will be a chance of 0.99 (99%) to detect adverse effects with 5% incidence. The compensation for 10% drop-out was calculated, resulting in a sample size of at least 112 subjects.

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Phase II

3.2.1 Population

Patient with split-thickness skin graft (STSG) donor site wound in the division of plastic and reconstructive surgery, King Chulalongkorn Memorial Hospital

3.2.2 Samples

Patient with split-thickness skin graft (STSG) donor site wound in the division of plastic and reconstructive surgery, King Chulalongkorn Memorial Hospital from July 2020 to July 2021 who passed all inclusion and exclusion criteria

3.2.3 Inclusion and exclusion criteria

Inclusion criteria

1. Adult patients aged more than 18 years old
2. The split-thickness skin graft is harvested.
3. Donor sites are on the thigh area.
4. Patients who can communicate with the Thai language
5. Patients who are willing to attend the study and sign the informed consent

Exclusion criteria

1. Skin graft had been previously harvested in the same area.
2. Patients with mental disorders or immunocompromised diseases
3. The patient who cannot or is not willing to follow the protocol
4. Known sensitivity or allergy to sericin, chlorhexidine, fish, and collagen
5. Pregnancy or lactation

Termination criteria

1. Wound with signs of clinical infection (enhanced secretion, pain, and positive culture)

3.2.4 Sample size calculation

This study investigated time (days) to complete healing between STSG donor sites treated by sericin dressing with collagen and treated by control dressing (Bactigras®) in 2 dependent samples (equally divided wound). The sample size was calculated from the below formula.

$$N = \frac{(Z_\alpha + Z_\beta)^2 {S_p}^2}{D^2}$$

The study of Lohsiriwat et al. in 2009 [89] determined the efficacy of anionic silver-containing hydrofiber dressing (Aquacel) compared with the paraffin gauze dressing on STSG donor sites. Complete epithelialization time in Aquacel treated group (7.90 ± 2.47 days) was significantly lower than in the paraffin gauze dressing group (11.20 ± 3.52 days), p = 0.031.

Confidence level is considered at 95% (α = 0.05) one-tailed. Power is considered at 90% (β = 0.1).

Z_α = 1.645 Z_β = 1.282

N = sample size per group S_p^2 = pooled variance

D = effect size; minimal clinically important difference in time to complete healing of STSG donor site is considered at 1.9 days [71].

Calculate pooled variance; $S_p{}^2 = \frac{S_1{}^2 + S_2{}^2}{2} = \frac{2.47^2 + 3.52^2}{2} = 9.24565$

Sample size; $N = \frac{(Z_\alpha + Z_\beta)^2 S_p{}^2}{D^2} = \frac{(1.645 + 1.282)^2\ 9.246}{1.9^2}$

$= 21.94 \approx$ 22 STSG donor sites

Calculate for 25% drop out rate

$N = \frac{22}{1 - 0.25} = 29.33 \approx$ 30 STSG donor sites

Therefore, 30 STSG donor sites ($\leq$ 30 patients) were enrolled in this study.

3.3 Random allocation

Phase I

The skin at the back area of each healthy volunteer was divided into two sites. Each site was randomly allocated into sericin dressing with collagen group (A) or control group, Bactigras® (B), by simple randomization using a computer-generated sequence. One participant received both dressings. Then, the same type of dressing was replaced at the identical site after removal at each appointment throughout the study.

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Phase II

STSG donor sites were divided into two equal areas. Each area was randomly allocated into sericin dressing with collagen or control groups by simple randomization using a computer-generated sequence.

3.4 Study procedure

Phase I

1. The eligible healthy volunteers were informed about the study details and asked to sign a consent form before starting the study.
2. At the first visit, the demographic data were collected from each volunteer. The back of each volunteer was divided into 2 sites. The photographs of particular skin were taken, and the erythema and melanin levels at the studied areas were measured using Mexameter. Then, the sericin dressing with collagen and Bactigras® were randomly attached to each back site before covering with adhesive tape (Fixomull®) for 72 hours. One participant received both dressings applied on different sites.
3. At every appointment, the photographs of particular skin were taken within 30 minutes after dressing removal. The erythema and melanin level at the studied areas were measured using Mexameter.
4. At the second visit, the existing dressing was removed. The fresh identical dressings were replaced at the same position continually for 72 hours, called induction phase.
5. After removing the dressings at the third visit, no dressing was attached for 7-10 days, the resting period.
6. Photographs and skin color were collected at the fourth visit. Then, the dressings were attached again to their identical position for 72 hours before removing at the fifth visit.
7. Three dermatologists evaluated all photographs without identifying the type of dressing using the human repeat insult patch test (HRIPT) scoring scale (Appendix B).

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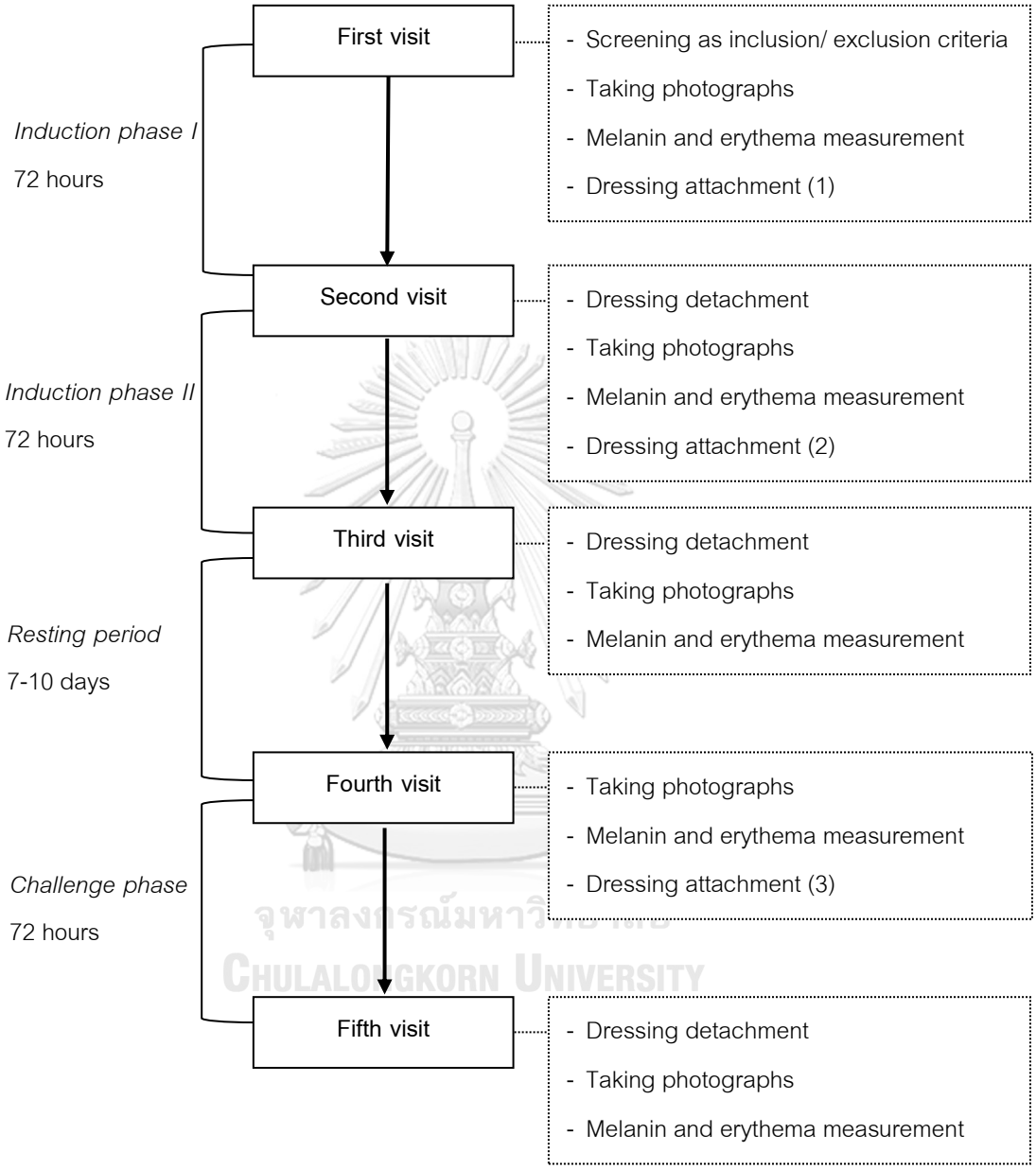
Flow chart: phase I



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Phase II

1. At Out-Patient-Department of plastic and reconstructive surgery, King Chulalongkorn Memorial Hospital, eligible patients received all information about the study, including objective, methodology, and possible risks and benefits from the researcher who had no involvement with their treatment. Patients could freely ask and receive their own time for consideration before signing a consent form.
2. The patients who gave the signed consent form were screened as inclusion and exclusion criteria. The demographic data and baseline of blood chemistry (liver function and renal function) were recorded.
3. The split-thickness skin graft was harvested along with the standard procedure. Firstly, the patient was administered general anesthesia. Then, the donor site skin was scrubbed with 10 %v/v povidone-iodine followed by 5 %v/v chlorhexidine gluconate solution. After that, a split-thickness skin graft was harvested from the thigh area using a Zimmer® dermatome (Zimmer, Inc., Warsaw, IN, USA). The wound at the donor site was covered with adrenaline-soaked gauze for a few minutes to stop bleeding. Photographs and detail of the wound were taken before dividing the wound equally into two sites. Each site was randomly allocated into either sericin dressing with collagen group (treatment group) or Bactigras® group (control group). Every wound of each patient received both sericin dressing with collagen and Bactigras® as the primary dressing. If the first site received a treatment dressing, the other site would receive a control dressing.
4. The size of both primary dressings was prepared in equal size (10x10 cm^2). The practitioner adjusted the size and number of dressings in order to cover the wound completely. Then, the wound was covered with the secondary dressing as the standard procedure.
5. After covering for 3 and 7 days, the secondary dressing was removed to examine the surrounding tissue and wounds (if possible). Moreover, the patient was asked to evaluate pain at each site using VAS on days 1, 2, 3, 4, 5, 7, and 14.
6. The primary dressing would not be changed unless there was excessive exudate or any sign of a worse reaction. If there were any signs of infection, the wound would be swabbed or biopsied as the clinician's consideration for the microbiological test.

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7. The systemic adverse effect on renal and hepatic functions was monitored from a blood sample collected 7 days after dressing application. If there were any signs of adverse effects, the researcher would evaluate the patient using Naranjo's algorithm.
8. After covering for 7 days, the complete healing of the wound was examined every day by removing the secondary dressing and investigating for spontaneous detachment of primary dressing without pain. Time to complete healing was recorded after the clinician considered for full epithelialization.
9. Patients would stay in the hospital around 1-2 weeks, mainly depending on the healing of their defects that causing STSG or until the clinician has a decision that patients can be discharged.
10. The patients discharged before the detachment of primary dressing were asked to monitor the detachment of wound dressing and record the date if the dressing detached before the follow-up.
11. At the first follow-up (1-2 weeks after discharge), the secondary wound dressing was removed by the clinician to examine the adherence of the primary dressing to the wound. The primary dressing would be removed if it had no adherence and pain during dressing removal. The date of time to complete healing was recorded for the wound with full epithelialization. Then, the scar's color, TEWL, and epidermal hydration were evaluated using Mexameter®, Tewameter®, and Corneometer®, respectively. The primary dressing would not be removed if the dressing still adhered to the wound or the patient had pain during the removal. The secondary dressing was re-applied, and these patients were asked to monitor and record the date of detachment continually.
12. After complete healing for 1, 3, and 6 months, the scar quality after wound healing was evaluated by a clinician who could not identify the previously applied dressing using subjective method (VSS) and by the researcher using objective method (Mexameter®, Tewameter®, and Corneometer®). At 6 months, the additional measurements, i.e., the elasticity and patient satisfaction, were measured using Cutometer and POSAS (patient part).

Conditions for objective measurements

All measurements, including scar color (Mexameter®), TEWL (Tewameter®), hydration level (Corneometer®), and skin elasticity (Cutometer®) were performed in the same closed room that controlled for the light, temperature, and relative humidity. Patients were asked to avoid applying creams or moisturizers on the STSG donor area for at least 8 hours before the measurements. Each measurement was taken at the center of each site. The scar color and TEWL were repeatedly measured 10 and 25 times, respectively. The hydration level was measured in at least 5-times with an interval of 5 seconds. According to the manufacturer guideline, if two values differ from 4 a.u., an additional sixth measurement would be conducted to balance an average. Cutometer® with an aperture of 2 mm was used at constant negative pressure (450 mbar). The suction time and relaxation time were 2 seconds, which were automatically repeated 3 times. All devices were calibrated according to the manufacturer guideline every month.

3.5 Operational definitions

Complete healing time

The number of days since starting treatment until the primary dressing can spontaneously detach from the wound with full re-epithelialization (at least 95% re-epithelialization) without any pain and drainage

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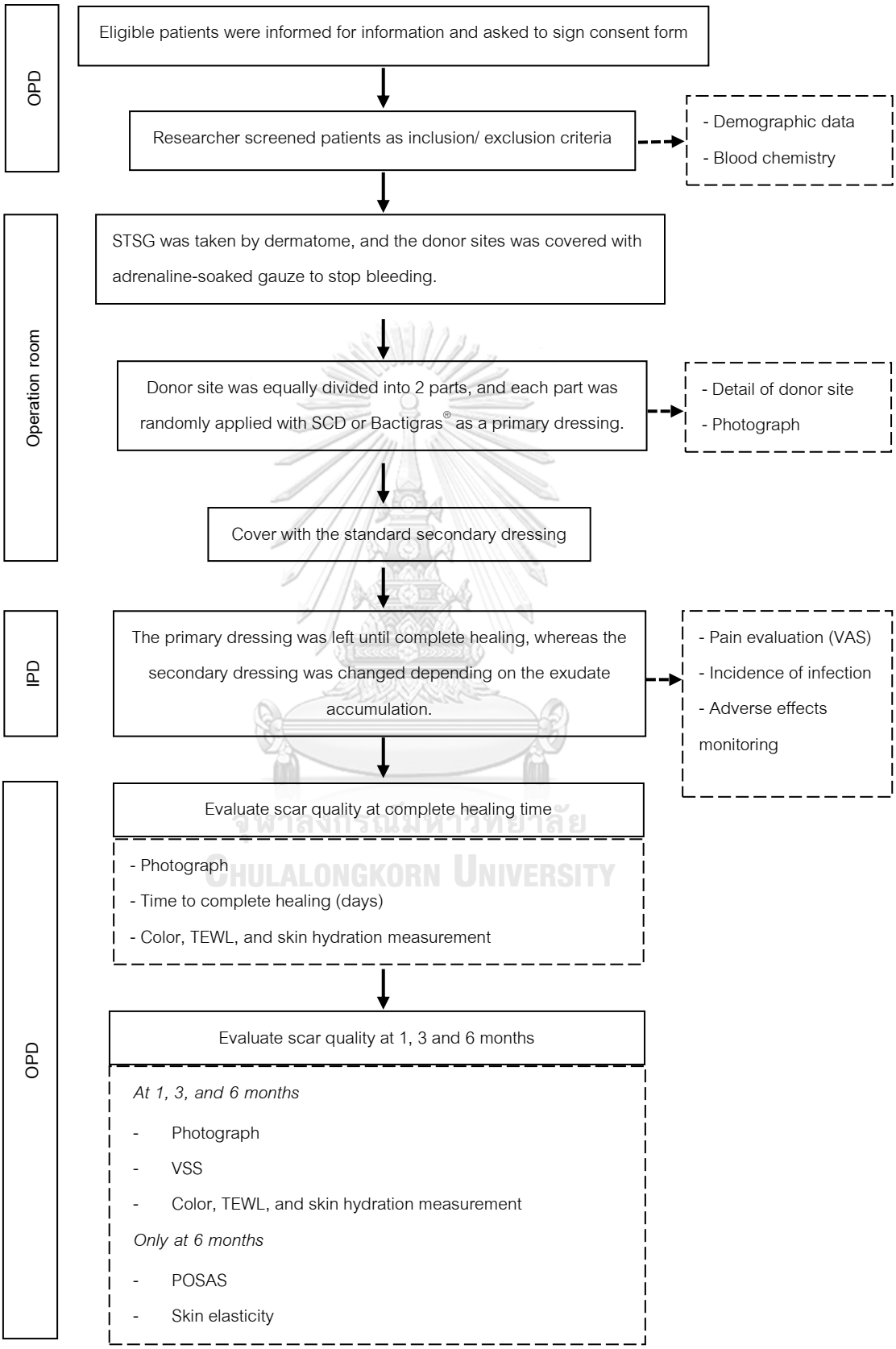
Infection wound

The wound which has purulent secretion or some of the inflammatory reactions (erythema, warmth, pain, tenderness, or induration) and has positive result from the microbiological test

Adverse effects

The adverse effects were the unexpected medical problems that happen during treatment, including the local adverse effects, e.g., rash or allergic reactions determined by the clinicians and the systemic adverse effects, which are the change of the results from blood chemistry into the abnormal range.

Flow chart: phase II



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3.6 Data collection

Phase I

Demographic data of healthy volunteers were collected before starting the study. At every visit before attachment or after detachment of dressings, the photographs of the studied area were taken and the measurements for melanin and erythema level were measured by Mexameter®.

Phase II

The data collection time of phase II is displayed in Table 7.

Table 7 Data collection time

Collection time	Data
At baseline (day 0)	1. Patient demographic data 2. Baseline blood chemistry 3. Wound characteristic: cause, location, size, depth 4. Photograph
Any day after receiving treatment	1. Adverse effect with Naranjo score and signs of infection (if any) 2. Microbiological tests (if there is any sign of infection)
On day 1-7 and 14	1. Pain score (VAS)
Within 7 days	1. Blood chemistry
At complete healing time	1. Time to complete healing (days) 2. Photograph 3. Scar quality - Trans-epidermal water loss (TEWL) by Tewameter® - Melanin and erythema level by Mexameter® - Epidermal hydration by Corneometer®
After complete healing for 1, 3, and 6 months	1. Photograph 2. Scar quality *at 1, 3, and 6 months*: TEWL, melanin and erythema level, epidermal hydration, and VSS *Only at 6 months:* Skin elasticity by Cutometer® and patient part of Patient and Observer Scar Scale (POSAS) by patient

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3.7 Materials/ Tools

3.7.1 Wound dressings

Experimental dressing: Sericin dressing with collagen (SDC) was prepared by cast dying method under sterile condition and controlled for the quality by Bioactive Resources for Innovative Clinical Applications Research Unit, Pharmaceutical Sciences, Chulalongkorn University. The evaluation for the properties of dressing was shown in Appendix O. To control the quality between batches, samples of SDC from each batch were randomly evaluated for the protein content by BCA Protein Assay Reagent. Gamma radiation (25 kGy) was used for sterilization by the Thailand Institute of Nuclear Technology (Nakornnayok, Thailand). The sterilization of dressing

Control dressing: Bactigras® (Smith & Nephew Co., Ltd, London, UK), which is the standard wound dressing for treating STSG donor sites

3.7.2 Data record forms

1. Demographic data of healthy volunteer record form (Appendix A)
2. Human repeat insult patch test (HRIPT) scoring scale (Appendix B)
3. Skin color measured by Mexameter record form (Appendix C)
4. Demographic data of patient record form (Appendix D)
5. Adverse effect record form (Appendix E)
6. Naranjo's Algorithm record form (Appendix F)
7. Wound characteristics, microbiological test, and pain score record form (Appendix G)
8. Pain evaluation record (Appendix H)
9. Scar quality (VSS) record form (Appendix I)
10. Scar quality evaluated by objective devices record form (Appendix J)
11. Scar quality (POSAS) record form (Appendix K)

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3.7.3 Objective scar measurement

1. Cutometer® MPA 580 (Courage + Khazaka electronic GmbH, Germany)
2. Mexameter® MX 18 (Courage + Khazaka electronic GmbH, Germany)
3. Tewameter® TM 300 (Courage + Khazaka electronic GmbH, Germany)
4. Corneometer® CM 825 (Courage + Khazaka electronic GmbH, Germany)

3.8 Data analysis and interpretation

The data were analyzed by using SPSS version 13.0 (SPSS. Co., Ltd., Bangkok Thailand). The significance level was defined at α = 0.05. The demographic data will be presented as frequency, percent, mean, and standard deviation.

Phase I

Repeated measures ANOVA was used for determining the significant change of the melanin and erythema levels. For erythema score and edema evaluated by dermatologists, Friedman's test was used to analyze the statistical difference, followed by the Wilcoxon Signed Ranks test. The significant differences of other elevated responses (papules, vesicles, and bullae) were analyzed by Cochran's Q test followed by the McNemar test.

Phase II

The outcomes comparisons are presented in table 8.

Table 8 Variables and statistical analyses in phase II

Hypotheses	Variables	Statistical analyses
1. The sericin dressing with collagen group has a lower complete healing time for treating the STSG donor site than the Bactigras® group.	IV: Type of dressing DV: Complete healing time	Wilcoxon signed-rank test
2. The sericin dressing with collagen group has a lower VSS score compared with the Bactigras® group.	IV: Type of dressing DV: VSS scores	Friedman's test and Wilcoxon signed-rank test
3. The sericin dressing with collagen group has a lower POSAS score compared with the Bactigras® group.	IV: Type of dressing DV: POSAS scores	Wilcoxon signed-rank test
4. The sericin dressing with collagen group has a lower melanin level compared with the Bactigras® group.	IV: Type of dressing DV: Melanin level	Repeated measure ANOVA adjusted by Bonferroni

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Table 8 Variables and statistical analyses in phase II (cont.)

Hypotheses	Variables	Statistical analyses
5. The sericin dressing with collagen group has a lower erythema level compared with the Bactigras® group.	IV: Type of dressing DV: Erythema level	Friedman's test and Wilcoxon signed-rank test
6. The sericin dressing with collagen group has lower TEWL compared with the Bactigras® group.	IV: Type of dressing DV: TEWL	Friedman's test and Wilcoxon signed-rank test
7. The sericin dressing with collagen group has a higher hydration level compared with the Bactigras® group.	IV: Type of dressing DV: Hydration level	Friedman's test and Wilcoxon signed-rank test
8. The skin elasticities in sericin dressing with collagen and Bactigras® groups were comparable with normal skin.	IV: Type of dressing DV: Cutometer parameters	Repeated measures ANOVA adjusted by Bonferroni or Friedman's test and Wilcoxon signed-rank test
9. The sericin dressing with collagen group has a lower pain score on the STSG donor site treatment than the Bactigras® group.	IV: Type of dressing DV: Pain score	Friedman's test and Wilcoxon signed-rank test
10. There is no difference in the incidence of infection between the STSG donor site treated with sericin dressing with collagen and Bactigras®.	IV: Type of dressing DV: Incidence of infection	Chi-square test
11. There is no adverse effect obtained from the application of sericin dressing with collagen and Bactigras®.	IV: Type of dressing DV: Incidence of adverse effect	Chi-square test

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3.9 Ethical Consideration

All participants received all information about the study until they had well understood it. Moreover, they have the freedom to attend or not attend to the project. All information was kept confidential data with no data in the report that can refer to the participants. Phase I (COA No. 496/2018) and phase II (COA No. 662/2020) of the study were approved by the Ethics Review Committee for Research Involving Human Research Subjects, Institutional Review Board, Faculty of Medicine, Chulalongkorn University (Appendix L).

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CHAPTER IV
RESULTS

4.1 Phase I

There were 113 healthy volunteers enrolled in this study. One participant was excluded because of having an underlying disease indicated in the exclusion criteria. Because of personal reasons, e.g., traveling problem, six participants resigned from the study after the first application and absented in the second appointment. Three participants lost dressing before the next appointment resulting in withdrawal. One hundred and three volunteers completed the entire study. The demographic data of healthy volunteers are presented in Table 9.

The per-protocol analysis was utilized for comparing the results between SDC and Bactigras® group. Figure 3 shows the process of the patch test conducted on the skin of healthy volunteers. The levels of erythema and melanin measured by Cutometer® (Mexameter mode) had no significant difference between sericin dressing with collagen (SDC) and Bactigras®, as shown in Figure 4 and Table 10. The erythema levels of both dressings (approximately 240 units) did not significantly differ between before and after application. The levels of melanin (approximately 200 units) after applying both dressings decreased significantly compared with the first appointment. Tables 11 and 12 display the results from the evaluation of dermatologists, demonstrating the results following the Human repeat insult patch test (HRIPT) scoring scale in Appendix B. The erythema scores and the edema scores in the SDC group were significantly higher than the Bactigras® group after the first, second, and third application. It was found that most of the participants (around 90%) had no edema. For the other elevated responses, there was no significant difference in papules between the two dressings, and there were no vesicles and bullae in both groups.

Moreover, the intention-to-treat analysis was also conducted. The results showed no significant difference in both melanin and erythema level between groups. Compared to the first visit, significant reductions in the melanin level at all later visits were found in the SDC group. There was no significant change in erythema level. The melanin level in the Bactigras® group at later visits was significantly lower than the first visit, except for the second visit. The erythema levels in the Bactigras® group significantly decreased at the third and fifth visit, compared to the first visit. Utilizing

intention-to-treat analysis, the skin responses evaluated by the dermatologists revealed similar results with the per-protocol analysis. Overall, the findings from the intention-to-treat analysis seem to comply with the per-protocol analysis.

Table 9 Demographic data of healthy volunteers (n=103)

Characteristics	Min	Max	Mean±SD
Age (year)	18	65	34.36±12.74
BMI (kg/m^2)	16.02	34.38	22.36±3.70
Characteristics	**Category**	**Number of participants**	**%**
Gender	Male	26	25.24
	Female	77	74.76
Occupations	Officer	49	47.57
	Student	27	26.21
	Labor	24	23.30
	Business owner	3	2.91
Underlying diseases	None	88	85.44
	Diabetes mellitus Type II	2	1.94
	Hypertension	3	2.91
	Cancers	-	-
	Dyslipidemia	-	-
	Others	10	9.71
Alcohol intake history	No	85	82.52
	Yes	18	17.48
Smoking history	No	93	90.29
	Yes	10	9.71
Caffeine intake	No	48	46.60
	Yes	55	53.40
Known allergy	No	93	90.29
	Yes	10	9.71

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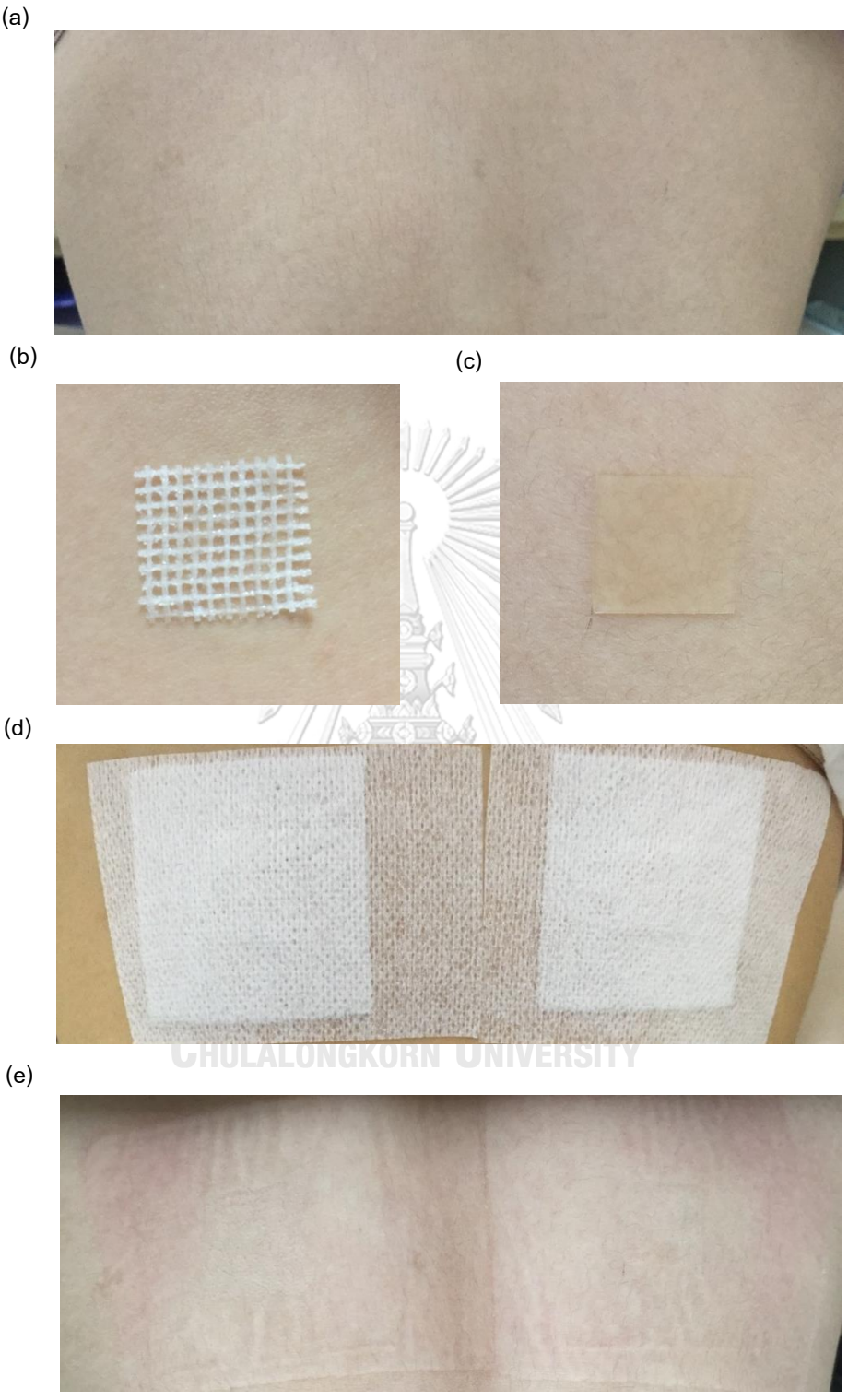

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Figure 3 (a) Skin at the back area of healthy volunteer, (b) Applied Bactigras®, (c) Applied sericin dressing with collagen, (d) Covered with Fixomull®, (e) After dressing removal

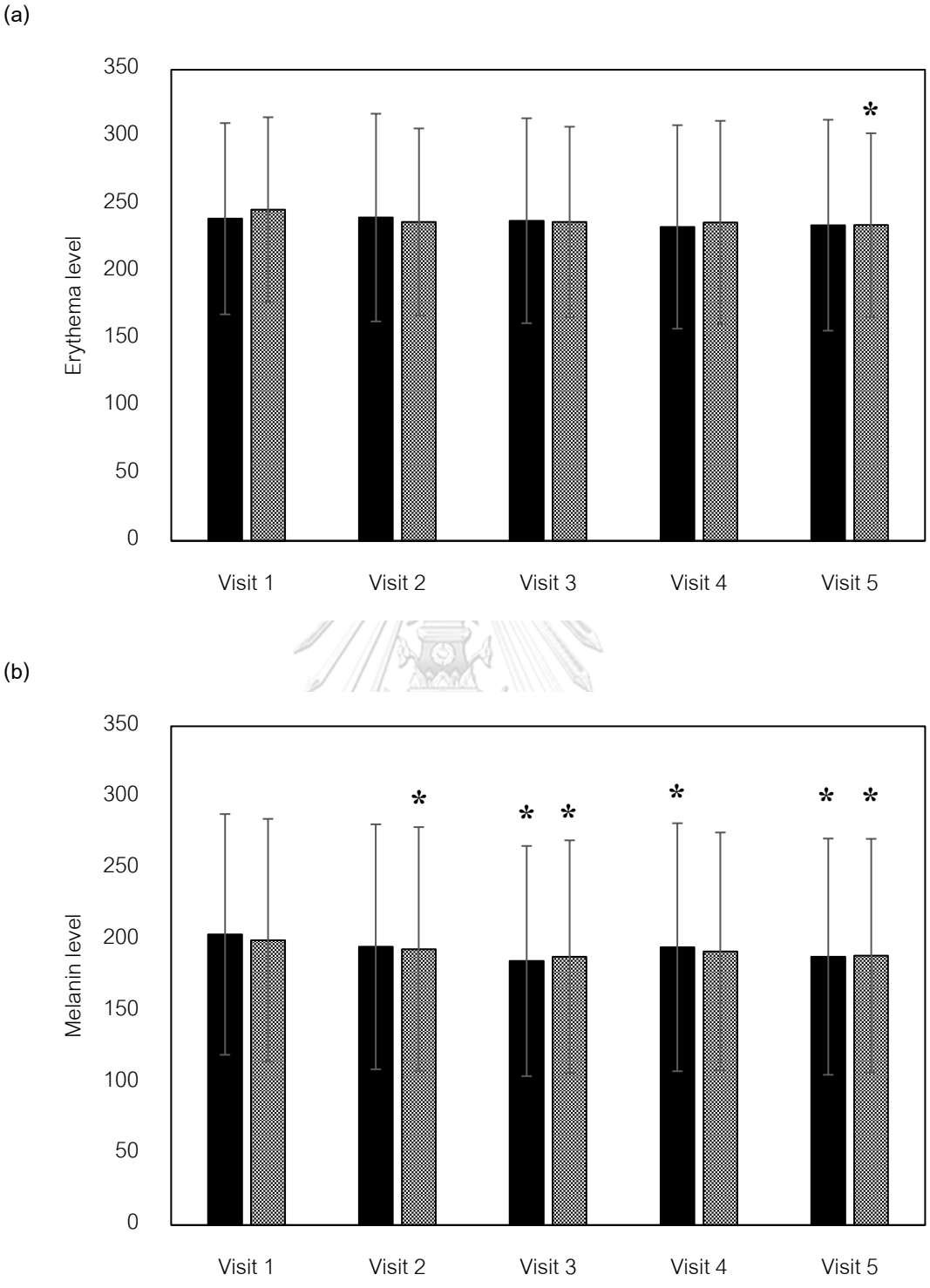

Figure 4 Levels of (a) erythema and (b) melanin measured by Cutometer® (Mexameter mode) after applying sericin dressing with collagen (SDC) (black bars) and Bactigras® (dotted bar) at each visit * Significant differences at $p<0.05$, compared with the first visit

Table 10 Levels of erythema and melanin measured by Cutometer® (Mexameter mode) (n=103)

Time	Erythema (Mean ±SD)		Melanin (Mean ±SD)	
	SDC	Bactigras®	SDC	Bactigras®
Visit 1	239.33 ± 71.04	246.07 ± 68.64	203.94 ± 84.45	199.88 ± 85.15
Visit 2	240.17 ± 77.15	236.88 ± 69.52	195.23 ± 85.97*	193.63 ± 85.61
Visit 3	237.84 ± 76.13	236.91 ± 70.76	185.26 ± 80.76*	188.27 ± 81.69*
Visit 4	233.21 ± 75.58	236.51 ± 75.53	194.95 ± 87.01*	192.05 ± 83.42
Visit 5	234.48 ± 78.36	234.58 ± 68.25*	188.32 ± 82.89*	189.04 ± 81.97*

* Significant differences at $p<0.05$, comparing with the first visit

Table 11 Percentage of erythema score evaluated by three dermatologists after the first, second, and third application (n=103)

Erythema scale	Sericin dressing with collagen			Bactigras®		
	First	Second	Third	First	Second	Third
No	55.3%*	44.7%*	52.4%*	79.6%	81.6%	81.6%
Mild[a]	36.9%	51.4%	43.7%	19.4%	18.4%	18.4%
Moderate[b]	7.8%	3.9%	3.9%	1.0%	0.0%	0.0%
Severe[c]	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

* Significant differences at $p<0.001$, comparing between two dressings

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Table 12 Percentage of elevated response evaluated by three dermatologists after the first, second, and third application (n=103)

Elevated response	Sericin dressing with collagen			Bactigras®		
	First	Second	Third	First	Second	Third
Edema						
No	89.3%*	91.3%†	88.4%‡	96.1%	100.0%	99.0%
Mild	9.7%	6.8%	9.7%	3.9%	0.0%	1.0%
Moderate	1.0%	1.9%	1.9%	0.0%	0.0%	0.0%
Severe	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Papules						
No	95.1%	94.2%	95.1%	94.2%	91.3%	93.2%
Exist	4.9%	5.8%	4.9%	5.8%	8.7%	6.8%
Vesicles						
No	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Exist	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Bullae						
No	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Exist	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%

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* Significant differences at p=0.021, comparing between two dressings

† Significant differences at p=0.005, comparing between two dressings

‡ Significant differences at p=0.003, comparing between two dressings

4.2 Phase II

The demographic data of patients and the characteristics of STSG donor sites are presented in Table 13 and Table 14, respectively. Twenty-one patients were enrolled in the study, aged between 19 to 77 years, and the average age was around 51 years. One-third of the patients were female. The total number of donor sites was 30 wounds. The highest number of studied donor sites per one patient was 3 wounds.

Table 13 Patients' demographic data (n=21)

Characteristics	Min	Max	Mean±SD
Age (year)	19	77	50.71±16.66
BMI (kg/m^2)	19.49	32.00	24.35±4.09
Characteristics	**category**	**Number of patients**	**%**
Gender	Male	14	66.67
	Female	7	33.33
Number of studied wounds in one patient	1	14	66.67
	2	5	23.81
	3	2	9.52
Number of comorbidities in one patent	No	5	23.80
	1	8	38.10
	>1	8	38.10
Comorbidity	None	5	-
	Diabetes mellitus Type II	7	-
	Dyslipidemia	5	-
	Cancers	13	-
	Cardiovascular diseases	7	-
	Others	7	-
Alcohol intake history	No	17	80.95
	Yes	4	19.05
Smoking history	No	14	66.67
	Yes	7	33.33
Known allergy	No	15	71.43
	Yes	6	28.57
Hypertrophic scar history	No	12	57.14
	Yes	9	42.86

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Table 14 Donor sites' characteristics (n=30)

Characteristics		Number	(%)
Cause of STSG	Excision of tumors	19	63.33
	Diabetic wounds	4	13.33
	Injuries from accidents	4	13.33
	Burns	2	6.67
	Others	1	3.33
Characteristics	Min	Max	Mean±SD
Wound size (cm^2)	24	128	79.12±24.62
Wound depth (inches)	8/1000	16/1000	10.23±1.52/1000

4.2.1 Complete healing time

Complete healing time in sericin dressing with collagen (SDC) group (15.00 ± 7.00 days) was significantly lower than Bactigras® (control) group (16.00 ± 8.00 days), Table 15. Figure 5 shows the appearance of donor sites before, during, and after treating with SDC and control dressing. SDC had a transparent appearance allowing the inspection for wounds without the removal. The appearance of detached wound dressing at complete healing time is presented in Figure 6. SDC had no adherence with the absorbent gauze, which is the secondary dressing, whereas Bactigras® adhered with the secondary dressing.

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Table 15 The median of complete healing time in each group

Group	Complete healing time (days) Median ± IQR (range)
Sericin dressing with collagen	15.00 ± 7.00 (7-36)*
Bactigras®	16.00 ± 8.00 (10-36)

*Significant difference (p = 0.015, one-tailed), calculated by a Wilcoxon signed-rank test.

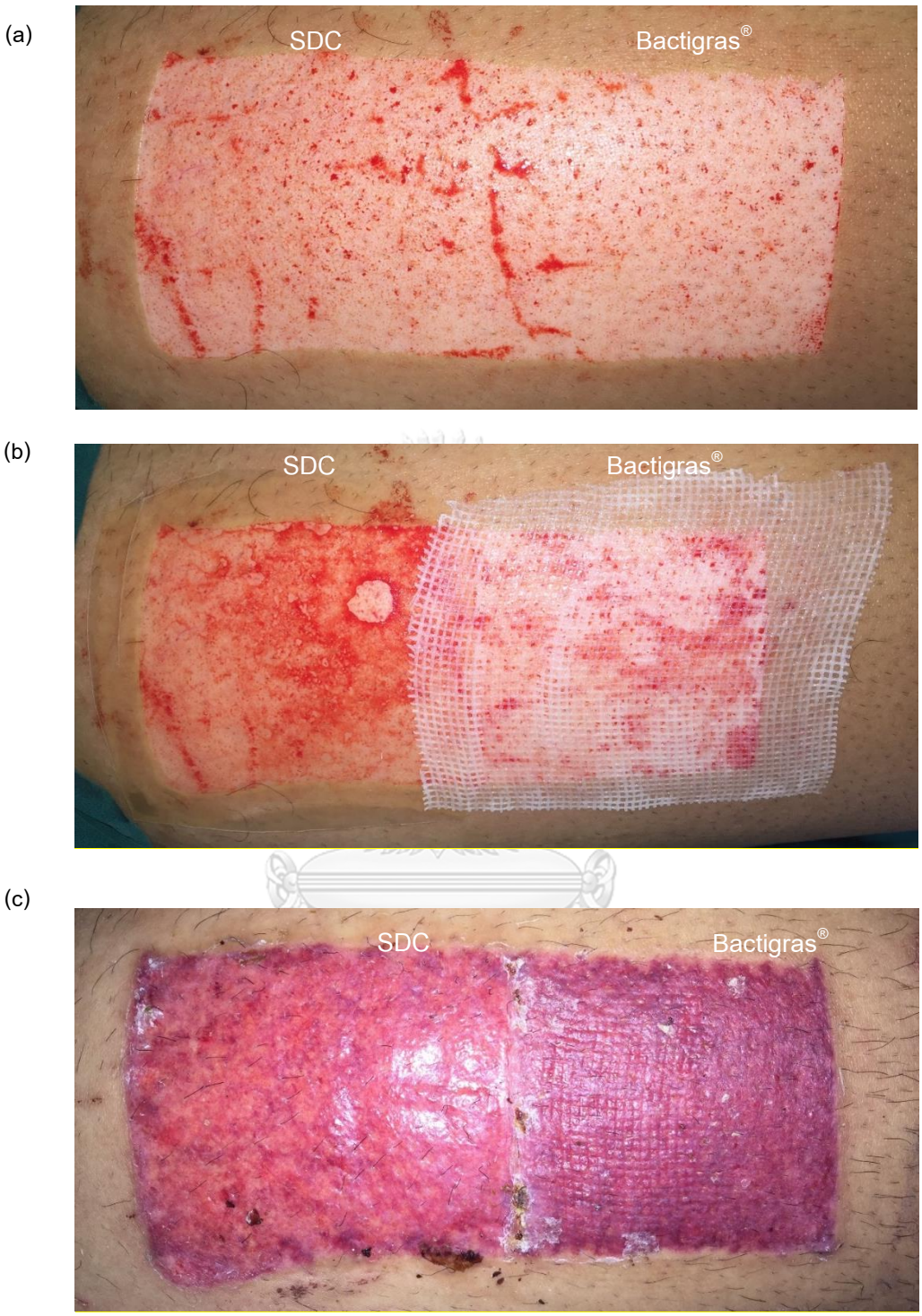

Figure 5 The appearance of STSG donor sites (a) after harvesting, (b) after covering with the primary dressing (SDC and Bactigras®), and (c) at complete healing time, and the appearance of detached primary dressing (SDC and Bactigras®)

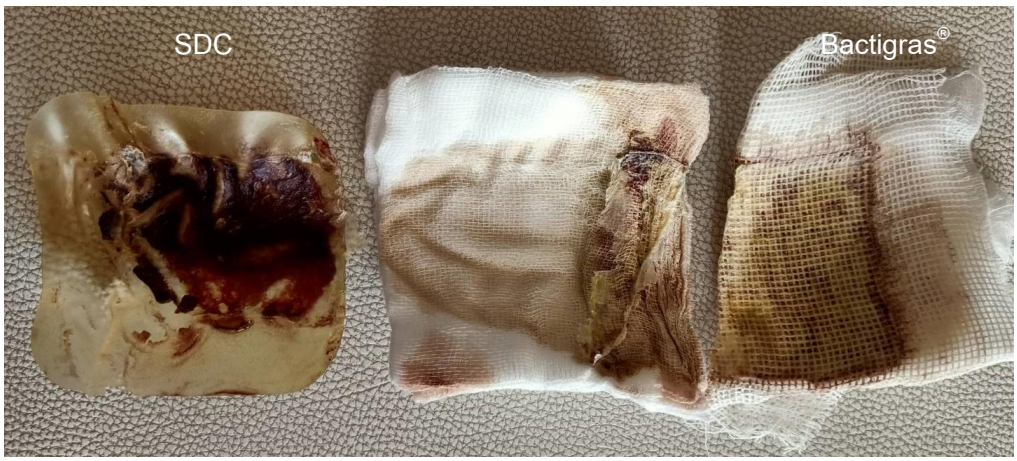

Figure 6 The appearance of SDC and Bactigras® after detachment

4.2.2 Scar quality

After complete healing, two patients with four wounds (two wounds per patient) had a recurrent injury on both of SDC and Bactigras® side before the first follow-up (1 month). These wounds were continually monitored for progression, and it was found that all wounds healed later. The data from the measurements of these wounds were not included in the scar quality outcomes. Demographic data of the remaining patients (19 patients with 26 wounds) had no significant difference with the data of all patients. When compared the healing time of the remaining patients, the SDC group still had significantly lower healing time (median ± IQR, range: 15.00 ± 7.75, 7-36) than the Bactigras® group (median ± IQR, range: 17.00 ± 8.25, 10-36). After complete healing for 0, 1, 3, and 6 months, the scar quality of donor sites treated with both dressings was examined by subjective and objective methods. One patient at 3 months and two patients at 6 months lost to follow-up because of traveling problems, and one patient with 2 wounds deceased before the last follow-up (6 months).

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Subjective method

There were two subjective measurements used in the current study to examine the effects of treatment from the clinician and patient perspective. Vancouver scar scale (VSS) was evaluated by the clinician, and the patient assessed the POSAS (patient part).

4.2.2.1 Vancouver scar scale (VSS)

Vancouver scar scales (VSS), consisting of pliability, height, vascularity, and pigmentation, were utilized for evaluating scar quality after complete wound healing for 1, 3, and 6 months.

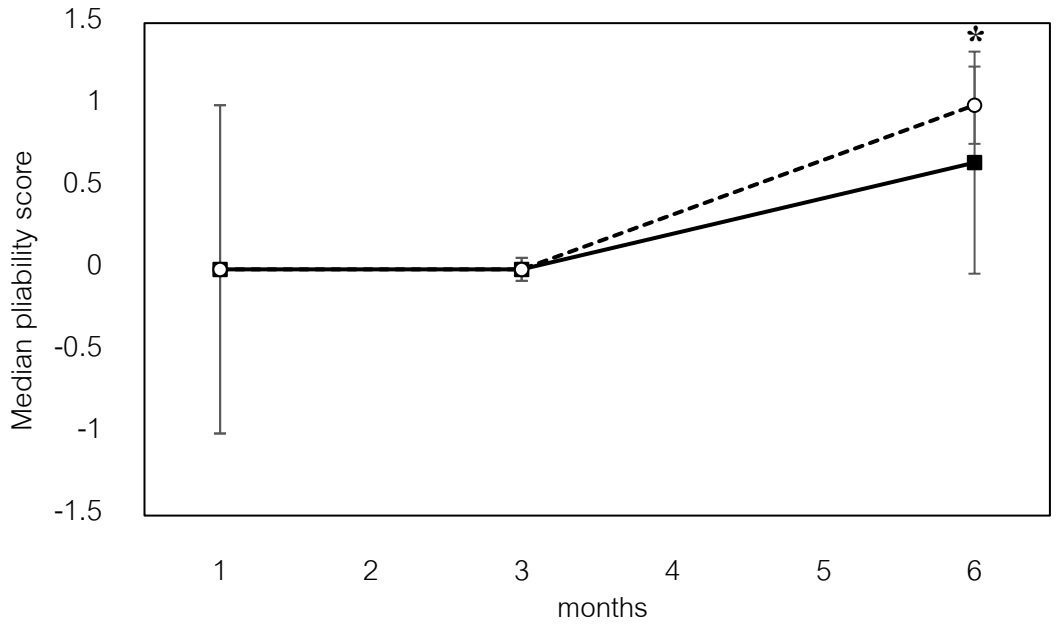

Figure 7 Pliability score (median±IQR) of STSG donor sites wound treated with sericin dressing with collagen (SDC) (solid line; —■—) Bactigras® (control; dashed line; --○--) at 1, 3, and 6 months after complete healing

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The pliability score of STSG donor sites treated with Bactigras® was significantly higher than SDC at 6 months (p = 0.003) (Figure 7). The pliability of the Bactigras® group at 3 months was also significantly changed compared with at the first month. Compared to at 3 months, the pliability at 6 months of donor sites treated with both dressings significantly increased.

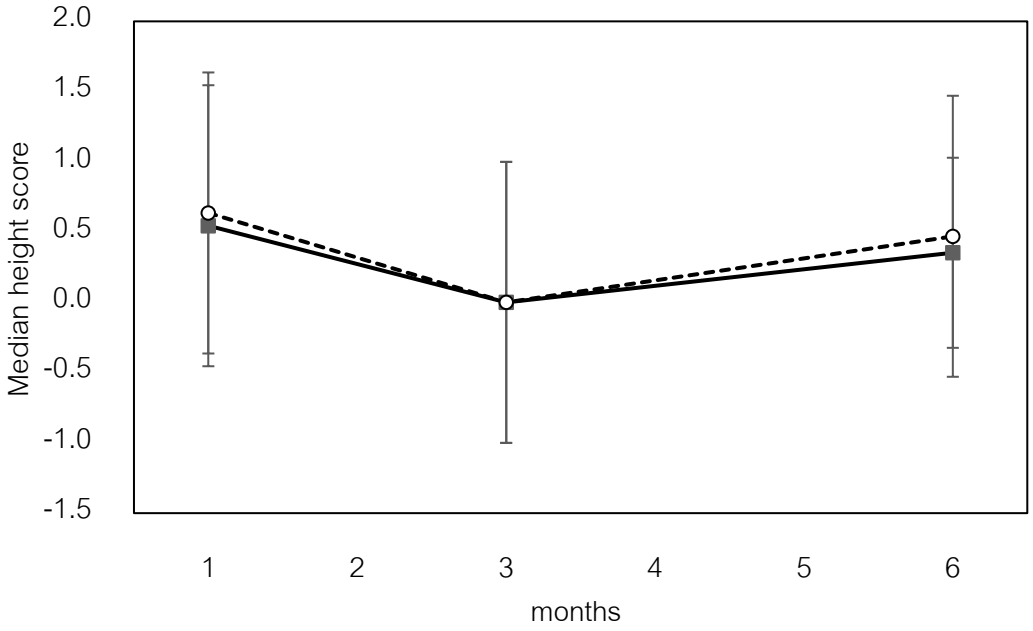

Figure 8 Height score (median±IQR) of STSG donor sites wound treated with sericin dressing with collagen (SDC) (solid line; —■—) Bactigras® (control; dashed line; --o--) at 1, 3, and 6 months after complete healing

There was no significant difference in the height scores between the Bactigras® group and SDC group at all time points. The height scores of STSG donor sites in both groups had no significant change during the 6-month follow-up (Figure 8).

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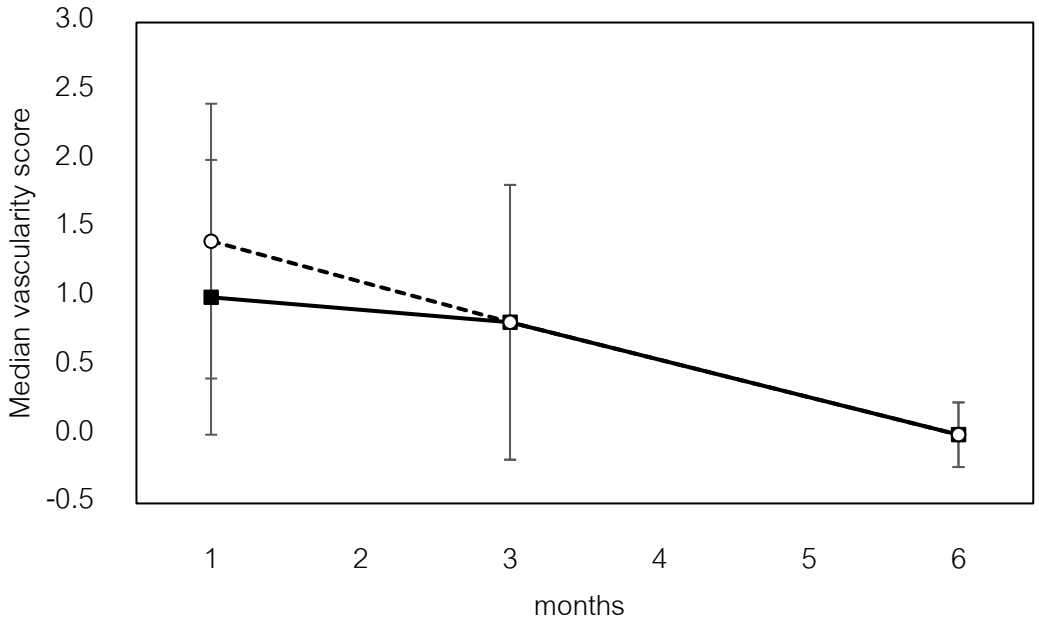

Figure 9 Vascularity score (median±IQR) of STSG donor sites wound treated with sericin dressing with collagen (SDC) (solid line; —■—) Bactigras® (control; dashed line; --○--) at 1, 3, and 6 months after complete healing

The median vascularity score between the two groups was not significantly different at all time points. The vascularity score in the SDC and Bactigras® groups significantly decreased at 6 months compared to 1 and 3 months. The vascularity score at 3 months in the Bactigras® group was significantly lower than at 1 month, Figure 9.

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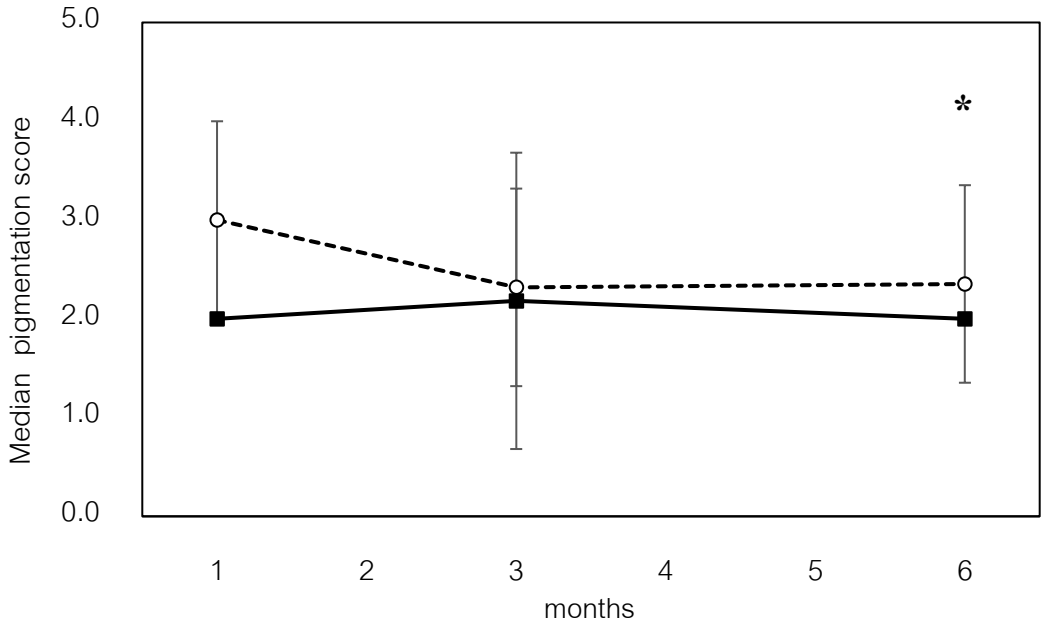

Figure 10 Pigmentation score (median±IQR) of STSG donor sites wound treated with sericin dressing with collagen (SDC) (solid line; —■—) Bactigras® (control; dashed line; --○--) at 1, 3, and 6 months after complete healing

SDC group had a significantly lower pigmentation score at 6 months when compared to the Bactigras® group (p = 0.004). When compared between time points, the scar pigmentation in both groups had no significant change during the 6-month follow-up (Figure 10).

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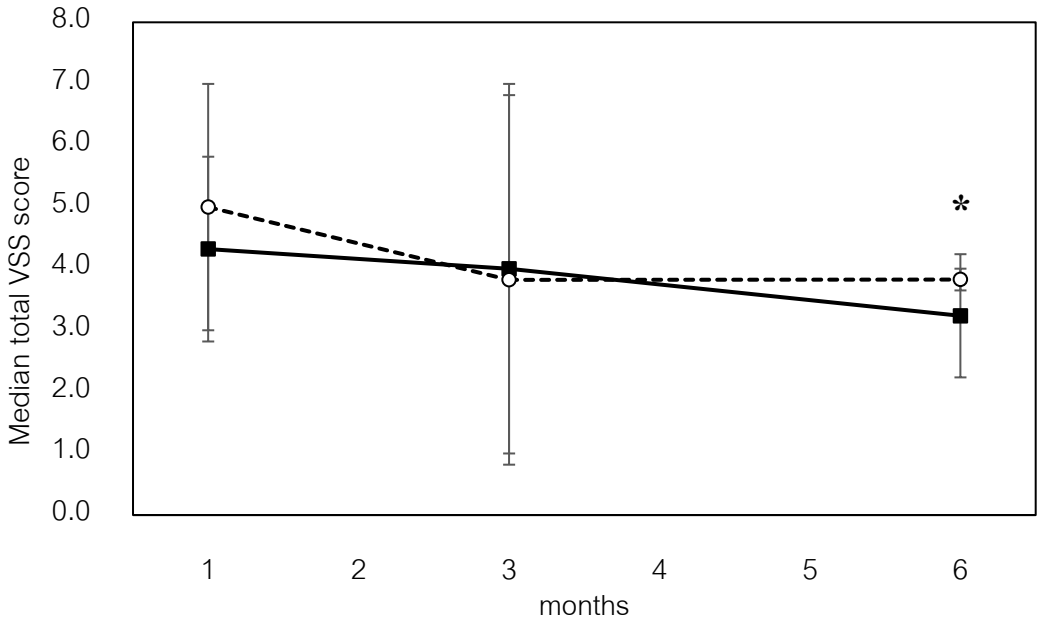

Figure 11 Total VSS score (median±IQR) of STSG donor sites wound treated with sericin dressing with collagen (SDC) (solid line; —■—) Bactigras® (control; dashed line; --○--) at 1, 3, and 6 months after complete healing

The total score of VSS is stated in Figure 11. It was found that the total VSS score in the SDC group was significantly lower than the Bactigras® group at 6 months (p = 0.011). The total VSS score in the Bactigras® group at 3 months was significantly lower than at 1 month. The total VSS scores in both groups at 6 months were significantly reduced compared with the total score of each group at 1 month.

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4.2.2.2 Patient and Observer Scar Assessment Scales (POSAS)

Patients were asked to evaluate their donor site scar at 6 months after complete healing using the patient part of POSAS.

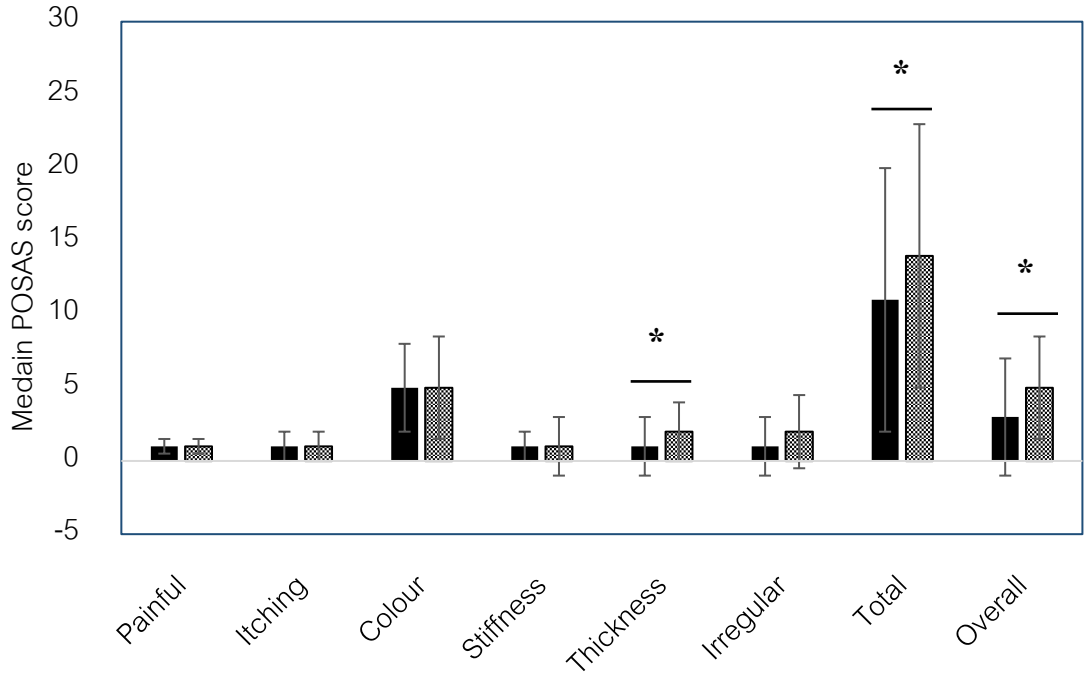

Figure 12 POSAS scores evaluated by the patient at 6 months of STSG donor sites wound treated with sericin dressing with collagen (SDC) (black bars) and Bactigras® (dotted bar) (n=21)

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Figure 12 presents the POSAS score evaluated by patients at 6 months after complete healing. The results showed that the median scores of the thickness (p=0.016), total scores (p=0.025), and overall opinion (p=0.020) in the donor sites treated with SDC were significantly lower than those treated with Bactigras®, calculated by a Wilcoxon signed-rank test.

Objective method

Mexameter® was used to determine the scar color at donor sites reporting as the melanin and erythema level. The skin barrier function, determined from transepidermal water loss (TEWL), was measured by Tewameter®, and the skin hydration was examined by Corneometer®. These parameters were measured at 0, 1, 3, and 6 months after complete healing. The results were calculated as the ratios compared with the adjacent normal skin. For skin elasticity, Cutometer® was used for the evaluation at only 6 months.

4.2.2.3 Scar color (Melanin and erythema level)

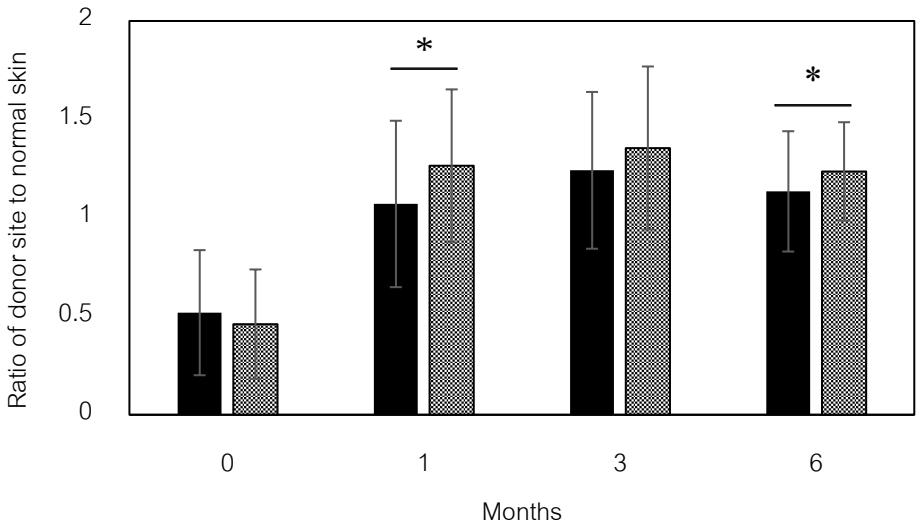

Figure 13 Ratio of melanin level (mean±SD) at STSG donor sites treated with sericin dressing with collagen (SDC) (black bars) and Bactigras® (dotted bars) to at normal skin at 0 (healing time), 1, 3, and 6 months

Figure 13 shows the ratio of melanin levels at 0, 1, 3, and 6 months. When considered from overall time points, it was found that the ratio of melanin level in the SDC group was significantly lower than the Bactigras® group, calculated by repeated measures ANOVA (p=0.011). Moreover, the ratio of melanin level at 1 and 6 months of SDC group was significantly lower than Bactigras®; p=0.000372 and p=0.031, respectively. The ratio of melanin level at complete healing time was significantly lower than other time points, and the ratio of melanin level from both dressings did not significantly change between 1, 3, and 6 months.

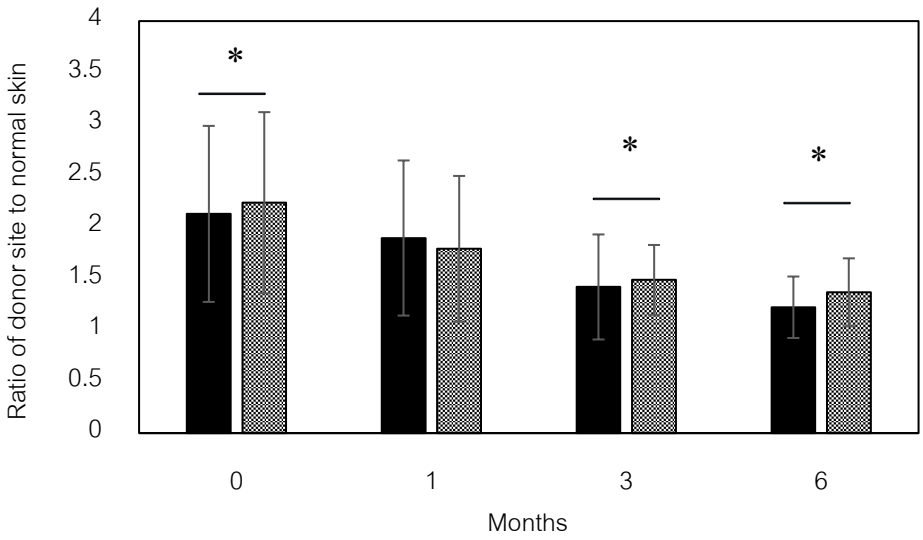

Figure 14 Ratio of erythema level (median±IQR) at STSG donor sites treated with sericin dressing with collagen (SDC) (black bars) and Bactigras® (dotted bars) to at normal skin at 0 (healing time), 1, 3, and 6 months

The ratios of erythema level in STSG donor sites treated with SDC were significantly lower than Bactigras® at healing time, 3 months, and 6 months (p=0.002, p=0.012, and p=0.012, calculated by Wilcoxon Signed Ranks Test) Figure 14. For both groups, the highest ratio of erythema level was found at complete healing time. Compared to at complete healing time, there was no significant decrease in the erythema ratio at 1 month. In contrast, the ratios significantly decreased at 3 months and 6 months for the SDC group. The ratio of erythema level in the Bactigras® group significantly decreased at 1, 3, and 6 months compared with the ratio at complete healing time.

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4.2.2.4 Transepidermal water loss (TEWL)

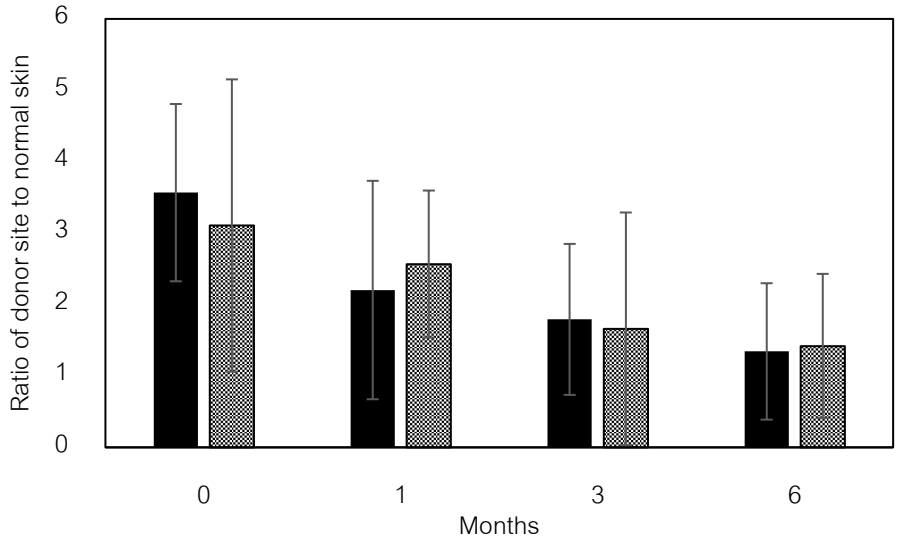

Figure 15 Ratio of transepidermal water loss; TEWL (median±IQR) at STSG donor sites treated with sericin dressing with collagen (SDC) (black bars) and Bactigras® (dotted bars) to at normal skin at 0 (healing time), 1, 3, and 6 months

Figure 15 displays transepidermal water loss determined by Tewameter® at complete healing time, 1, 3, and 6 months after complete healing. There was no significant difference in the ratio of TEWL between the SDC and Bactigras® groups at all time points. The ratio of TEWL in the SDC group at 1 month and 6 months significantly decreased compared with the ratio at complete healing time. In contrast, the ratio of TEWL in the Bactigras® group significantly reduced at 6 months compared to the ratio at complete healing time.

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4.2.2.5 Skin hydration

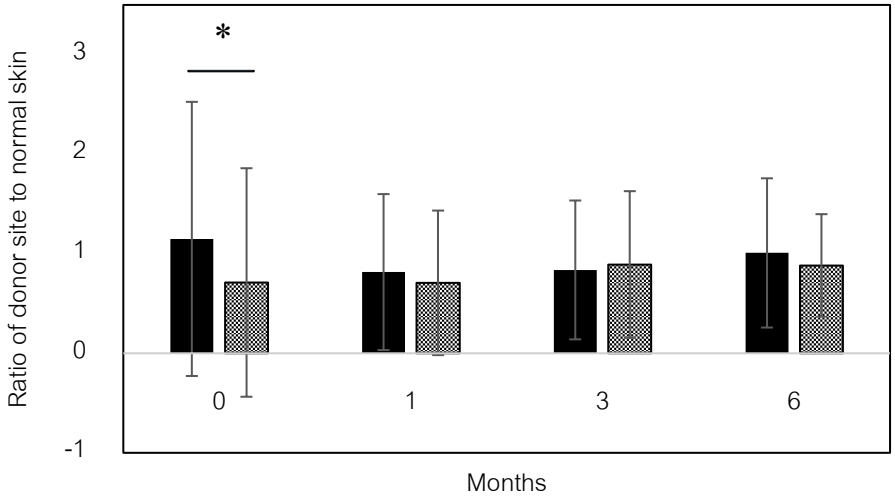

Figure 16 Ratio of hydration level (median±IQR) at STSG donor sites treated with sericin dressing with collagen (SDC) (black bars) and Bactigras® (dotted bars) to at normal skin at 0 (healing time), 1, 3, and 6 months

Skin hydration was measured by Corneometer® after complete wound healing for 1, 3, and 6 months, Figure 16. SDC group had a significantly higher ratio of hydration level than the Bactigras® group at complete healing time (p = 0.001). In contrast, there was no significant difference between groups in other time points. Moreover, the ratio of hydration level in the SDC group was significantly reduced at 1 month compared with the ratio at complete healing time (p = 0.032). There was no significant difference in hydration ratio between other time points in both groups.

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4.2.2.6 Skin elasticity

The Cutometer parameters obtained from intact adjacent skin, SDC group, and Bactigras® group were compared (Table 16). When compared between the SDC group, Bactigras® group, and normal skin, statistical differences were found in parameters R0, R2, R3, and R8. However, the post hoc analysis found a significant difference in only R2 and R8 and found no difference in R0 and R3. The parameters R2 and R8 in both the SDC and Bactigras® groups significantly differed compared with normal skin. However, there was no significant difference between the SDC group and the Bactigras® group.

Table 16 The comparison of Cutometer parameters at 6 months

Parameters	p-values (compare 3 groups)	Pairwise comparison		
		SDC VS Bactigras®	SDC VS Normal skin	Bactigras® VS Normal skin
R0	**0.035**	0.318±0.127 VS 0.315±0.127 (p = 1.000)	0.318±0.127 VS 0.376±0.081 (p = 0.143)	0.315±0.127 VS 0.376±0.081 (p = 0.097)
R1	0.267	-	-	-
R2	**0.010**	0.748±0.127 VS 0.783±0.112 (p = 0.596)	**0.748±0.127 VS 0.852±0.078* (p = 0.004)**	**0.783±0.112 VS 0.852±0.078* (p = 0.047)**
R3	**0.036**	0.349±0.130 VS 0.346±0.130 (p = 1.000)	0.349±0.130 VS 0.408±0.082 (p = 0.138)	0.346±0.130 VS 0.408±0.082 (p = 0.106)
R4	0.120	-	-	-
R5	0.819	-	-	-
R6	0.705	-	-	-
R7	0.560	-	-	-
R8	**0.010**	0.230±0.170 VS 0.184±0.188 (p = 0.812)	**0.230±0.170 VS 0.310±0.133* (p = 0.002)**	**0.184±0.188 VS 0.310±0.133* (p = 0.004)**
R9	0.626	-	-	-

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4.2.3 Pain control

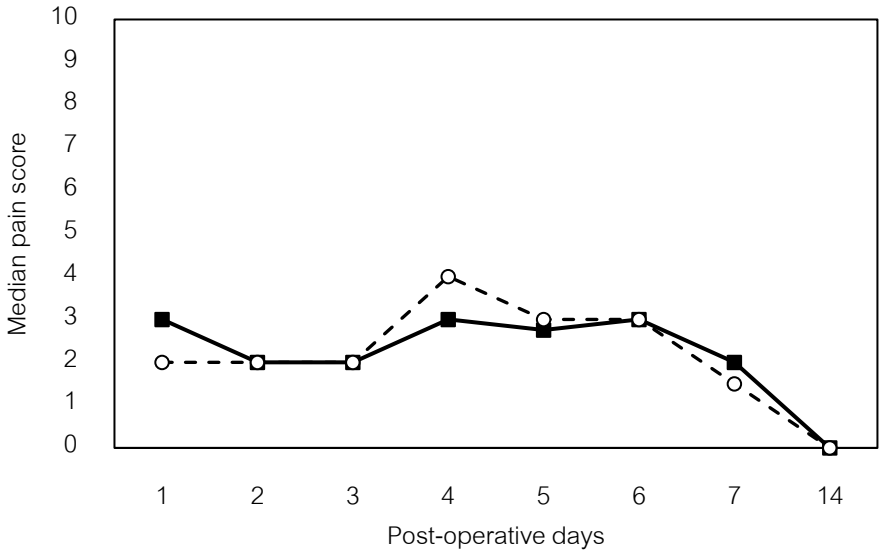

Figure 17 Pain score (median±IQR) of STSG donor sites wound treated with sericin dressing with collagen (SDC) (solid line; —■—) Bactigras® (control; dashed line; --o--) at post-operative days

The pain score evaluated by VAS was presented in Figure 17. There was no significant difference in the pain score between the SDC and Bactigras® groups at all time points. At 14 days after the operation, the median pain scores were zero in both groups.

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4.2.4 Infection control

The donor sites of all patients in both groups had no signs of infection, so the microbiological test was not performed. The average body temperature of all patients on pre-operative day, operative day, and post-operative day (7 days) was around 37°C. The highest average body temperature was found after operation for 1 and 2 days (Figure 18).

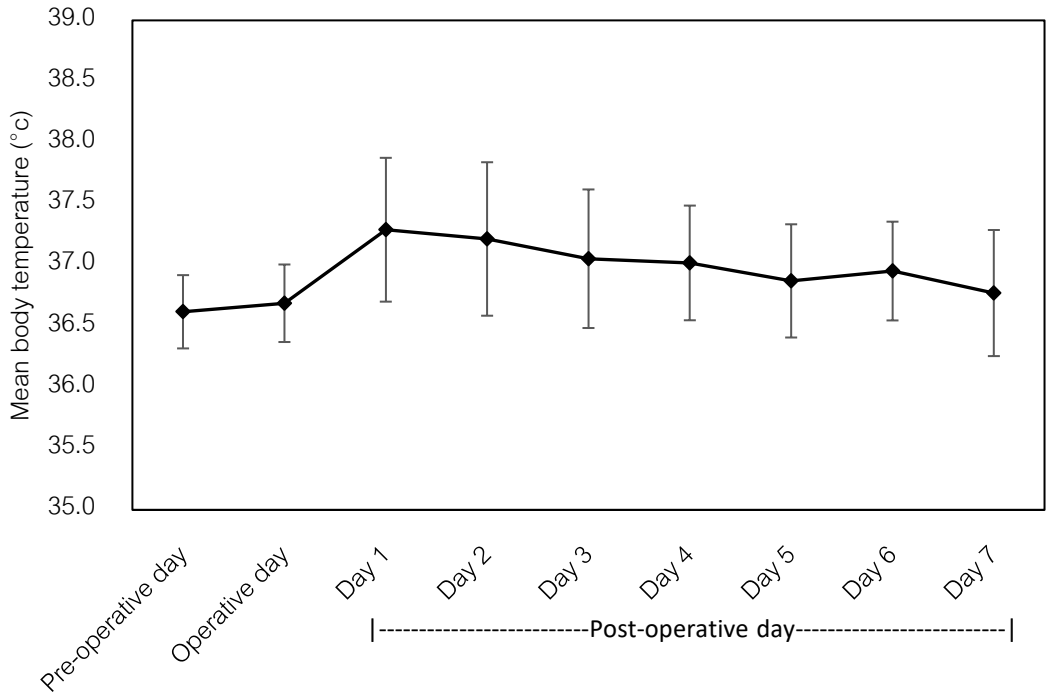

Figure 18 Mean body temperature of patients before and after operative day

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4.2.5 Adverse effects

There were no local adverse effects found in any patients. The systemic adverse effects on hepatic and renal functions were determined from the blood samples of patients before the operative day and 7 days after the operative day. It was found that the average values of all parameters were in the normal range before and after dressings application (Table 17).

Table 17 Blood chemistry data

Biochemistry	Normal range	Day 0	Post-op 1 week
BUN (mg/dL)	7 - 20	12.94±4.83	13.69±6.90
Creatinine (mg/dL)	0.50 - 1.00 (F), 0.70 - 1.20 (M)	0.80±0.29	0.83±0.32
Bilirubin (Total) (mg/dL)	0.20 - 1.20	0.90±0.53	0.59±0.26
AST (U/L)	5 - 35	23.70±8.19	30.00±19.92
ALT (U/L)	0 - 40	17.20±9.85	19.00±8.34
ALP (U/L)	40 - 120	65.11±14.53	70.40±27.65

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CHAPTER V
DISCUSSION AND CONCLUSIONS

5.1 Discussion

Split-thickness skin graft (STSG) is the necessary operation for treating extensive skin defects. The wound at the donor site after graft harvesting is an open wound with moderate exudate. Morbidities associated with STSG donor sites, including pain, pruritus, discomfort, unpleasant scar, and impaired quality of life, have been reported in many patients [8, 9]. Wound dressing that can relieve these problems has become an essential medical device. The sericin dressing with collagen (SDC) is the hydrogel with a transparent appearance, providing a moist environment and releasing bioactive substances to the wound bed. Therefore, this dressing can give advantages to wound healing, and it might become the alternative dressing for STSG donor site treatment. The clinical safety of dressing was determined in the healthy volunteers in phase I clinical study. Then, the clinical efficacy and safety of SDC in STSG donor sites treatment were determined in phase II of this study.

5.1.1 Phase I clinical study

The safety of sericin dressing with collagen was evaluated and compared with Bactigras® in the normal skin of healthy volunteers (phase I). Both the per-protocol and intention-to-treat analyses were conducted, and the agreement between analyses seems to be found. Mexameter® was used for measuring the color of skin reporting as melanin and erythema level. The melanin level is the darkness of the skin, while the erythema level displays the redness of the skin. Both groups had no change in erythema levels compared between the levels measured before and after applying the dressing. In contrast, the melanin levels in both groups significantly reduced after applying the dressing. When compared between dressings, the levels of erythema and melanin showed no significant difference between groups. The results from Mexameter might suggest the comparable safety between SDC and Bactigras®. Nevertheless, the erythema score in the SDC group evaluated by three dermatologists was significantly higher than the control group, which was seemly

inconsistent with the results from Mexameter. Although most SDC applying sites (around 90%) had no edema, the edema score in the SDC group was also significantly higher than the control group. When comparing with the study of Siritientong et al. [32] that evaluated the safety of scaffold sericin dressing, there was no erythema in 44.7-55.3% of participants in this study, which was seemly higher than in the study of Siritientong, et al. (30.6-59.4%). Moreover, the percentage of edema responses in the SDC group in this study (8.7-11.6%) were less than the responses in the sericin group in the study of Siritientong (14.5-21.5%); whereas the percentage of edema responses in the Bactigras® group in the study of Siritientong (11.2-22.7%) were higher than this study (1.0-3.9%). The higher scores of erythema and edema found from the evaluation of dermatologists may contribute to the difference in dressing thickness. Because the SDC is thicker than Bactigras®, the application of SDC might result in higher pressure on volunteers' skin, and the erythema and edema may be observed more obviously. For other elevated responses, there was no significant difference in the existence of papules responses between the SDC (4.9-5.8%) and Bactigras® (5.8-8.7%) group. Moreover, there was no incidence of vesicles and bullae in both groups. The results of these elevated responses in the current study seem to be similar to the study of Siritientong et al.

According to ICDRG criteria [149, 150], most of the results (around 90%) in the SDC group can be interpreted as negative and doubtful reactions. The remaining results (around 10%) can be interpreted as weak reactions. In addition, all responses were not persistent. The responses resolved spontaneously within a few hours after removing the dressings without using any medicine. The findings in healthy volunteers can infer that the sericin dressing with collagen is seemly safe compared to Bactigras®, a commercial dressing. Therefore, the dressing could be applied to the wound of the patient in order to determine its clinical efficacy.

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5.1.2 Phase II clinical study

Complete healing time

The clinical efficacy and safety of sericin dressing with collagen (SDC) were determined in STSG donor site treatment, compared with the commercial dressing (Bactigras®). The primary outcome is time to complete healing in STSG donor site treatment. The STSG donor site is a sterile wound with controlled depth and size. Therefore, it could be equally divided into two comparable

sides. Applying two dressings on each side of the same STSG donor site could diminish the confounding effects from the intrinsic factors in individual patients, including age, gender, and comorbidities. Therefore, it allows a credible comparison of the effects between treatments [70, 71].

In the current study, the SDC group had a significantly lower time to complete healing when compared to the Bactigras® group. The healing acceleration could contribute to the moist environment obtained from hydrogel [12, 14, 15] and the beneficial effects on wound healing from sericin and collagen hydrolysate. It has been widely known that a moist environment brings advantages to wound healing [85, 86], including in STSG donor site healing [64]. Hydrogel has high water content. It could be a moisture donor to the wound bed and concurrently absorber for the exudate [12, 13], providing the moisture balance at the wound site. Moreover, hydrogel could promote angiogenesis, reduce pain [16] and inflammation, stimulate the growth and proliferation of fibroblasts and endothelial cells, and prevent scab formation and the subcutaneous migration of epidermal cells [85]. For sericin, it can decrease the inflammation in wound bed because of its anti-oxidant [199] and anti-inflammatory effects [25], and sericin could also activate the proliferation, migration, and attachment of several mammalian cell lines [200], such as fibroblast [96, 201] and keratinocyte [97] leading to the increase of collagen synthesis [96] and re-epithelialization [97]. In addition, the beneficial effects of collagen hydrolysate on wound healing process [36, 38, 43, 119] consist of stop bleeding [37], decrease inflammation [36], activate fibroblast [202, 203], give nutritional support [43], promote collagen synthesis, inhibit matrix metalloproteinases (MMPs) release, decrease collagen and elastin degradation [204], and provide moisture retention [118]. Overall positive effects might harmoniously enhance the healing of STSG donor sites.

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Several dressings containing collagen hydrolysate have been recently developing [40, 119, 123]; however, the effects on wound healing were seemly reported in only cell lines and animal models [123, 134]. In contrast, the beneficial effects of undenatured collagen dressing in the clinical trials were exhibited on several types of wound, including chronic leg and foot ulcer [124], partial thickness skin graft donor site [42, 43], burns wounds [125], diabetic wounds [126], and pressure ulcers [41]. While the acceleration of human wound healing was displayed in patients who orally received CH as a dietary supplement [44, 135], the benefit from topical administration of dressing containing collagen hydrolysate in the human wound is seemly still the gap nowadays [49-51].

Therefore, the comparison with other studies for the clinical efficacy of topical collagen hydrolysate seems to be limited. Besides, the findings in this study might fill the gap of knowledge.

Compared to the previous studies of sericin dressing, the reduction of healing time found in this study seems to concur with the results from the study of Siritientong et al., 2014 [32] and Hasatsri et al., 2015 [108]. The median of complete healing time in sericin dressing group VS Bactigras® group in the study of Siritientong et al. and Hasatsri et al. were 12.0±5.0 VS 14.0±5.2 days and 11.0±6.0 VS 14.0±6.0 days, respectively. The findings might confirm that sericin dressing can enhance wound healing, especially for STSG donor sites. Nevertheless, the sericin dressing could not show the advantage on complete healing time in the study of Napavichayanun et al., 2018, which reported the comparable healing time between the sericin and control groups at 19± 5 days [109].

Factors affecting wound healing such as age, comorbidities, and gender may contribute to the difference of findings. It has been known that the younger people would have faster healing, and the delayed healing is usually found in the elderly [205, 206]. The average age of patients in the study of Siritientong et al. and Hasatsri et al. was around 37-39 years, and it was 50 years in this study. Whereas, the average age of patients in the Napavichayanun et al. study was 60 years, which was seemly higher than other studies. Comparing studies, the longest time to complete healing was found in the study of Napavichayanun et al., and sericin dressing could not show the benefit on wound healing over Bactigras®. It was found that the complete healing time in the current study was seemly higher than the studies of Siritientong et al. and Hasatsri et al. for a few days; however, the SDC in this study still possibly enhances wound healing when compared to Bactigras®. Even in healthy patients, an increase in age could influence mostly entire healing processes [207]. In the hemostasis phase, the elderly seem to have an increased platelet aggregation and interaction [208]. The early increased neutrophils, delayed monocytes infiltration, increase in mature macrophages, and impaired macrophage functions found in the inflammation phase lead to increased inflammation in elderly patients. Moreover, the delay of angiogenesis, collagen deposition, and re-epithelization are also found in the proliferation phase. In the re-modeling phase, the collagen deposition is reduced and less organized [206]; however, there was a report for the accelerated maturation in healthy old volunteers leading to scar improvement compared with the young individuals [207].

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Therefore, the age of patients might be one factor influencing the difference in healing outcomes between studies.

The comorbidities such as diabetes mellitus, cancer, cardiovascular disease, renal failures, and dyslipidemia could also impair wound healing [205, 209]. The comorbidities could interfere the normal wound healing at various steps of healing. Diabetes could negatively affect wound healing in every phase, including delaying the hemostasis, affecting the migration of cells, altering the proliferation phase, and influencing the maturation phase. Renal failures could also influence numerous points of healing, which are interfering hemostasis, continuing inflammation, generating free radicals, impairing collagen function, and disturbing keratinization, depending on the stage of disease [209]. Moreover, dyslipidemia also negatively affects wound healing with or without diabetes [210], impairing angiogenesis [211] and re-epithelialization. The supply of oxygen and nutrition might also reduce contributing to the delay in wound healing in patients who have cardiovascular diseases [212]. Moreover, the medications such as chemotherapy and corticosteroids used for treating cancer could impede the proliferation phase and fibroplasia. While low-dosage aspirin used for prevention in cardiovascular disease is suspected to negatively impact wound healing [213]. The majority of patients in the study of Siritientong et al. (64.3%) and Hasatsri et al. (73.9%) had no comorbidity. In contrast, two-thirds of patients in the study of Napavichayanun et al. had at least one comorbidity. For the current study, around 76% of patients had at least one comorbidity that could affect wound healing. Although the details of comorbidities from each study could not be directly compared, the patients in this study seem to have the highest overall comorbidities. Besides, the comorbidities of patients in this study and the study of Napavichayanun et al. were seemly higher than the other two studies. It might suggest that the comorbidities may influence the effects of sericin dressing on wound healing.

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In addition, gender could also be one of the factors affecting wound healing, especially in the elderly. Around 57% of patients are males in the studies of Siritientong et al. and Hasatsri et al.. In contrast, two-thirds and seventy-six percentage of patients in this study and the Napavichayanun et al. study are males, respectively. It has been widely known that aged males have a higher risk of abnormal wound healing than aged females, depending on their sex hormone levels. Estrogen provides positive effects on wound healing via downregulating macrophage migration inhibitory

factor [214], inducing fibroblast migration [215], enhancing keratinocyte proliferation, inhibiting apoptosis, and decreasing the protease levels [216]. On the contrary, androgens seemly correlate with delayed healing. However, the considerable decrease of estrogen in postmenopausal women could also lead to impaired wound healing [216]. Therefore, the higher ratio of males and elderly patients might concurrently bring about the longer complete healing time found in this study and the study of Napavichayanun et al.

For the other factors, including BMI of patients and depth of donor sites, it was found that these factors seem to be comparable between the studies, whereas the alcohol consumption and smoking that could also impair wound healing moderately varied among the studies. However, their negative effects might be minimized as the patients could not consume alcohol and cigarette during hospitalization. Moreover, the negative effects of alcohol intake and smoking depend on the amount and duration of consumption, which is difficult to compare between the studies.

Besides the intrinsic factors of patients, the addition of substances into the sericin dressings might also influence the effects on wound healing. Polyhexamethylene biguanide (PHMB) was added into the sericin dressing of Napavichayanun et al. to provide infection control. Although the safety of dressing was shown in both in vitro and in vivo studies [217], PHMB might still interfere with wound healing [218]. For the current study, collagen hydrolysate was added to sericin dressing. Based on the literature [52, 53], sericin and collagen seemly provide synergistic effects on wound healing. Therefore, the benefit in wound healing found in this current study might be enhanced because of the addition of collagen hydrolysate, even though most patients are males with relatively older ages and various comorbidities.

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Accordingly, the sericin dressing with collagen in the current study possibly accelerates STSG donor site healing, supporting the previous studies. The moist environment provided by the dressing could be one factor positively affecting wound healing. The effects of sericin may be influenced by the intrinsic factors of patients and the composition of the wound dressing. The addition of collagen into sericin dressing might enhance the beneficial effects of sericin. The faster healing decreases patient suffering and seems to give advantages to patients' quality of life [9]. The phases of wound healing overlap, and the collagen deposition occurs concurrently with the

proliferation phase. Therefore, the SDC application could influence scar formation. The examination for the scar quality after complete healing might give more information about the effects of SDC on STSG donor site healing and the long-term outcomes from SDC application.

Scar outcomes

The scar characteristics were evaluated at 0, 1, 3, and 6 months after healing to estimate the effects of dressing on scar quality that may vary depending on time [177]. For the first month, the color of the scar can represent the inflammation during the healing process and the vascular formation in the scar. At 3 months, the progressions of scar were evaluated for a second time because most of the signs of pathological scar are usually apparent. The maturation scar can be observed at 6 months because the maximum strength of the scar is usually established. Moreover, the persistent scar symptoms such as pain and itching at 6 months may indicate the chronic injury of the scar. Based on the systematic review in STSG donor sites [8], the data in long-term evaluation at 3 or 6 months was seemly limited. Therefore, the evaluation of scar quality at different times up to 6 months in this study might provide more information for the effects of dressing in STSG donor sites. The subjective and objective methods could provide different advantages and disadvantages in the assessment of scar quality. Therefore, both of subjective and objective method are recommended to be concurrently evaluated in order to examine the results from several aspects [196]. Moreover, determining scar quality by multiple methods can increase the possibilities to compare the results with the other studies that use various methods.

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The Vancouver Scar Scale (VSS) considers the scar quality from 4 parameters: vascularity, thickness, pliability, and pigmentation. The normal skin would be scored as “0”, so the lower score means the better scar quality. Although there was no significant difference in vascularity and thickness item between the two groups, the pliability and pigmentation of donor site treated with SDC were significantly lower than control at 6 months. Moreover, the total VSS score in the SDC group was also significantly lower than the control group at 6 months. It can suggest that SDC probably improves the pliability and darkness of scars, leading to better scar quality. The findings support that moist dressing could improve the scar quality evaluated by VSS, as previously reported [81]. A lower VSS score was also found in the sericin group compared to the Bactigras® group in the previous

study [109], whereas the benefit on scar quality of undenatured collagen dressing seems to be less reported [43, 136].

Due to VSS could be used for longitudinal monitoring, the comparison between time points could indicate the changes of scar characteristics in both groups. The resolution of vascularity can be used as the indicator for scar maturation [157]. The results showed that vascularity in both groups significantly reduced during the follow-up period, and the scores were seemly equal to normal skin at 6 months. Therefore, it can suggest the progression of scar maturation in both groups at the sixth month. The height of the scar seems similar to normal skin along 6 months. The height abnormality might indicate pathological scars [165], which could be investigated during 6 months. The findings, therefore, suggest that the hypertrophic scar or keloid was rarely found in both groups. Compared to the third month, both groups had significantly higher pliability at 6 months, which was higher than normal skin. The higher pliability score than normal skin seems to concur with the literature. The maximum strength of scar was generally found at 6 months [157], and scar could be generally harder than normal skin up to several years [174]. Moreover, the pliability represents the stiffness of skin that could affect the function of skin, such as movement and the protection ability [173]. The pliability is influenced by the collagen organization, viscous ground substance, and elastic fibers [59]. The lower pliability found in the SDC group compared with the control group possibly indicate the better structure and organization of those substances and better mechanical properties of scar in the SDC group. The pigmentation scores of both groups were not significantly changed during 6 months, and the SDC group had the better pigmentation in the sixth month. The primary cause of dyspigmentation seems to be inflammation. The findings might suggest the lower inflammation in the SDC group. The pigmentation could negatively impact the satisfaction of patients [8]. Therefore, the improved pigmentation might enhance the overall opinion of patients on their scar quality. Furthermore, the significant decrease of total VSS score in both groups between time points can indicate the improved scar quality during the 6 months. Each sub-score of VSS would be compared and discussed with the patient's evaluation and device measurement results later.

The impact of STSG donor site scarring on patients seems to be underestimated by healthcare providers [65]. Therefore, the patient scale of POSAS was included in this study to examine patients' perspective and satisfaction on their scars, that is, hardly measured by observer

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assessment and objective method. The lower POSAS (patient part) score represents the better scar quality from the patient's perspective. The results showed that the SDC group had a better score in thickness, total score, and overall opinion than the control group. In contrast, the pain, itching, color, stiffness, and irregular scores were comparable between groups. The findings in this study seem to concur with the study of Draaijers et al. (2014), indicating that patients' opinions on their scar could be mainly influenced by the itching and thickness [63]. Although the itching between the two groups was similar in the current study, the patients appraised the better thickness in scars previously treated with SDC. The lower thickness might influence the better overall satisfaction of patients in the SDC group. When considering the other items, which were insignificant different, the pain, itching, stiffness, and irregular items in both groups were rated at a pretty low score. In contrast, the color score seems to be moderate. Itching could be induced by the dryness of the scar [219], and chronic pain and itching after 6 months could also reflect the persistent inflammation in the wound bed or the psychological problems in patients [153]. At the same time, the abnormal stiffness and irregular surface might represent the incomplete collagen organization [178]. Therefore, there was only a few persistence of these problems for the patients at 6 months, except for the color. The findings seem to concur with the previous study reporting that color was the least appreciated outcome in patients with STSG donor sites [65]. The problem in color may be persistent for up to several years in the opinion of the patient [197]. Moreover, most patients in this study were elderly males, and they may have less concern about their scars, according to the literature [65]. It may decrease the difference in POSAS score between groups as most POSAS items were rated as the minimal difference from normal skin by patients in the current study. However, the better total POSAS score and overall opinion score were found in the SDC group. It possibly reflects the higher satisfaction of patients in the donor sites treated with SDC that might finally improve their quality of life. A better total POSAS score was also found in the moist dressing group in the previous study [197]. Due to the scar outcome evaluated by the patients was not included in the clinical trial of sericin dressing conducted by Siritientong et al. [32], Hasatsri et al [108], Napavichayanun [109], and the patients' perspective could also vary depending on their age, gender, and other factors [65], the comparison with those studies seems to be limited. The average overall opinion score in the current study was 3 and 5 for the SDC and Bactigras® groups, respectively. The mean of overall opinion at 12 months was reported

at around 3.2 in the previous study [65]. Typically, the scar quality improves during the increasing time. It might suggest that the scar in the SDC group at 6 months seems better or comparable to the scar at 12 months in the previous study. In contrast, the scar in Bactigras® might still be worse, regardless of different demographic data.

The total score from both POSAS and VSS at 6 months is seemingly in accordance as the lower total scores were found in the SDC group from both assessment scales, suggesting that SDC could improve the scar quality compared to Bactigras® whether in the clinician or patient's perspective. However, it appears that there were some inconsistencies in some related sub-scale evaluated by the patient (POSAS) and clinician (VSS) in the current study. These issues would be discussed with the related outcome measured by the objective measurement, including the possible effects of the dressing application on the scar characteristics.

The clinically important difference might be stated from the statistically significant difference obtained from the scar assessment scales. However, the insignificant statistical difference did not indicate that it is not clinically significant different [196]. The objective measurements are seemly the sensitive detector for determining the progression of the scar. Therefore, the objective results should be compared with the subjective questionnaires to gather the overall effects of treatment. The scar characteristics, color (melanin and erythema level), barrier function, hydration, and elasticity of scar were objectively determined by Mexameter®, Tewameter®, Corneometer®, and Cutometer®, respectively. The related scar characteristics measured by several methods would be compared after the discussions of each objective result.

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Mexameter® was used for objectively evaluating the scar color, including melanin and erythema levels. Melanin level or the darkness of skin can represent the post-inflammatory of the wound. Although dyspigmentation might not be harmful, this parameter seems to be the commonly concerned outcome of patients that may negatively affect their quality of life [161]. At each time, melanin levels at both sides were calculated as the ratios compared with the individual melanin levels at the adjacent normal skin. The results showed that the donor sites treated with SDC had lower overall melanin levels than Bactigras®, considering from all time points. The significantly lower melanin levels in the SDC group were also found at 1 and 6 months compared with Bactigras®. The

findings probably suggest that SDC improves the darkness of scar when compared to the Bactigras®, and the improvement could be found since the first month and possibly still exist in the sixth month. The change between time points was considered. The melanin levels at complete healing time in both groups were significantly lower than other time points and were seemly lower than the normal skin. Then, the pigmentation increased and became higher than normal skin since the first month. After that, the melanin levels in both groups had no significant change when compared between 1, 3, and 6 months. According to the literature, dyspigmentation tends to be a problematic issue in Asians than Caucasians that may normalize after 12 months [158, 159]. Although the melanin level of STSG donor sites at 6 months was still higher than the normal skin, the melanin level in the SDC group at 6 months was relatively closer to the normal level.

The erythema level obtained from the Mexameter® represents the absorption intensity of hemoglobin contained in the skin. Three mechanisms could cause erythema in scars: inflammation, vascularization, and epidermis defect [154]. The results in the current study showed that the highest level of erythema in both groups was found at complete healing time. Compared to complete healing time, the ratios in the SDC group were significantly reduced at the third and sixth months. Significant reductions were also found from the first month until the sixth month for the Bactigras® group. It was also found that the SDC group had a significantly lower erythema level than the Bactigras® group since at the complete healing time until 6-month follow-up, except for at 1 month. The redness at the early stage could contribute to inflammation and increased vascular formation, which may persist in the first month [154, 155]. During the re-modeling process of normal scar, the elastin replacement and decrease of excess vascular lead to the decrease of erythema level along the time, which could be used for monitoring the maturation of scar [154, 157]. The individual scar may have different maturation. For example, the vascularization of burn shows a continuous decrease at 3 months [156]. Therefore, the lower erythema level at the complete healing time of the SDC group might result from lower inflammation during the healing process. The insignificant change of scar redness in the SDC group at 1 month may result from increased vascularization instead of inflammation [155]. Moreover, the lower erythema level in the SDC group found at 3-6 months after healing might also suggest that the STSG donor sites treated with SDC seem to move on to the maturation faster than Bactigras®.

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Skin hydration is one of the physiological parameters, which might be challenging to measure by subjective method [167]. Skin hydration is considered from the water content in both of epidermis and dermis. The ability to control hydration mainly depends on the stratum corneum (SC) arrangement and the natural hygroscopic agent within the corneocytes. The optimal hydration could influence the desquamation of skin [168], leading to the smoothness and softness of the skin. While, the lack of hydration could lead to flaky skin, relating to the itching symptom [219]. Transepidermal water loss (TEWL), stratum corneum hydration, and dermal water content are related, so using more than one method are recommended for determining skin hydration. Tewameter® and Corneometer® were used to investigate TEWL and SC hydration in this study. The results were reported as the ratio to the normal skin to reduce the influencing factors (150), including the environmental conditions. Dermal water content can be determined by using Confocal Raman Spectroscopy and Near-Infrared Spectroscopy (NIRS). The first method is quietly complicated and expensive [220], whereas the second method still needs further validation [167]. Therefore, the investigation for dermal water content was not performed in the current study.

TEWL is calculated from the water vapor pressure gradient at the skin surfaces. It can be the indicator for the recovery of the skin barrier. The finding showed no significant difference in TEWL between SDC and Bactigras® at each time point. The skin barrier function controlling moisture evaporation between two groups might be comparable. Although the TEWL in both groups at 6 months was seemly higher than the normal skin, both groups tend to have a continuingly decrease of TEWL during the 6 months. It seemly concurs with the literature previously stating the improvement of TEWL along the time [158]. Compared to the ratio at complete healing time, the significant decrease of TEWL in the SDC group seems to be earlier found at 1 month. In contrast, a significant reduction of TEWL was found at 6 months in the Bactigras® group. The faster decrease of TEWL might indicate the rapid improvement of barrier function in donor sites treated with the SDC group.

The previous studies found a significant reduction of TEWL in the sericin dressing group compared to the control dressing, which was not found in this study. The recovery of TEWL can be influenced by several factors [158]. Besides, the recovery of the skin appendages such as the sweat gland is also crucial, and the water evaporation from secreted sweat could not be excluded from the

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measurement of TEWL [220]. Therefore, the consideration together with the water content in SC might help clarify the overall skin hydration.

In the current study, the Corneometer® was used for examining the SC hydration based on the capacitance method. At complete healing time, the higher hydration level of donor sites found in the SDC group might indicate the moist environment provided by the wound dressing [168]. The moist environment seems to be an appropriate environment for treating STSG donor sites [64]. It possibly facilitates healing and improves scar quality [170, 171]. The significantly higher hydration level in the SDC group could not be found after 1 month until 6 months, which may arise from the absence of dressing. Furthermore, the patients were encouraged to apply topical preparation on their skin after complete healing. Although the patients were asked to stop applying topical preparation at least 8 hours before measuring, their continued daily application could accumulate the scar's hydration [221]. It might conceal the effects of SDC in the long-term evaluation. Although the difference could not reach statistical significance, the SDC group tends to have a higher hydration level than the Bactigras® group at 1 and 6 months. Moreover, the higher hydration in the SDC group might meaningfully improve scar quality when considered from the minimal clinically important difference (MCID), which is 4% on scars for the Corneometer® [167]. Besides, the SC hydration at 6 months in the SDC group was seemly closer to the normal skin, possibly representing the recovery of epidermal function, including the restoration of appendages [172]. It is possible that the hydrated condition occurred during wound healing process could still affect the scar quality at later stage [12, 70, 168, 222], which might be also noticed from the other characteristics.

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Hydration in the stratum corneum could be the indicator for the epidermal function. According to the findings, SDC probably ameliorates the functions and organization of SC during the early healing process, and the effects possibly existed until the remodeling phase. The increased hydration may not only result from the hydrogel characteristic of SDC. Sericin, which contains high content of serine amino acid, is also an excellent moisturizing agent [56, 223]. It was previously reported that sericin could increase skin hydration in healthy volunteers [110]. Similarly, collagen hydrolysate showed its ability to absorb and retain the moisture in the skin [39, 40, 131, 132]. It is possible that SDC dressing might provide synergistic effects on skin hydration. At 6 months, the TEWL in both sides seems to be higher than the normal skin. In contrast, a comparable hydration

level between donor sites and normal skin was found, especially for the SDC group. Besides affecting the softness and smoothness of skin, the water holding capacity of SC also influences the protective function and the flexibility of skin [167-169]. The flexibility of skin could be objectively determined from the Cutometer outcomes.

The mechanical properties of scar, including the distension and elasticity, are seemly the most clinically relevant parameters. The recovery of biophysical properties could affect the function of the skin [173] and indicate pathological scar formation [185]. Typically, the maximal strength of the scar could be established at approximately 6 months. Cutometer was used for determining the skin elasticity by providing the negative pressure to deform the scar, and the noncontact optical measuring system detects the deformation of the scar. The output from Cutometer® contains different parameters representing the different aspects of scar deformation [186, 192]. The Cutometer parameters obtained from the SDC group, Bactigras® group, and normal skin were compared. The statistical differences were found in skin distensibility (R0), overall elasticity (R2), last maximal deformation (R3), and the final retraction (R8). In contrast, the other parameters including R1 (resilient distension), R4 (last residual deformation), R5 (net elasticity without viscous deformation), R6 (ratio of viscoelastic to elastic distension), R7 (ratio of immediate retraction to total distension), and R9 (hysteresis) were seemly comparable between three groups. The pairwise comparisons for the significant parameters were done by post hoc analysis. There was no significant difference between SDC and Bactigras® in all Cutometer parameters. Compared to normal skin, STSG donor sites treated with SDC and Bactigras® had significantly lower overall elasticity (R2) and final retraction (R8) than normal skin. However, the post hoc analysis cannot state the difference between groups in skin distensibility (R0) and the last maximal deformation (R3).

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The differences in viscoelastic properties between donor sites and normal skin could be indicated from Cutometer parameters. Although significantly faster wound healing was found in the SDC group, the effects of dressing on Cutometer parameters could not be seen. The findings seem to agree with the previous study [173], which also reported that the faster healing group could not show the better viscoelasticity measured by Cutometer®. However, the difference between donor sites and normal skin could be detected. Although the statistical significance could not be reached, the tendencies to improve scar viscoelasticity of moist dressing and undenatured collagen were also

mentioned in the clinical studies [136, 173]. In comparison, the effect of sericin on Cutometer parameters has not been determined in donor sites scar before [23, 32, 108, 109].

Overall elasticity (R2) is the ratio of total retraction to total deformation, calculated from Ua/Uf. It can represent the overall elasticity of the scar, including the viscous deformation. The value which is closer to 1 (100%) means the more elastic skin. The lower overall elasticity of both groups might attribute to the decrease of the final retraction (R8 or Ua) rather than the increase of Uf. The pairwise comparison cannot state the differences in skin distensibility (R0 or Uf) and the last maximal deformation (R3) between normal skin and donor sites scars. However, both of the skin distensibility (R0) and the last maximal deformation (R3) of donor site scars in both groups tends to be lower than the normal skin, meaning that the donor site scars at 6 months are seemly stiffer than the normal skin, following the literature [174].

The overall elasticity (R2), the final skin distension (R0 or Uf), the final retraction (R8 or Ua), and the last maximal deformation (R3) comprise both immediate deformation (elastic part) and delayed deformation (viscoelastic part) of skin. The immediate change could reflect the function of the solid structures (elastin and collagen), and the delayed change represents the viscous responses of the solid elastic structures combined with the fluid [224]. The elastic and viscoelastic parts of donor sites scar treated by both dressings may still differ from the normal skin because the donor site scars were still during the remodeling phase. Therefore, some mechanical properties were comparable to the normal skin, whereas the differences in some parameters still existed. It was previously reported that the elasticity of the scar could differ from normal skin up to several years [59].

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In the remodeling phase, the ordinary appearances of the scar in the early stage, which is the immature scar, are red and slightly raised. Immature scar contains inflammatory cells, fibroblasts, high numbers of blood vessels, increased fluid, and increased collagen. Then, the matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) play an essential role in remodeling granulation tissue. Collagen type I, which commonly presents in normal skin, replaces collagen type III, predominant in granulation tissue. After that, the elastin formerly absent in granulation tissue finally occurs in the scar, mainly influencing skin elasticity. The vascular cells and

myofibroblasts in granulation tissue also undergo apoptosis during the remodeling phase [225]. The lower elasticity of donor sites scar at 6 months might attribute to the incomplete re-establishing of elastin [186], the increased collagen synthesis [186], and the multiple direction arrangement of collagen fibers [157]. Furthermore, the R6 parameter (ratio of viscoelastic to elastic distension; Uv/Ue) was indicated as the most sensitive parameter to the epidermal hydration level [226]. The results showed that R6 in both groups was comparable with the normal skin at 6 months. It might suggest that the hydration levels at donor sites are close to the normal skin, complying with the results of Corneometer®.

In the current study, some scar characteristics, seemingly similar or related, were evaluated by several methods, whether objective or subjective. The results of scar characteristics, including color (darkness and redness), thickness, and the biomechanical properties of scar, from all measurements, were compared and discussed in this section to determine the overall outcomes. On the other hand, other scar characteristics that were evaluated by only a single measurement, i.e., pain, itching, TEWL, and hydration level, could not be directly compared.

The scar color, including the darkness and redness, were measured by VSS, POSAS, and Mexameter®. Some different outcomes from each assessment seem to be found. The results from VSS and Mexameter® seemly state the benefit of SDC in scar darkness, whereas the results from POSAS could not. The pigmentation evaluated by VSS representing the darkness of scar could be scored as 0 = normal, 1 = hypopigmentation, 2 = mixed pigmentation, and 3 = hyperpigmentation. Although the different degrees of hyperpigmentation could be observed between two sides, both sides would be similarly scored as hyperpigmentation when using VSS. The similar rated scores might result in no significant difference in some time points. While the scar color was scored as the rating scale indicating the intensity of color using POSAS, the results could not show a significant difference between groups. The patients were asked to generally assess the scar color in the term of “color” because the differentiation between darkness and redness might be too difficult for the patients [63]. Therefore, the patients’ evaluation results seemly contribute to the combining between darkness and redness, which seemly differ from the pigmentation item from VSS and the melanin level from Mexameter®. The melanin levels were objectively measured by Mexameter®, and it appears that the SDC group has an overall better shade of scar than the control group. Moreover,

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the melanin levels of the SDC group at 6 months are quite close to normal skin levels. The lighter skin formerly treated with sericin dressing at 0 and 1 month after complete healing was also mentioned in the previous studies [32, 109]. From all findings, it may suggest that SDC could normalize the pigmentation in the scar.

The common cause of hypo- and hyperpigmentation in the scar is the post-inflammatory effect [163], resulting from the release of free radicals during the inflammatory phase of wound healing [227]. Therefore, the anti-oxidant effects from sericin [25, 92] and collagen hydrolysate [118, 228] and the anti-inflammatory effects from sericin [25] might reduce the inflammation in the wound bed. Then, the reduction of inflammation leads to the improvement of scar quality [229]. Moreover, the anti-melanogenic activity of sericin [25] probably also decreases the melanin level in the scar. Besides, the moist environment obtained from hydrogel [12, 222]and moisturizing properties from both sericin [56, 223] and collagen hydrolysate [39, 40, 131, 132] could also reduce the inflammation during healing leading to better scar formation [222]. The moisture retention property of SDC was seemly supported by the higher hydration level in the SDC group found at complete healing time. Therefore, the combined effects from hydrogel characteristic of SDC, sericin, and collagen hydrolysate could become the possible causes for improving the scar darkness. Although hypo- and hyperpigmentation are seemly the aesthetic problems that are harmless to patients, they could lead to patient dissatisfaction that could negatively affect their quality of life [8, 161]. Therefore, the improvement of darkness found in the SDC group is seemly the favorable effect for the patient.

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For the scar redness, both VSS and POSAS showed no difference between dressings. On the contrary, the differences could be found from the erythema levels obtained from Mexameter®. Using VSS, the scar redness was evaluated in term of vascularity. The vascularity was rated as normal, pink, red, and purple. In contrast, when using POSAS, the redness is rated on a 10-point score in the color item (combining darkness and redness). Similar issues as mentioned in the darkness evaluation could also be found in the redness evaluation. The scars with a slight difference of redness might be rated as the same score using VSS, and the patient indirectly evaluated redness in the combining term of color when using POSAS. Besides the differences between assessments, the poor correlation between the redness measured by the device and scar assessment scales [186] might attribute to the different findings. The output obtained from Mexameter® are continuous data,

which generally have greater statistical power. It might imply that SDC possibly decreases the redness of scar compared with Bactigras®, but the differences between the two groups might be too small to be differentiated by using VSS and patient part of POSAS.

The results from both VSS and Mexameter® showed the agreement on reducing redness in scars during 6 months. The vascularity at 6 months in both groups was nearly similar to the normal skin, indicating scar maturation [157]. For POSAS, the redness was evaluated as the combining term of color at only 6 months, so the comparison between times could not be conducted. The median score of the color item was around 5 in both groups. The results could indicate the different color of scar when compared to normal skin that might be influenced by the darkness rather than only the redness. The erythematous scars usually cause pain and pruritus to the patients because of inflammatory stimulation. However, the findings showed the minimal pain and pruritus reported by patients. The results might suggest that the treated scar had only fewer problems in redness at 6 months. Moreover, the results from Mexameter® possibly further indicate that SDC seemly bring about the faster maturation because the SDC group had lower erythema levels at 3 and 6 months compared with the Bactigras® group. The redness of the skin could result from inflammation and the increased blood vessels in the scar [154]. As discussed before, SDC might reduce inflammation in the wound bed during the wound healing process. It might lead to lower redness at complete healing time in the SDC group. Compared with complete healing time, the minor redness change at 1 month in the SDC group might contribute to the blood vessels containing in scar [155] that usually decrease from 3 months [156]. Accordingly, SDC seemly reduce the inflammation while providing the normal angiogenesis in the wound bed, which probably leads to faster scar maturation at 3 and 6 months.

The height or thickness of the scar was assessed by using VSS and POSAS. The difference in thickness between groups was found in POSAS, whereas it could not be found in VSS. The thickness in POSAS was rated as a 10-point numeric scale that might provide finer data than the five-ordinal scale of VSS. Moreover, the increase in height/ thickness might indicate hypertrophic or keloid formation. Most of the scars in the current study seemly had normal thickness, leading to minimal differences between groups. Therefore, the difference in height sub-scale of VSS was not found. The objective device was not used for thickness measurement in this study. However, the

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results from POSAS could still show the favorable effect on scar thickness of SDC over Bactigras®, especially in patients' perspective.

The raised scar could be commonly found in the early stage of scar maturation because of the accumulation of collagen and fluids [225]. Therefore, the scar thickness might indirectly represent the collagen organization in the scar [154]. The flat scar would appear after maturation [157], whereas the hypertrophic and keloid have persistently raised thickness. The pathological scars are seemly caused by the prolonged inflammation in the scar. Typically, the hypertrophic scar would regress at 6 months, whereas the continued progression over 6 months could be found in keloids [177-179]. The lower thickness of scar in the SDC group evaluated by POSAS probably indicates the normality of scar at 6 months, especially for the remodeling of ECM [61]. The decrease of inflammation and the faster maturation possibly found in the SDC group, as mentioned above, might bring about better scar thickness. Besides, the thickness from POSAS seems to be the crucial outcome affecting the patients' opinion on their scars [63]. It is possible that the better thickness of scar in SDC group can positively affect the patient satisfaction as well as their quality of life [230].

VSS, POSAS, and Cutometer® determined the biomechanical properties of scar in term of pliability, stiffness, and Cutometer parameters, respectively. Although the SDC group had a lower complete healing time, the SDC group could not show better results in Cutometer parameters and stiffness (POSAS) over the Bactigras® group. However, the results from the pliability item of VSS possibly still stated the superiority of SDC. Therefore, it should not be concluded that SDC has no effects on scar elasticity. It was previously reported that the correlation between viscoelasticity measured by Cutometer® and healing time was not found [173]. Moreover, skin elasticity varies depending on factors, including the age of the patient and skin location [231], and the clinically important difference is seemly difficult to determine [196]. Those points limited the consideration by using the MCID of Cutometer®. The previous study reported the stiffness score rated by patients with STSG donor sites. Using POSAS, almost all patients scored the stiffness of their donor sites scar as no and minimal differences to normal skin at 12 months [65]. Moreover, the assessment at 3 to 360 months also showed no significant correlation between the stiffness and the opinion of patients [63]. It might suggest that patients pay less attention to the stiffness leading to no difference between the

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results evaluated by POSAS. Besides, other possible reasons for the different outcomes between measurements will be discussed.

For the evaluation method, the clinician assessed the VSS pliability by wrinkling a skin fold. In contrast, Cutometer® generates suction from negative pressure to vertically deform the skin. The stiffness item of POSAS seemly depends on the patients' perspective. The different measuring principles might be one of the factors that influence the outcomes. In addition, the aperture of 2 mm of Cutometer® used in this study mainly measures the mechanical properties of the epidermis and partially papillary dermis in the small area [192]. In comparison, the relatively wider and deeper area would be assessed for the pliability item of VSS that might increase the differentiation between two areas treated with different dressings. Moreover, Cutometer® seemly has the limitation on measuring the scar with high stiffness leading to lower reliability [191]. The review on objective scar measurement also states a weak to moderate correlation between VSS pliability, POSAS stiffness, and Cutometer parameters [182, 191]. Some studies also showed the inconsistent outcomes between these methods [173, 232, 233].

Although the maximum strength of the scar should usually reach at least 6 months after injury, the remodeling process possibly continues for several years, varying in individuals [157, 161]. Therefore, the time of evaluation could affect the results measured by Cutometer. The results from previous studies showed the better elasticity outcomes measured by cutometer in Matriderm® group in 3-4 months [234] and 2- year follow-up [233]; however, the effect was not found in 1-year follow up [235]. Moreover, scar maturation in older patients might be faster than in younger patients. The patients attending this study were quietly elderly, so the differences might be minimal at 6 months [236].

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According to the above mentioned, it appears that SDC might improve the elasticity of scars in clinicians' perspective. However, the elasticity evaluated by patients and devices seems to be unable to indicate the difference between groups. The skin elasticity is considerably affected by the collagen and elastin organization [186] and the water content in the skin [59, 167]. The collagen organization and the SC hydration in the SDC group were seemly improved, as discussed above.

Therefore, it could lead to better pliability evaluated by clinicians involving both epidermis and deeper dermis.

According to overall findings, sericin dressing with collagen possibly enhances the STSG donor site healing and improves scar quality, i.e., the darkness, redness, thickness, epidermal functions, pliability, and patient satisfaction. Because the healing time might correlate with the scar quality [70, 164, 237], the better scar outcomes in the SDC group could attribute to the reduction of healing time. Although SDC could reduce complete healing time for only 1 day compared with Bactigras® in the current study, scar quality improvement was seemingly found in the SDC group. The results support the previous study stating that the decrease of healing time to only 1 day could also improve scar quality [70].

However, it was reported in the literature that the better scar quality could not be found in the faster healing group [164, 173]. The previous study also reported the beneficial effects of sericin on the scar quality, even though the decrease of healing time was not found [109]. The differences in each scar characteristic between groups might suggest the effects of SDC on STSG donor sites during the healing process leading to the improvement of the scar. The scar formation could not be affected by only one mechanism, cell, or factor. It is instead the result of the complex of interconnected processes [238]. The synergistic effects of the hydrogel properties of SDC [12, 222] and the pharmacological effects from sericin [25, 98] and collagen hydrolysate [118] seemly influence the individual scar characteristic that might consequently affect the other characteristics. The possible mechanisms of action from SDC application on the donor sites healing and scar formation were presented in the scheme.

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According to the literature, the effects from SDC could involve all phases of wound healing, including hemostasis, inflammatory, proliferation, and re-modeling phase. It is possible that sericin dressing with collagen could provide the moisture environment to wound bed because of the hydrogel [12, 14, 15] and the containing of sericin [56, 223], and collagen hydrolysate [39, 40, 131, 132]. The higher hydration level in the SDC treated site was found at complete healing time, which might confirm its hydrating effect in this study. At the same time, the earlier hemostasis might be obtained from collagen hydrolysate [37]. The hydrating and hemostatic effects could lead to the decrease of inflammation at the wound site [222], which may be concurrently reduced by the anti-

inflammatory effect of sericin [25] and the anti-oxidant effects from both sericin [25, 92] and collagen hydrolysate [118, 228]. The decrease of inflammation possibly results in the reduction of erythema at the early stage and the lower pigmentation of the SDC group [163]. In addition, the anti-melanogenic activity of sericin may also deplete pigmentation [25]. After that, the reduced inflammation might contribute to faster wound healing and the maturation of wounds treated with SDC. Both sericin and collagen could also increase proliferation and migration [96, 97, 200, 201], enhance collagen synthesis [96], and provide nutritional support [44], ameliorating the healing. The earlier maturation could be seen in the SDC group in the current study, determining from the resolution of erythema level because of the devascularization [157]. Besides the decrease of excessed vascular, the maturation of wounds also involves the reorganization of collagen and elastin, the accumulation of hygroscopic substances, and the recovery of skin appendages [156]. These processes affect the epidermal functions, i.e., TEWL and water holding capacity, the biomechanical properties of scar, the appearance of the scar, i.e., thickness and irregularity, and scar symptoms (pain and itching). The superiority of SDC on some scar characteristics, i.e., TEWL, Cutometer parameters, irregularity, pain, and itching, were not found in the SDC group. However, improvement in water holding capacity, pliability (VSS), and scar thickness were indicated in donor sites treated with SDC. Overall, the improvement of scar quality obtained from SDC application could be stated from overall measurements, including the clinician and patient evaluations and the objective measurements.

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To determine whether the changes in scar characteristics are clinically relevant, the minimally important change (MIC), which is “the smallest change in the score which patients, clinicians, or relevant others perceive as important” [186], was utilized. The statistical significance in the subjective measurement might be considered as the clinically important difference. However, no clinical significance could not be concluded because of the non-statistical significance [196]. Compared to the subjective method, the objective measurements possibly provide continuous quantitative data leading to the more obvious differentiation for the effects between treatments. Moreover, the physiological function of scars, e.g., TEWL and skin hydration, could be determined by only objective methods. However, the clinically important difference is difficult to specify for the objective measurement [196]. The scar quality evaluated by the patients, such as the patient part of POSAS, could be influenced by age, gender, and other factors [65]. Although the results from patient

and clinician evaluation may be inconsistent, the patient's perspective on their scars is still meaningful, and some scar characteristics, including pain and itching, could be assessed only by themselves. Accordingly, all measurements evaluated by clinician, patient, and device are recommended to be considered together. Based on our knowledge, this current study might be the first study reporting the scar outcome from sericin dressing application in the perspective of patients.

Pain control

The previous studies [32, 108, 109] displayed the agreement that sericin dressing could reduce the pain score in STSG donor sites compared with Bactigras®. Although this benefit could not be stated in the present study, the pain scores found in both groups were relatively low and comparable to the reported pain score in the sericin group in those studies [32, 108, 109]. Moreover, pain reduction from applying undenatured collagen dressing was also reported [42, 43]. However, the pain score is the subjective outcome from the individual patient, so the comparison with other studies seems limited. Most patients in this study underwent extensive surgeries such as an anterolateral thigh (ALT) free flap, mandibular reconstruction, and a local flap that required STSG for covering defects. Therefore, the patients received postoperative pain management, including opioids (morphine, fentanyl, and tramadol), NSAIDs (etoricoxib and celecoxib), and paracetamol, which were administered continuously in most of the patients or by using the patient-controlled analgesia (PCA) in some patients, especially in the first few days. Moreover, the patients could request analgesics as their needs during staying in the hospital. The analgesic administration would also relieve the pain at the donor site and possibly conceal the effects from each dressing. The confounding effect of analgesics on the pain evaluation was also mentioned in the systematic review. It could also lead to the variation of VAS pain score in donor sites reported in the several studies [8]. However, pain reduction during dressing application and removal might be expected from SDC because SDC is a hydrogel that is seemly non-adherent and possibly provides soothing and cooling effects to the wound bed [12, 13, 60, 79]. Moreover, the moisturizing, anti-inflammatory, and hemostatic effects from sericin and collagen hydrolysate [39, 40, 56, 131, 223] could encourage pain reduction. The maximum pain score at the studied wound might be monitored in future study to provide additional information about the effects of dressing on pain control.

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Infection and adverse effects

There was no incidence of infection on donor sites of all patients in both groups, and the average body temperature was seemly normal. The elevated body temperature on postoperative day 1 and day 2 may arise from the physiologic response to surgery. Some patients seem to develop a post-operative fever (> 38.3°C) [239] which is commonly found within 48 hours after surgery due to the release of inflammatory cytokines. It should be noted that SDC contains no antimicrobial agent in contrast to Bactigras® that consists of chlorhexidine; however, there was no incidence of infection on STSG donor sites treated with both dressings. Due to STSG being a sterile wound, the infection rate is generally low [8]. However, the colonization of bacteria, forming the biofilms, could also negatively affect wound healing. Therefore, the addition of antimicrobial substances into newly developed wound dressing might be considered. Moreover, sericin [25] and collagen hydrolysate [118] also have antimicrobial properties that probably provide additional effects on infection control. This finding agrees with the previous studies using sericin dressing for treating STSG donor sites [32, 108, 109].

The systematic adverse effects were monitored from blood samples of patients, and the local adverse effects were observed at the wound and surrounding area. The results showed no adverse effect after dressing application, and the safety of SDC seems to confirm the results from the phase I study and the previous studies.

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5.2 Limitations

1. The different appearances between sericin dressing with collagen and commercial dressing might limit the blinding after uncovering the secondary dressing. The patients were blinded during evaluation for the pain score as the secondary dressing covered the primary dressings. In contrast, the patients may perceive the type of dressings while uncovering dressing at complete healing time. However, they were asked to evaluate their wounds after complete healing for 6 months. Therefore, patients might focus on their scar quality, and the recognition of patients for the type of dressing might less affect the evaluation of patients. The evaluation by the clinician was utilized 1 month after complete wound healing so the assessor would not notice which type of dressing was formerly applied at each site.

2. The sample size was calculated for the primary objective, which is complete healing time. Moreover, the loss of follow-up patients during long-term evaluation brings about a lower number of patients. The statistical significance might not be found in some scar assessments due to less of sample size. However, the sample size for the objective measurements might be challenging to be calculated as the MCID is hard to be stated.

3. According to the literature, collagen hydrolysate could be absorbed into the blood resulting in the healing enhancement, and some amount of absorbed collagen probably affects the control site. Therefore, the comparison for the effects of collagen might be influenced. However, the positive effects of collagen hydrolysate on the treated side were seemly found in the current study.

4. The measurement for viscoelasticity properties at adjacent normal skin may not be the best control skin because skin elasticity varies depending on the location and position. The identification and measurement at the precise location before collecting graft might better compare the properties after complete healing. However, it seems to be impractical, increasing the difficulty of the operation.

5. Some scar characteristics were not examined due to device limitation, including sebum production, dermis hydration, and scar perfusion. Moreover, the structure of healed wound could not be visualized in the current study, unlike the results obtained from scar biopsy.

6. The analgesic medications were continuously administrated in some patients. It possibly conceals the effects of dressing on pain control.

7. After applying to the patient's wound, some limitations of SDC were found. The exudate accumulation was found underneath the SDC in some wounds. However, after complete wound healing, no maceration of wound edge or other local side effects. In addition, the hardness of SDC dressing relatively increased with the absence of exudate. However, no local side effect caused by SDC was detected from all STSG donor sites in this study.

5.3 Conclusions

Sericin dressing with collagen (SDC) was successfully fabricated by the simple cast drying method. The bioactive substances, i.e., sericin and collagen hydrolysate, were incorporated into polyvinyl alcohol hydrogel, providing the sustained release of proteins. Therefore, the obtained dressing could provide both the moist environment and bioactive properties to the wound bed. The sericin dressing with collagen showed its safety in healthy volunteers compared with the commercial dressing, Bactigras®. In split-thickness skin graft donor site treatment, the sericin dressing with collagen possibly facilitates wound healing. Moreover, the improved scar quality evaluated by the clinician, patients, and objective devices could be found in the STSG donor sites treated with sericin dressing with collagen compared to Bactigras®. Although the sericin dressing with collagen showed no superior effects over Bactigras® on pain control, the pain scores in both groups were relatively low. The infection control between sericin dressing with collagen and Bactigras® was comparable, and the safety from SDC application seems to be established. The application of sericin dressing with collagen possibly leads to the desired short-term and long-term outcomes that could result from the synergistic effects between the hydrogel characteristic and the pharmacological effects from sericin and collagen hydrolysate. Accordingly, the sericin dressing with collagen may fill the gap of current dressings and could become an alternative dressing in split-thickness skin graft donor site treatment. Future studies in other wound types may be conducted to clarify its clinical efficacy further.

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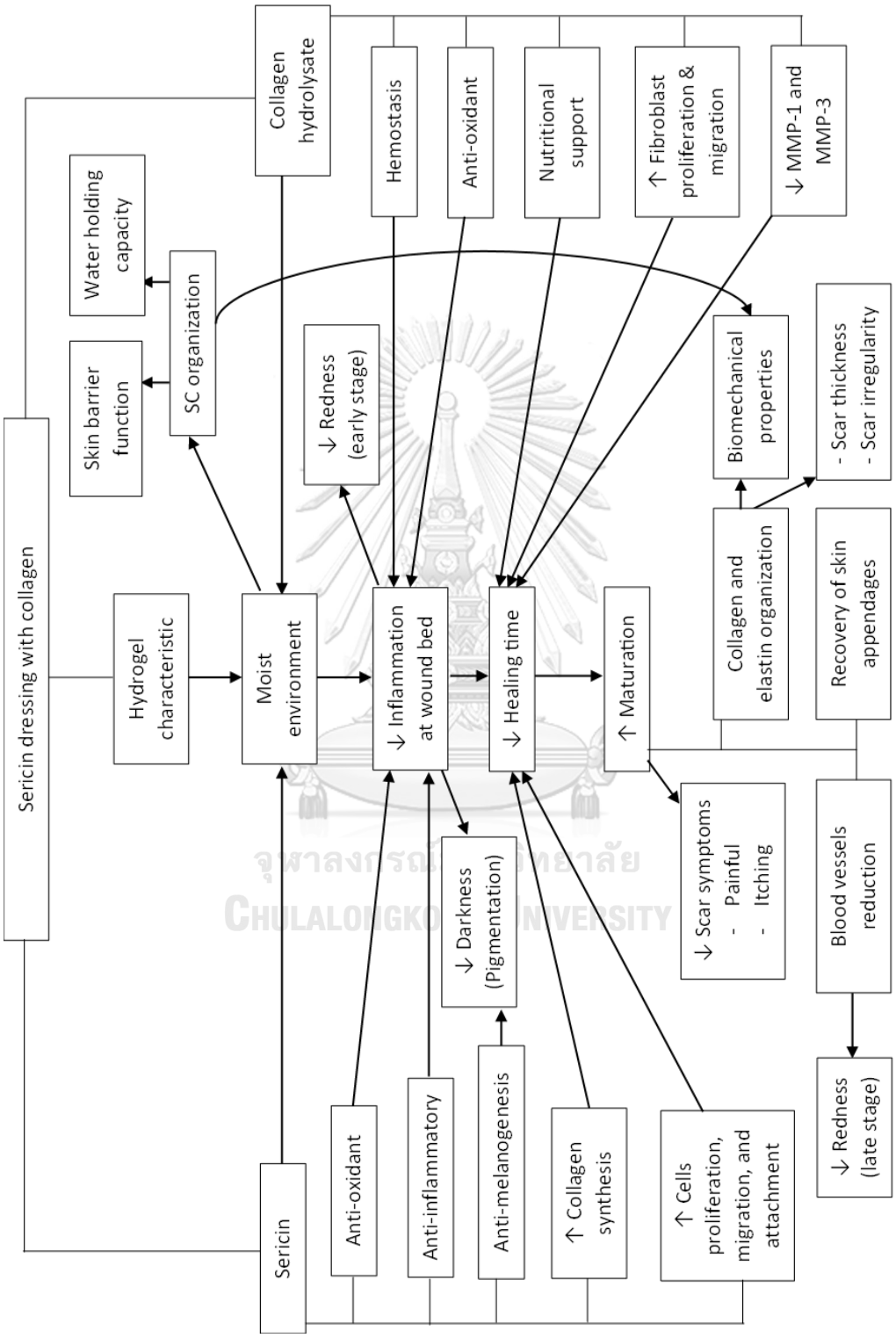
Scheme The possible mechanisms of action from sericin dressing collagen application



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APPENDICES

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Appendix A

แบบบันทึกข้อมูลพื้นฐานของอาสาสมัคร

ตัวแปร	หมายเหตุ
ส่วนที่ 1 ข้อมูลอาสาสมัคร	
ข้อมูลพื้นฐาน	
หมายเลขที่ □□□□	
เพศ □1. ชาย □2. หญิง	SEX □
อายุ.....ปี.....เดือน เกิด วันที่.....เดือน.....ปี.....	AGE □□
อาชีพที่ทำเป็นประจำและใช้เวลาเป็นส่วนใหญ่** □1. ว่างงาน □2. แม่บ้าน/พ่อบ้าน □3. เกษตรกร/ประมง □4. ผู้ใช้แรงงาน/รับจ้าง □5. นักเรียน/นักศึกษา □6. ข้าราชการ □7. ตำรวจ/ทหาร □8. พนักงานรัฐวิสาหกิจ □9. พนักงานบริษัท □10. ค้าขาย/ธุรกิจส่วนตัว □11. อื่นๆ (ระบุ).....	OCC □□
น้ำหนักตัว ..... กิโลกรัม***	WT □□
ความสูง..... เซนติเมตร***	HT □□□
ประวัติโรคประจำตัว □1. มี ระบุ..... ยาที่ใช้รักษา ..... □2. ไม่มี (ปฏิเสธโรคประจำตัว) *ยาหรือแผ่นแปะที่ใช้ภายใน 2 สัปดาห์ก่อนเข้าร่วมการวิจัย* □ *1. มี ระบุ*..... *ยาที่ใช้รักษา* ..... □ *2. ไม่มี*	
ประวัติการแพ้ (ยา/ อาหารเสริม/ สารเคมี) □ *มี ระบุ*..... □ *ไม่มี*	
ดื่มแอลกอฮอล์**** □ ดื่ม ระบุความถี่ □□ ครั้ง/สัปดาห์ □ ไม่ดื่ม □ เลิกดื่มมาแล้วนาน	Alc □
ดื่มชา/กาแฟ**** □ ดื่ม ระบุความถี่ □□ แก้ว/วัน □ ไม่ดื่ม	Caffeine □
สูบบุหรี่**** □ สูบ/เคยสูบ □□ มวน/วัน นาน.....ปี □ ไม่สูบ □ เลิกสูบมาแล้วนาน	Smoking □

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Appendix B

เกณฑ์คะแนนประเมินอาการไม่พึงประสงค์โดยแพทย์ผิวหนัง

Erythema scale:

This scale is used only for grading degree of erythema (redness). A score on this scale will be assigned following every application of a patch.

0 No visible erythema.

1 Mild erythema (faint pink to definite pink).

2 Moderate erythema (definite redness).

3 Severe erythema (very intense redness).

Designations for Elevated Responses:

Edema, papules, vesicles, and bullae, if present, are graded as independent responses.

E Edema - definite swelling. (0-4 ; no edema – severe edema)

P Papules - many small, red, solid elevations; surface of reaction has granular feeling.

V Vesicles - small, circumscribed elevations having translucent surfaces so that fluid is visible (blister-like). Vesicles are no larger than 0.5 cm in diameter.

B Bullae - vesicles with a diameter > 0.5 cm; vesicles may coalesce to form one or a few large blisters that fill the patch site.

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เลขที่อาสาสมัคร ☐☐☐☐

เกณฑ์คะแนนประเมินอาการไม่พึงประสงค์โดยแพทย์ผิวหนัง

ตำแหน่งที่ 1

ลักษณะ	คะแนนประเมินสภาพผิวหนัง					คะแนนประเมิน
	ครั้งที่ 1	ครั้งที่ 2	ครั้งที่ 3	ครั้งที่ 4	ครั้งที่ 5	
Erythema						0-3
Edema						0-4
Papules						มี (1) /ไม่มี (0)
Vesicles						มี (1) /ไม่มี (0)
Bullae						มี (1) /ไม่มี (0)

ตำแหน่งที่ 2

ลักษณะ	คะแนนประเมินสภาพผิวหนัง					คะแนนประเมิน
	ครั้งที่ 1	ครั้งที่ 2	ครั้งที่ 3	ครั้งที่ 4	ครั้งที่ 5	
Erythema						0-3
Edema						0-4
Papules						มี (1) /ไม่มี (0)
Vesicles						มี (1) /ไม่มี (0)
Bullae						มี (1) /ไม่มี (0)

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Appendix C

เลขที่อาสาสมัคร ☐ ☐ ☐ ☐

แบบฟอร์มการวัดความแดงของผิวหนังด้วยเครื่อง Cutometer® **ด้วยโหมด** Mexameter

ตำแหน่งที่ ☐ 1 ☐ 2

ความแดงของผิวหนัง (วัดตำแหน่งละ 10 ซ้ำ)	**ครั้งที่** 1 (Baseline)	**ครั้งที่** 2	**ครั้งที่** 3	**ครั้งที่** 4	**ครั้งที่** 5
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
Mean					
SD					

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Appendix D

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แบบบันทึกข้อมูลพื้นฐานของผู้เข้าร่วมงานวิจัย

ส่วนที่ 1 ข้อมูลส่วนบุคคล

1. เพศ ❒ ชาย ❒ หญิง
2. อายุ.....ปี
3. น้ำหนัก.....กิโลกรัม ส่วนสูง.....เซนติเมตร
4. สถานภาพ ❒ โสด ❒ คู่ ❒ หย่า/หม้าย/แยก
5. อาชีพ.....
6. การจ่ายค่ารักษาพยาบาล
   ❒ ประกันสุขภาพถ้วนหน้า (30บาท) ❒ ประกันสังคม ❒ ชำระเอง
   ❒ เบิกจ่ายตรงสวัสดิการข้าราชการ ❒ ประกันสุขภาพ ❒ อื่นๆระบุ.....
7. โรคประจำตัว/โรคร่วม
   1. ..... ระยะเวลาที่เป็น.....ปี
   ยาที่ใช้ .....
   .....
   2. ..... ระยะเวลาที่เป็น.....ปี
   ยาที่ใช้ .....
   .....
   3. ..... ระยะเวลาที่เป็น.....ปี
   ยาที่ใช้ .....
   .....
8. ยาอื่นหรือผลิตภัณฑ์เสริมอาหารที่ทานอยู่ในปัจจุบัน (นอกจากยาสำหรับโรคประจำตัว)
   .....
   .....
   .....
   .....
9. การดื่มสุรา: ❒ ดื่ม .....แก้ว/วัน/เดือน ❒ ไม่ดื่ม ❒ เลิกดื่มมาแล้วนาน ..... เดือน/ปี
   การสูบบุหรี่ : ❒ สูบ .....มวน/วัน ❒ ไม่สูบ ❒ เลิกสูบมาแล้วนาน ..... เดือน/ปี
10. ประวัติการแพ้ ยา อาหาร สารเคมี
    สิ่งที่แพ้.....ลักษณะอาการ.....
    การแก้ไข.....อาการเกิดขึ้นมาแล้วเมื่อ..... เดือน/ปี
    การเกิดซ้ำ มี/ ไม่มี เมื่อ .....เดือน/ปี ลักษณะอาการ.....
11. ประวัติการเกิด hypertrophic scar หรือ keloid
    ❒ ไม่มี ❒ มี บริเวณ ..... สาเหตุของแผล .....

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ส่วนที่ 2 ข้อมูลเกี่ยวกับความเจ็บป่วยและบาดแผล

Code

1. สาเหตุของการปลูกถ่ายผิวหนัง

   .....

2. วันที่เกิดเหตุ ..... วันที่เข้ารับการรักษา

   .....

3. ประวัติการรักษา

   .....

   .....

   .

4. จำนวนบาดแผล donor site ทั้งหมด ..... แผล แบ่งเป็น ..... ตำแหน่ง

5. ตำแหน่งและขนาดของบาดแผล (บันทึกตำแหน่งลงในรูป)

   1) ..... ขนาด ..... ความลึก..... นิ้ว ☐ แผ่นทดลอง ☐ แผ่นควบคุม วันที่แผลหาย ..... (..... วัน)

   2) ..... ขนาด ..... ความลึก..... นิ้ว ☐ แผ่นทดลอง ☐ แผ่นควบคุม วันที่แผลหาย ..... (..... วัน)

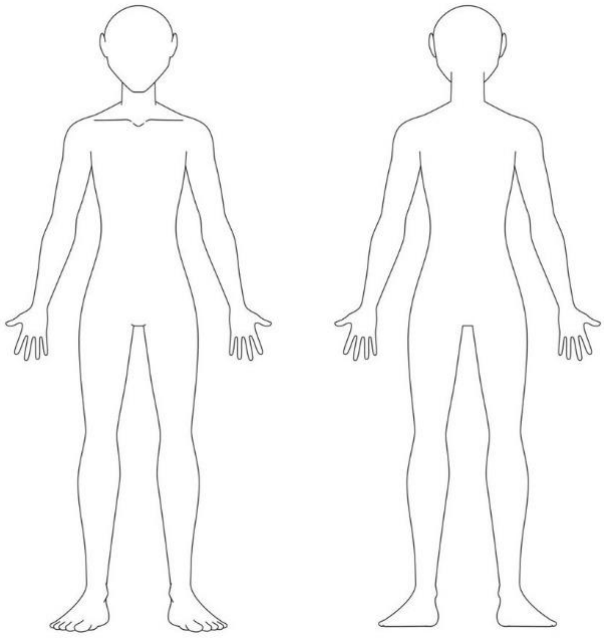
   3) ..... ขนาด ..... ความลึก..... นิ้ว ☐ แผ่นทดลอง ☐ แผ่นควบคุม วันที่แผลหาย ..... (..... วัน)

   4) ..... ขนาด ..... ความลึก..... นิ้ว ☐ แผ่นทดลอง ☐ แผ่นควบคุม วันที่แผลหาย ..... (..... วัน)

   5) ..... ขนาด ..... ความลึก..... นิ้ว ☐ แผ่นทดลอง ☐ แผ่นควบคุม วันที่แผลหาย ..... (..... วัน)

   6) ..... ขนาด ..... ความลึก..... นิ้ว ☐ แผ่นทดลอง ☐ แผ่นควบคุม วันที่แผลหาย ..... (..... วัน)

บันทึกรูปร่าง ตำแหน่งบาดแผลและแผ่นปิดแผลที่ใช้



ด้านหน้า ด้านหลัง

Appendix E

แบบรายงานอาการไม่พึงประสงค์จากการใช้แผ่นปิดแผลหรือยาอื่น ๆ

ขอให้ท่านช่วยบันทึกอาการไม่พึงประสงค์ อาการเจ็บป่วยต่างๆ ที่เกิดขึ้นกับผู้ป่วยในระหว่างการวิจัยลงในตารางด้านล่าง

☐ บริเวณผิวหนังที่ติดแผ่นปิดแผล ☐ แผ่นเซริซิน ☐ แผ่นในท้องตลาด ☐ แผ่นทั้ง 2 ชนิด

☐ ผู้ป่วยมีการใช้ยาอื่นๆ ระบุ ....

วัน/เดือน/ปี เวลา	อาการไม่พึงประสงค์ที่พบ	การแก้ไข/ยาที่ใช้ (ชื่อยาและ จำนวนที่ใช้)	ผลจากการแก้ไข/ใช้ยา	วันที่หาย/ดีขึ้น
สาเหตุ ☐แผ่นปิดแผล _________ ☐ ยา ___________ ☐ ไม่แน่ใจ ☐ อื่นๆ _______ Naranjo score ____________				
สาเหตุ ☐แผ่นปิดแผล _________ ☐ ยา ___________ ☐ ไม่แน่ใจ ☐ อื่นๆ _______ Naranjo score ____________				
สาเหตุ ☐แผ่นปิดแผล _________ ☐ ยา ___________ ☐ ไม่แน่ใจ ☐ อื่นๆ _______ Naranjo score ____________				
สาเหตุ ☐แผ่นปิดแผล _________ ☐ ยา ___________ ☐ ไม่แน่ใจ ☐ อื่นๆ _______ Naranjo score ____________				

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แบบประเมินการเกิดอาการไม่พึงประสงค์ Naranjo's Algorithm

อาการไม่พึงประสงค์.....**วันที่เกิดอาการ**.....**วันที่ประเมิน**.....

แผ่นปิดแผลที่ใช้ □ **แผ่นเซริซิน** □ **แผ่นในท้องตลาด**□ **แผ่นทั้ง 2 ชนิด** □ **ยาอื่น ๆ** .....

คำถาม	**ใช่**	**ไม่ใช่**	**ไม่ทราบ**	**คะแนน**
1. เคยมีสรุปหรือรายงานการปฏิกริยานี้มาแล้วหรือไม่	+1	0	0	
2. อาการไม่พึงประสงค์นี้เกิดขึ้นภายหลังจากได้รับยาที่คิดว่าเป็นสาเหตุหรือไม่	+2	-1	0	
3. อาการไม่พึงประสงค์นี้ดีขึ้นเมื่อหยุดยาดังกล่าว หรือเมื่อให้ยาต้านที่จำเพาะเจาะจง (specific antagonist) หรือไม่	+1	0	0	
4. อาการไม่พึงประสงค์ดังกล่าวเกิดขึ้นอีกเมื่อเริ่มให้ยาใหม่หรือไม่	+2	-1	0	
5. ปฏิกริยาที่เกิดขึ้นสามารถเกิดจากสาเหตุอื่น (นอกเหนือจากยา) ของผู้ป่วยได้หรือไม่	-1	+2	0	
6. ปฏิกริยาดังกล่าวเกิดขึ้นอีก เมื่อให้ยาหลอกหรือไม่	-1	+1	0	
7. สามารถตรวจวัดปริมาณยาได้ในเลือด (หรือของเหลวอื่น) ในปริมาณความเข้มข้นที่เป็นพิษหรือไม่	+1	0	0	
8. ปฏิกริยารุนแรงขึ้น เมื่อเพิ่มขนาดยาหรือลดความรุนแรงลงเมื่อลดขนาดยาหรือไม่	+1	0	0	
9. ผู้ป่วยเคยมีปฏิกริยาที่เหมือนหรือคล้ายคลึงกันนี้มาก่อน ในการได้รับยาครั้งก่อน ๆ หรือไม่	+1	0	0	
10. อาการไม่พึงประสงค์นี้ ได้รับการยืนยันโดยหลักฐานที่เป็นรูปธรรม (objective evidence)หรือไม่	+1	0	0	
รวม				

ระดับคะแนน	คะแนนมากกว่าหรือเท่ากับ 9	Definite	ใช่แน่
	คะแนนเท่ากับ 5-8	Probable	ใช่
	คะแนนเท่ากับ 1-4	Possible	อาจจะใช่
	คะแนนน้อยกว่าหรือเท่ากับ 0	Doubtful	น่าสงสัย

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Appendix G

Code

แบบบันทึกลักษณะบาดแผล ผลการวิเคราะห์เชื้อ และความเจ็บปวด

Date	ตำแหน่ง	ลักษณะบาดแผล (% epithelialization)	Body temp (°c)	การเก็บตัวอย่าง	Microbiological test	VAS score

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Appendix H

Code

แบบบันทึกความเจ็บปวดประจำวัน

ตำแหน่ง	ตำแหน่ง ....
วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10	วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10
วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10	วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10
วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10	วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10
วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10	วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10
วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10	วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10
วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10	วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10
วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10	วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10
วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10	วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10
วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10	วันที่ ....../...... /...... (หลังผ่าตัด .... วัน) No Pain — Moderate Pain — Worst Pain 0 1 2 3 4 5 6 7 8 9 10

Appendix I

Code

แบบบันทึกลักษณะของแผลหลังจากการหาย (Scar quality)

Date	Site	Vascularity (0 = normal, 1 = pink, 2 = red, 3 = purple)	Height/thickness (0 = flat, 1 = 0-1 mm, 2 = 1–2 mm, 3 = 2-4 mm, 4 = >4 mm)	Pliability (0 = normal, 1 = supple, 2 = yielding, 3 = firm, 4 = banding, 5 = contracture	Pigmentation (0 = normal, 1 = hypopigmentation, 2 = mixed pigmentation, 3 = hyperpigmentation)	Note

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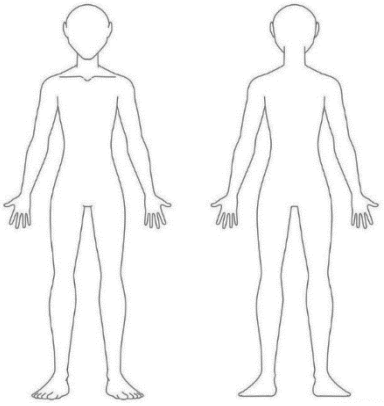
ระบุตำแหน่ง (A, B, C, D) **ของบาดแผล**



ด้านหน้า ด้านหลัง

Appendix J

Code

แบบบันทึกลักษณะของแผลหลังจากการหายจากการวัดด้วยเครื่อง Cutometer

วันที่	**ตำแหน่ง**	Mexameter		Tewameter (TEWL)	Corneometer (Hydration state)
		Melanin level	Erythema level		

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Appendix K

Code

POSAS Patient scale

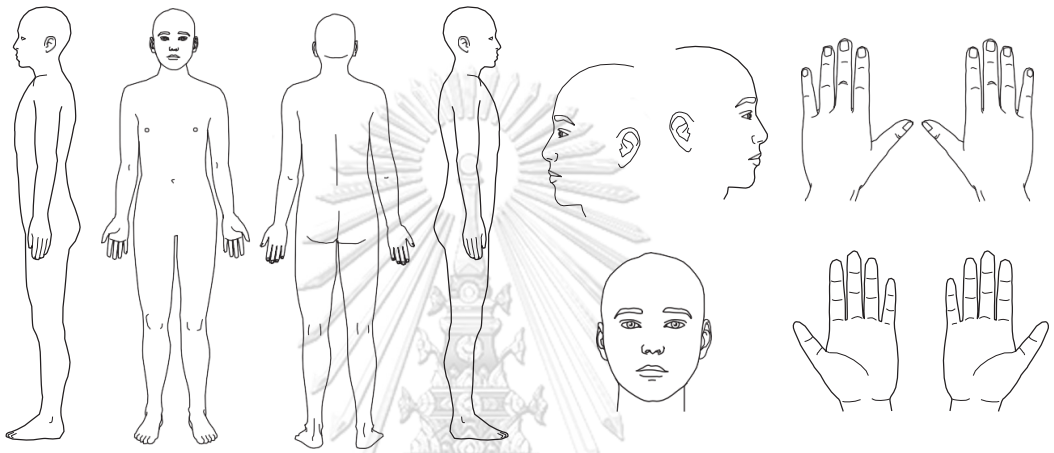
The Patient and Observer Scar Assessment Scale v 2.0 / EN

Date of examination:

Observer:

Location:

Research / study:



	1 = no, not at all … yes, very much = 10
has the scar been painful the past few weeks?	1 2 3 4 5 6 7 8 9 10
has the scar been itching the past few weeks?	1 2 3 4 5 6 7 8 9 10

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	1 = no, as normal skin … yes, very different = 10
is the scar color different from the color of your normal skin at present?	1 2 3 4 5 6 7 8 9 10
is the stiffness of the scar different from your normal skin at present?	1 2 3 4 5 6 7 8 9 10
is the thickness of the scar different from your normal skin at present?	1 2 3 4 5 6 7 8 9 10
is the scar more irregular than your normal skin at present?	1 2 3 4 5 6 7 8 9 10

	1 = as normal skin … very different = 10
what is your overall opinion of the scar compared to normal skin?	1 2 3 4 5 6 7 8 9 10

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Appendix L

Certificate of Ethical Approval

COA No. 496/2018

IRB No. 086/61

INSTITUTIONAL REVIEW BOARD

Faculty of Medicine, Chulalongkorn University

1873 Rama 4 Road, Patumwan, Bangkok 10330, Thailand, Tel 662-256-4493

Certificate of Approval

The Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, has approved the following study in compliance with the International guidelines for human research protection as Declaration of Helsinki, The Belmont Report, CIOMS Guideline and International Conference on Harmonization in Good Clinical Practice (ICH-GCP)

Study Title	: SAFETY OF SILK SERICIN DRESSING WITH COLLAGEN IN HEALTHY VOLUNTEERS
Study Code	: -
Principal Investigator	: Prof. Dr. Pornanong Aramwit, Pharm.D, Ph.D.
Affiliation of PI	: Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Chulalongkorn University.
Review Method	: Full board
Continuing Report	: At least once annually or submit the final report if finished.

Document Reviewed :

1. Research Proposal Version 3 Date 03/05/2018
2. Protocol Synopsis Version 2 Date 04/04/2018
3. Information sheet for research participant Version 3 Date 30/04/2018
4. Informed consent for participating volunteers Version 1 Date 30/01/2018

Approval granted is subject to the following conditions: (see back of this Certificate)

5. Appendix 1 Baseline Voluntary Recording Format Version 1 Date 30/01/2018
6. Appendix 2 Criteria for Adverse Drug Reactions Dermatitis Version 1 Date 30/01/2018
7. Cutometer® Skin Tone Formation with Mexameter Version 1 Date 30/01/2018
8. Public relations document Version 1 Date 30/01/2018
9. Curriculum Vitae and GCP Training
   - Prof. Dr. Pornanong Aramwit, Pharm.D, Ph.D.
   - Apirujee Punjataewakupt
   - Assoc. Prof. Apichai Angspatt, M.D., F.R.C.S.T.

Signature ..... Signature .....

(Emeritus Professor Tada Sueblinvong MD) (Assistant Professor Prapapan Rajatapiti MD, PhD)

Chairperson Member and Secretary

The Institutional Review Board The Institutional Review Board

Date of Approval : June 5, 2018

Approval Expire Date : June 4, 2019

Approval granted is subject to the following conditions: (see back of this Certificate)

All approved investigators must comply with the following conditions:

1. Strictly conduct the research as required by the protocol;
2. Use only the information sheet, consent form (and recruitment materials, if any), interview outlines and/or questionnaires bearing the Institutional Review Board's seal of approval ; and return one copy of such documents of the first subject recruited to the Institutional Review Board (IRB) for the record;
3. Report to the Institutional Review Board any serious adverse event or any changes in the research activity within five working days;
4. Provide reports to the Institutional Review Board concerning the progress of the research upon the specified period of time or when requested;
5. If the study cannot be finished within the expire date of the approval certificate, the investigator is obliged to reapply for approval at least one month before the date of expiration.
6. If the research project is completed, the researcher must be form the Faculty of Medicine, Chulalongkorn University.

* A list of the Institutional Review Board members (names and positions) present at the meeting of Institutional Review Board on the date of approval of this study has been attached. All approved documents will be forwarded to the principal investigator.

COA No. 662/2020

IRB No. 143/63

INSTITUTIONAL REVIEW BOARD

Faculty of Medicine, Chulalongkorn University

1873 Rama 4 Road, Patumwan, Bangkok 10330, Thailand, Tel 662-256-4493

Certificate of Approval

The Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, has approved the following study in compliance with the International guidelines for human research protection as Declaration of Helsinki, The Belmont Report, CIOMS Guideline and International Conference on Harmonization in Good Clinical Practice (ICH-GCP)

Study Title : CLINICAL EFFICACY OF SILK SERICIN DRESSING WITH COLLAGEN FOR SPLIT-THICKNESS SKIN GRAFT DONOR SITE TREATMENT

Study Code : -

Principal Investigator : Apirujee Punjataewakupt

Affiliation of PI : Faculty of Pharmaceutical Sciences, Chulalongkorn University.

Review Method : Full board

Continuing Report : Every 6 months.

Document Reviewed :

1. Research Proposal Version 2 Date 10/05/2020
2. Protocol Synopsis Version 2 Date 10/05/2020
3. Information sheet for research participant Version 3 Date 27/5/2563
4. Informed Consent Form Version 2 Date 12/05/2020
5. Case Record Form
   - Appendix 1 Basic Participant Record Form Version 2 Date 10/05/2020
   - Appendix 2 Laboratory Record Form Version 2 Date 10/05/2020

Approval granted is subject to the following conditions: (see back of this Certificate)

- Appendix 3 Report of adverse reactions from the use of wound dressings or other drugs Version 2 Date 10/05/2020
- Appendix 4 Date Reaction Assessment Form Naranjo's Algorithm Version 2 Date 10/05/2020
- Appendix 5 Wound Record Form Infection analysis results And the pain Version 2 Date 10/05/2020
- Appendix 6 Daily Pain Record Version 2 Date 10/05/2020
- Appendix 7 Record form for wound dressing sheets Version 2 Date 10/05/2020
- Appendix 8 Scar quality record form with VSS Version 2 Date 10/05/2020
- Appendix 9 Record form of wound after healing after Cutometer Version 2 Date 10/05/2020
- Appendix 10 Scar quality form for wound healing after POSAS Version 2 Date 10/05/2020

6. Budgets
7. Curriculum Vitae and GCP Training
   - Apirujee Punjataewakupt
   - Prof. Dr. Pornanong Aramwit, Pharm.D., Ph.D.
   - Assist.Prof. Apichai Angspatt, M.D.
   - Sarintorn Thomrongdullaphak, M.D.

Signature ..... Signature .....

(Emeritus Professor Tada Sueblinvong MD)
Chairperson
The Institutional Review Board

(Assistant Professor Prapapan Rajatapiti MD, PhD)
Member and Secretary
The Institutional Review Board

Date of Approval : June 4, 2020
Approval Expire Date : June 3, 2021

Approval granted is subject to the following conditions: (see back of this Certificate)

All approved investigators must comply with the following conditions:

1. Strictly conduct the research as required by the protocol;
2. Use only the information sheet, consent form (and recruitment materials, if any), interview outlines and/or questionnaires bearing the Institutional Review Board's seal of approval ; and return one copy of such documents of the first subject recruited to the Institutional Review Board (IRB) for the record;
3. Report to the Institutional Review Board any serious adverse event or any changes in the research activity within five working days;
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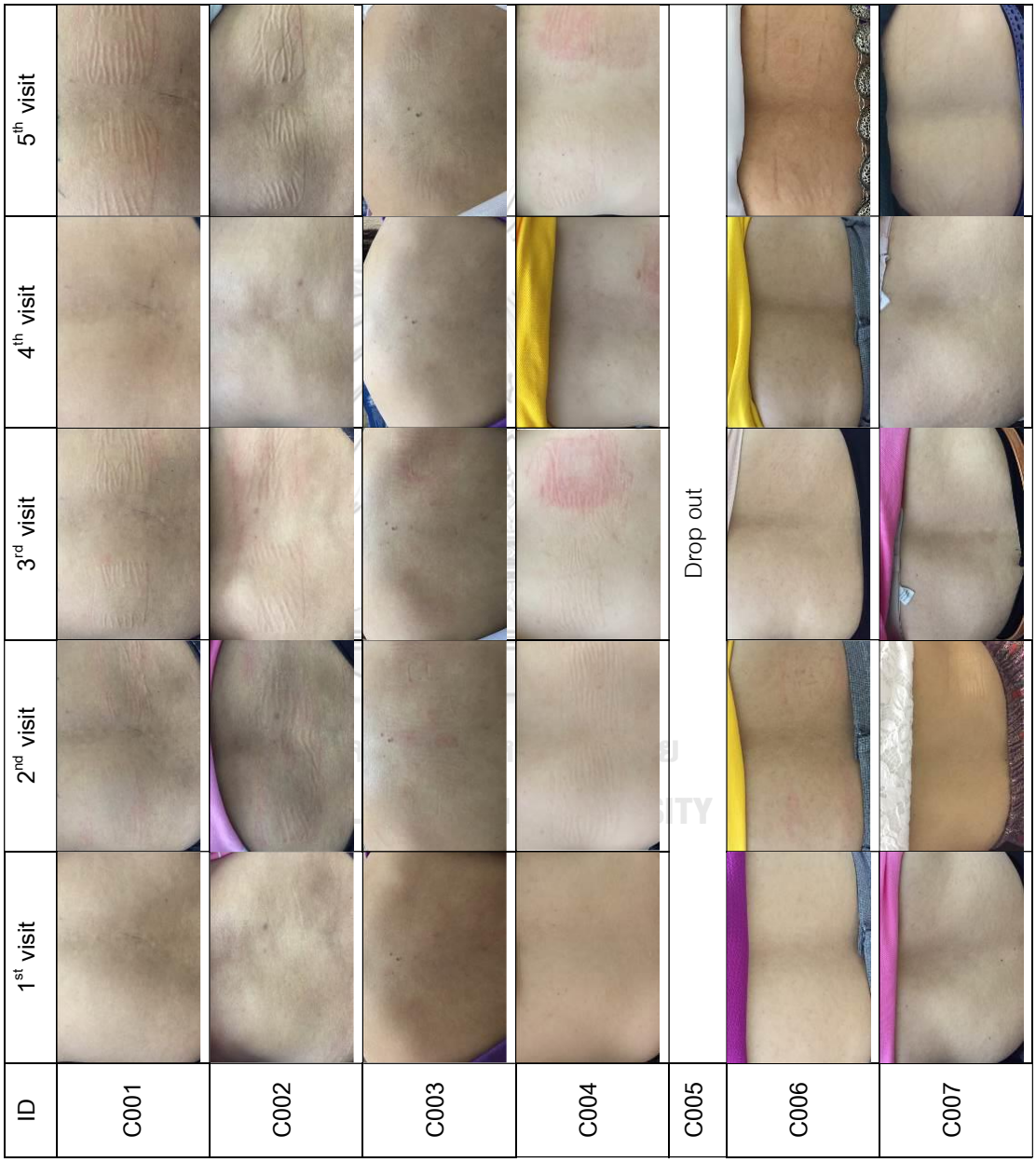
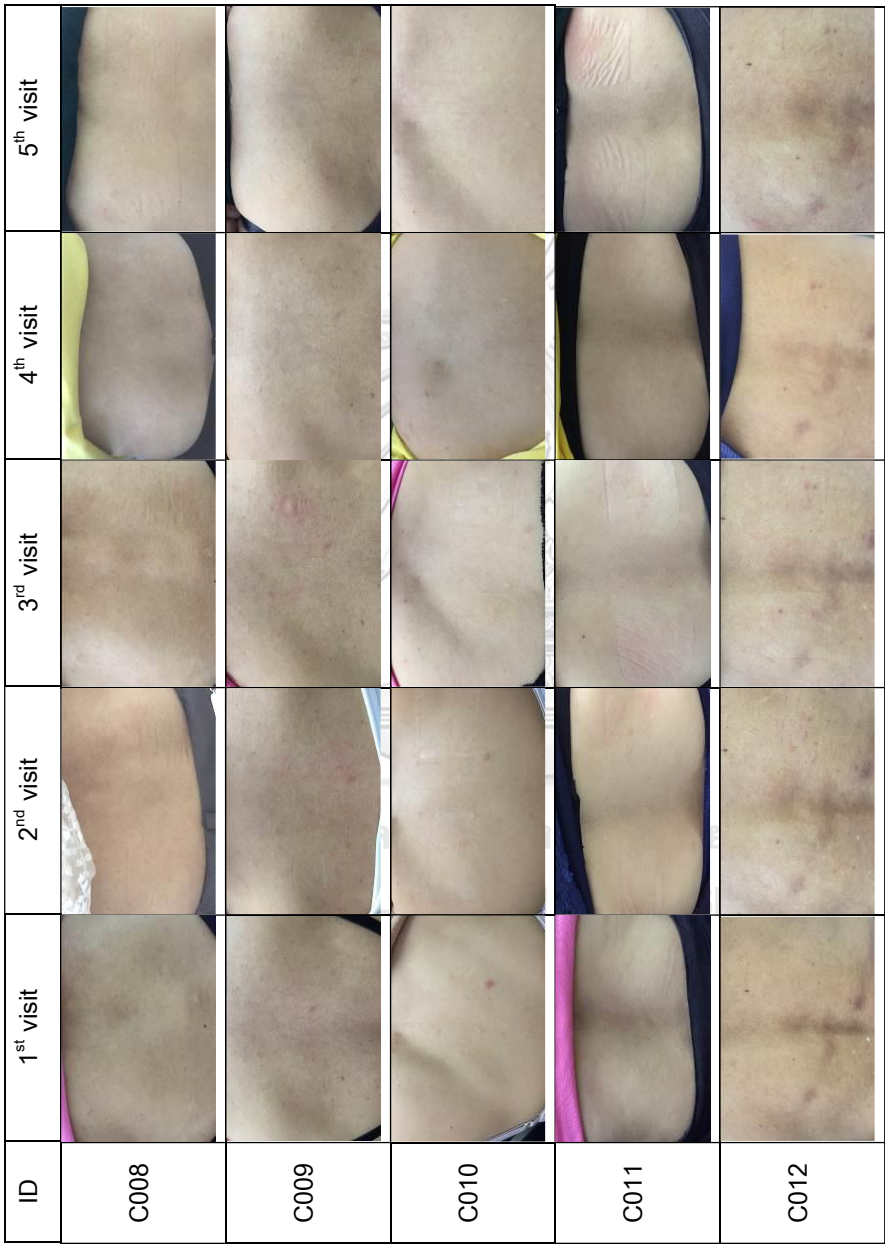
5. If the study cannot be finished within the expire date of the approval certificate, the investigator is obliged to reapply for approval at least one month before the date of expiration.
6. If the research project is completed, the researcher must be form the Faculty of Medicine, Chulalongkorn University.

* A list of the Institutional Review Board members (names and positions) present at the meeting of Institutional Review Board on the date of approval of this study has been attached. All approved documents will be forwarded to the principal investigator.

Appendix M

Pictures from patch test in healthy volunteers

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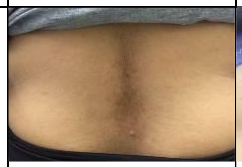
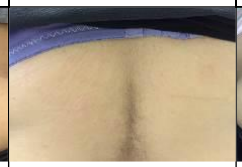
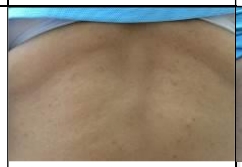
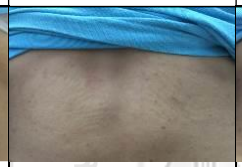
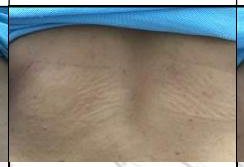
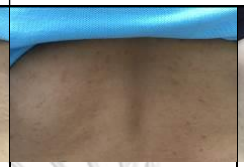
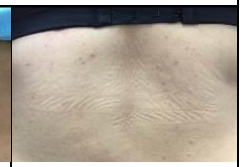
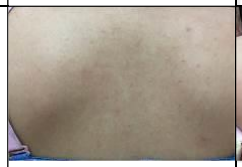
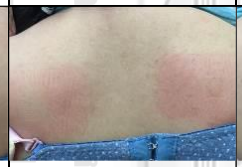
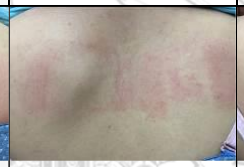
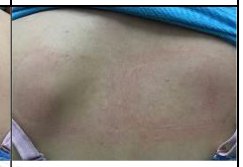
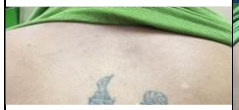
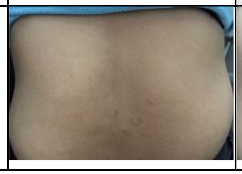
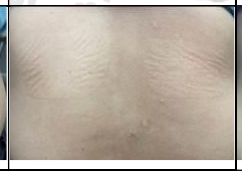
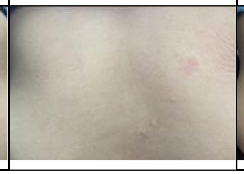
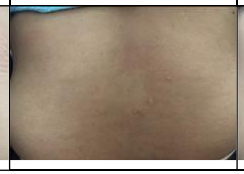
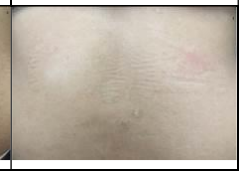
1st visit
2nd visit
3rd visit
4th visit
5th visit
C008
C009
C010
C011
C012

ID	1st visit	2nd visit	3rd visit	4th visit	5th visit
C013				Drop out	
C014					
C015					
C016					
C017					

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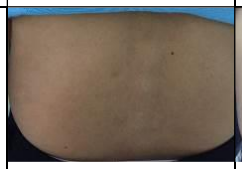
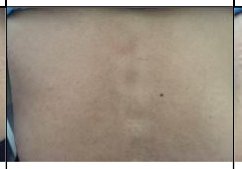
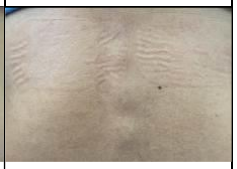
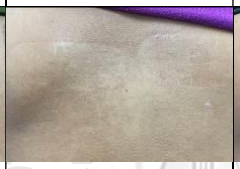
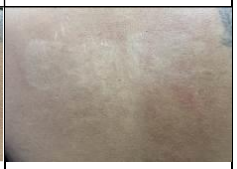
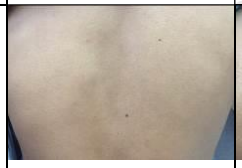
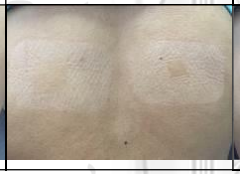
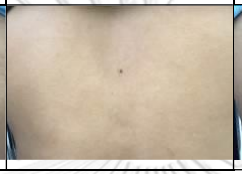
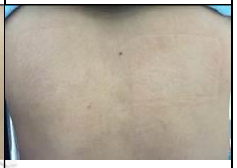
C018					
C019					
C020					
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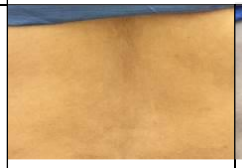
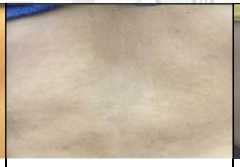
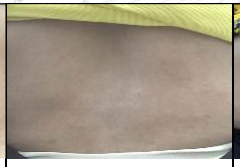
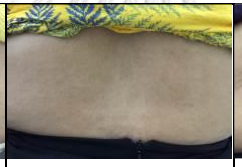
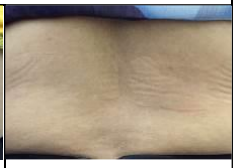
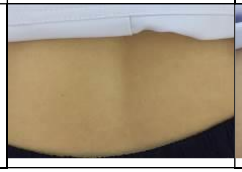
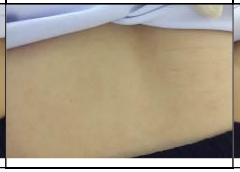
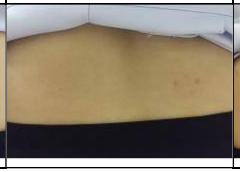
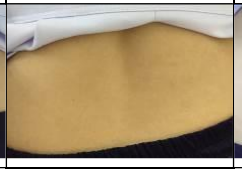
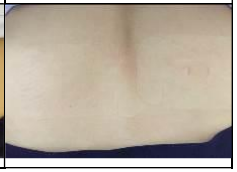
C022					
C023					

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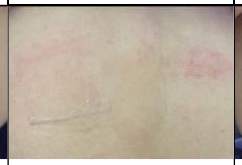
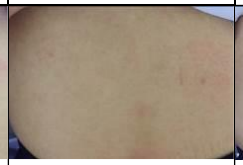
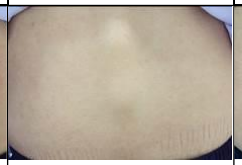
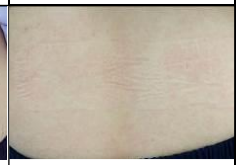
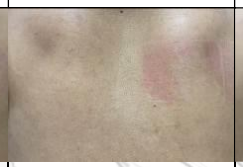
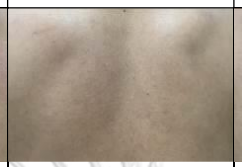
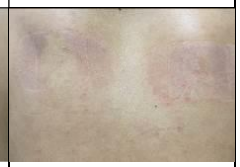
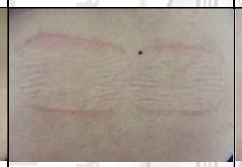
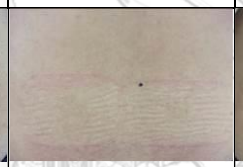
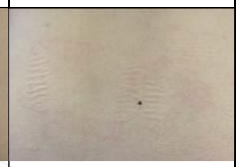
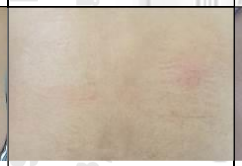
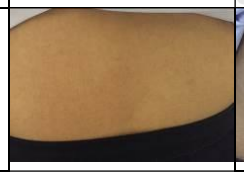
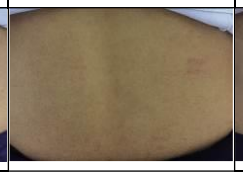
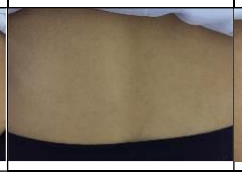
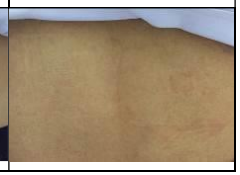
C024					
C025					
C026					
C027					
C028					

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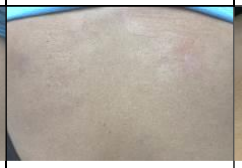
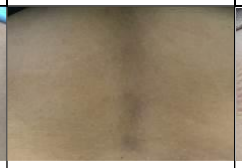
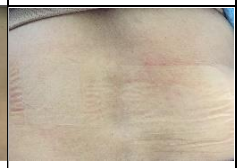
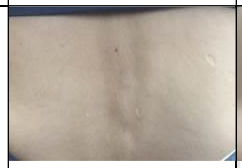
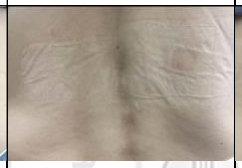
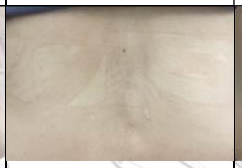
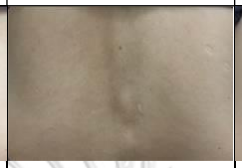
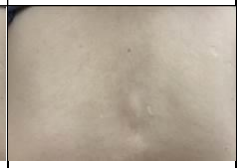
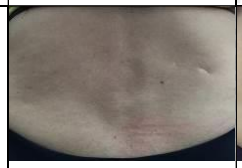
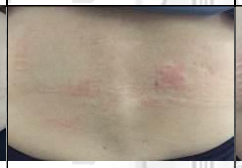
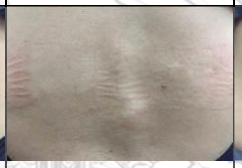
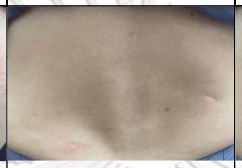
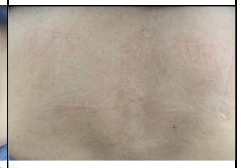
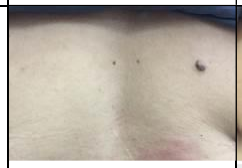
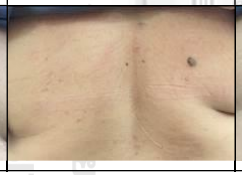
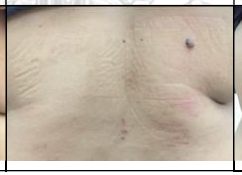
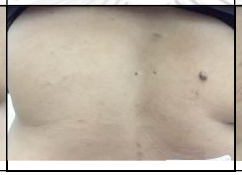
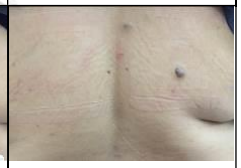
C029					
C030					
C031					
C032					
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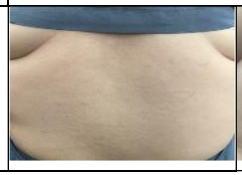
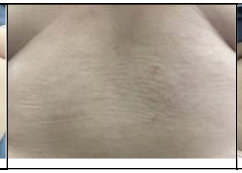
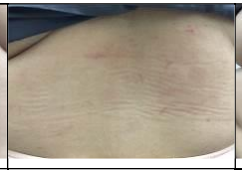
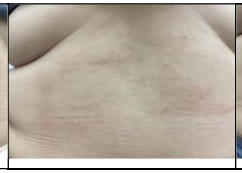
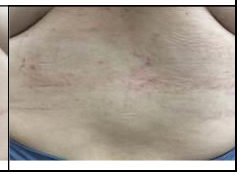
C034					

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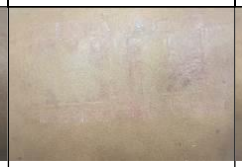
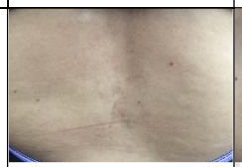
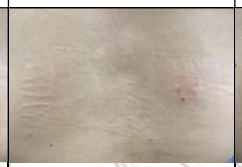
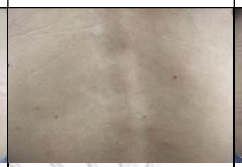
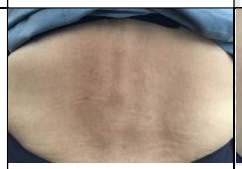
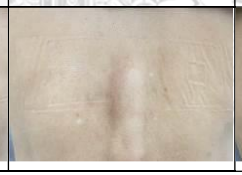
C035					
C036					
C037					
C038					
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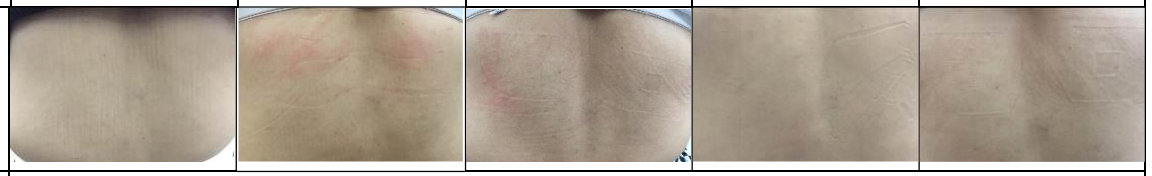
C041					
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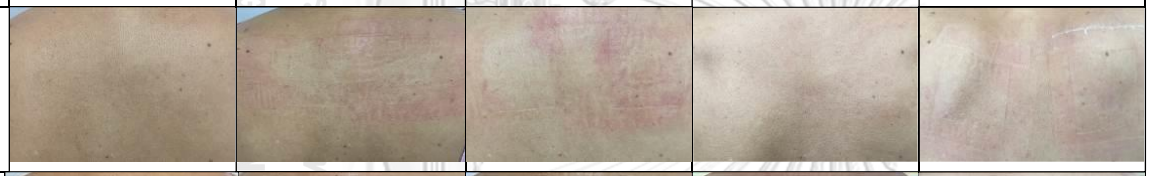
C044					
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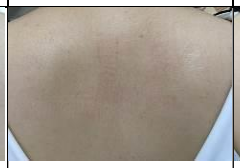
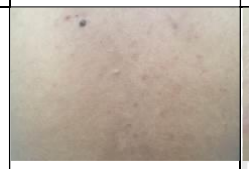
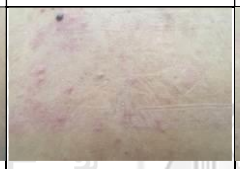
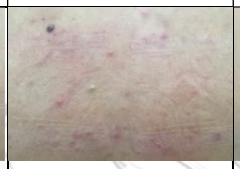
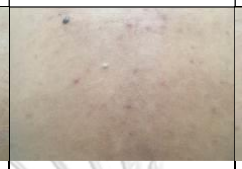
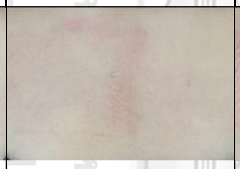
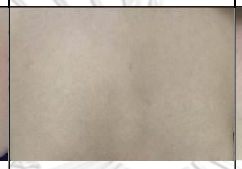
C046					
C047					
C048					
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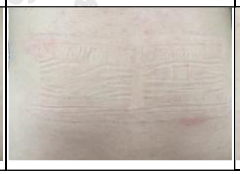
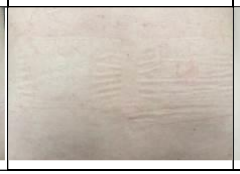
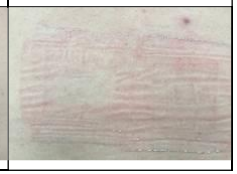
C050					

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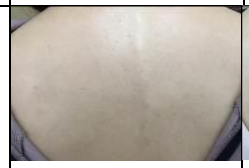
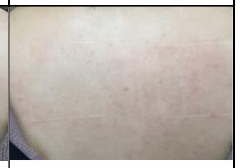
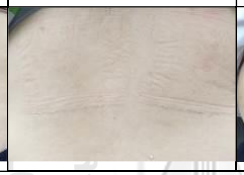
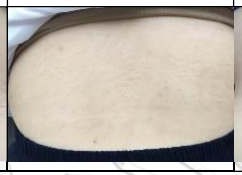
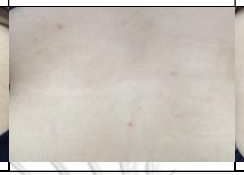
C051					
C052					
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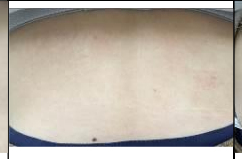
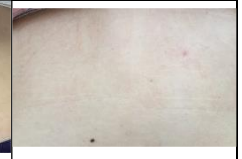
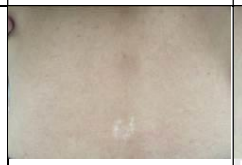
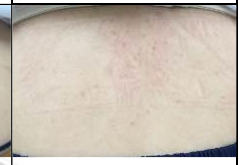
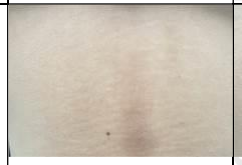
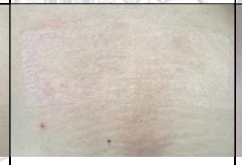
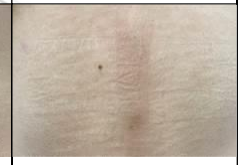
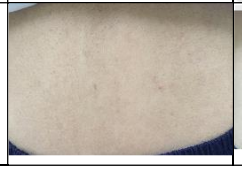
C056					
C057					
C058					
C059					
C060					

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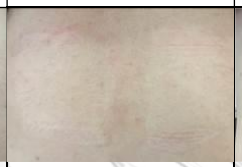
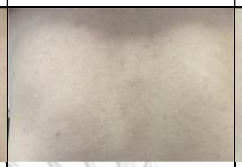
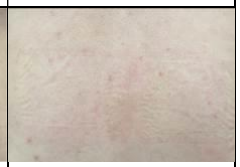
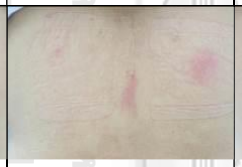
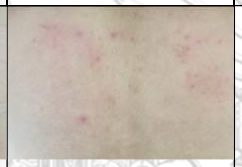
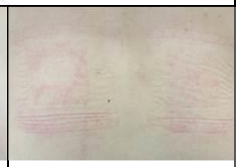
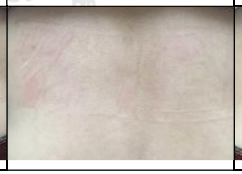
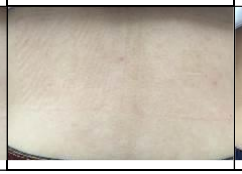
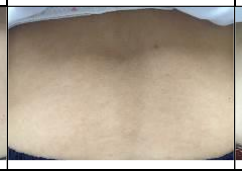
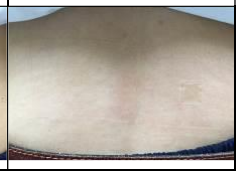
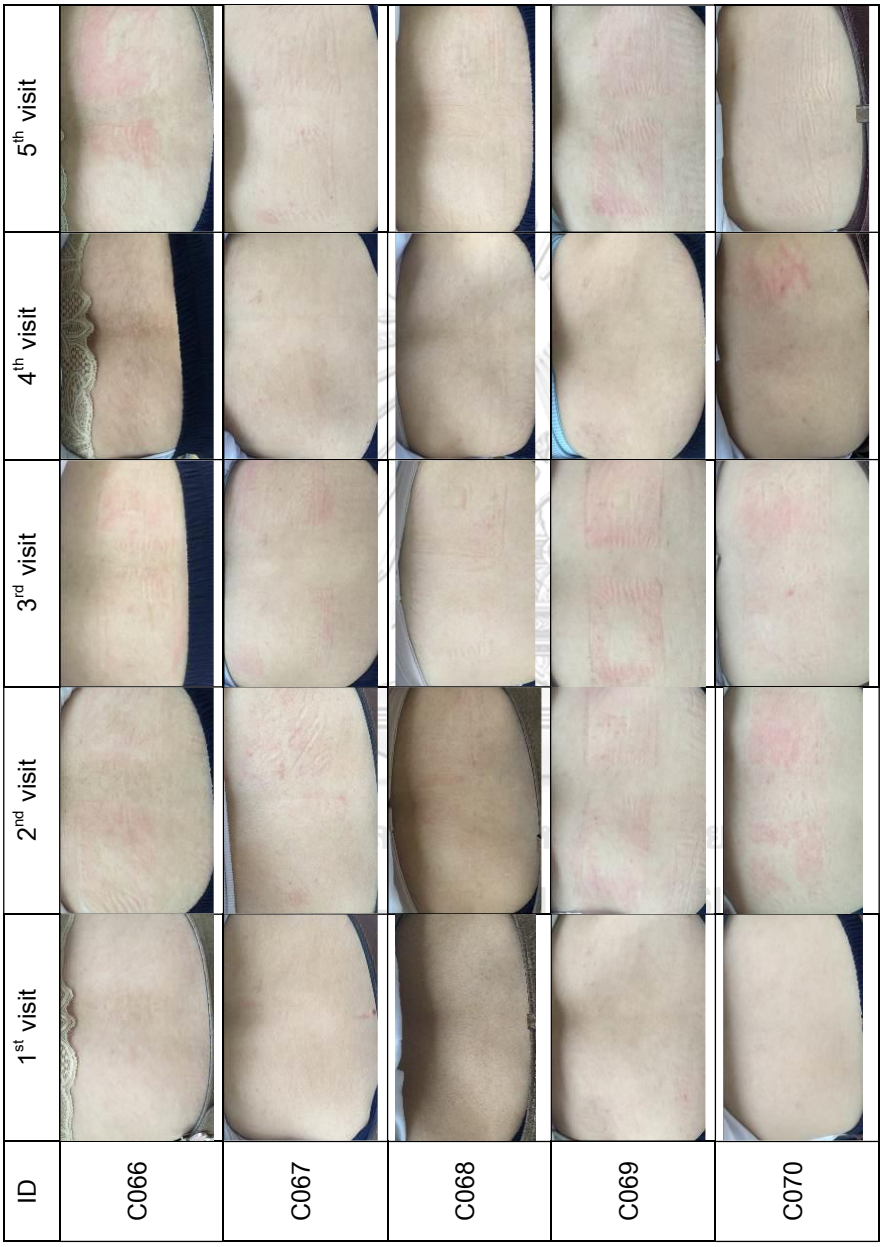
C062					
C063				Drop out	
C064					
C065					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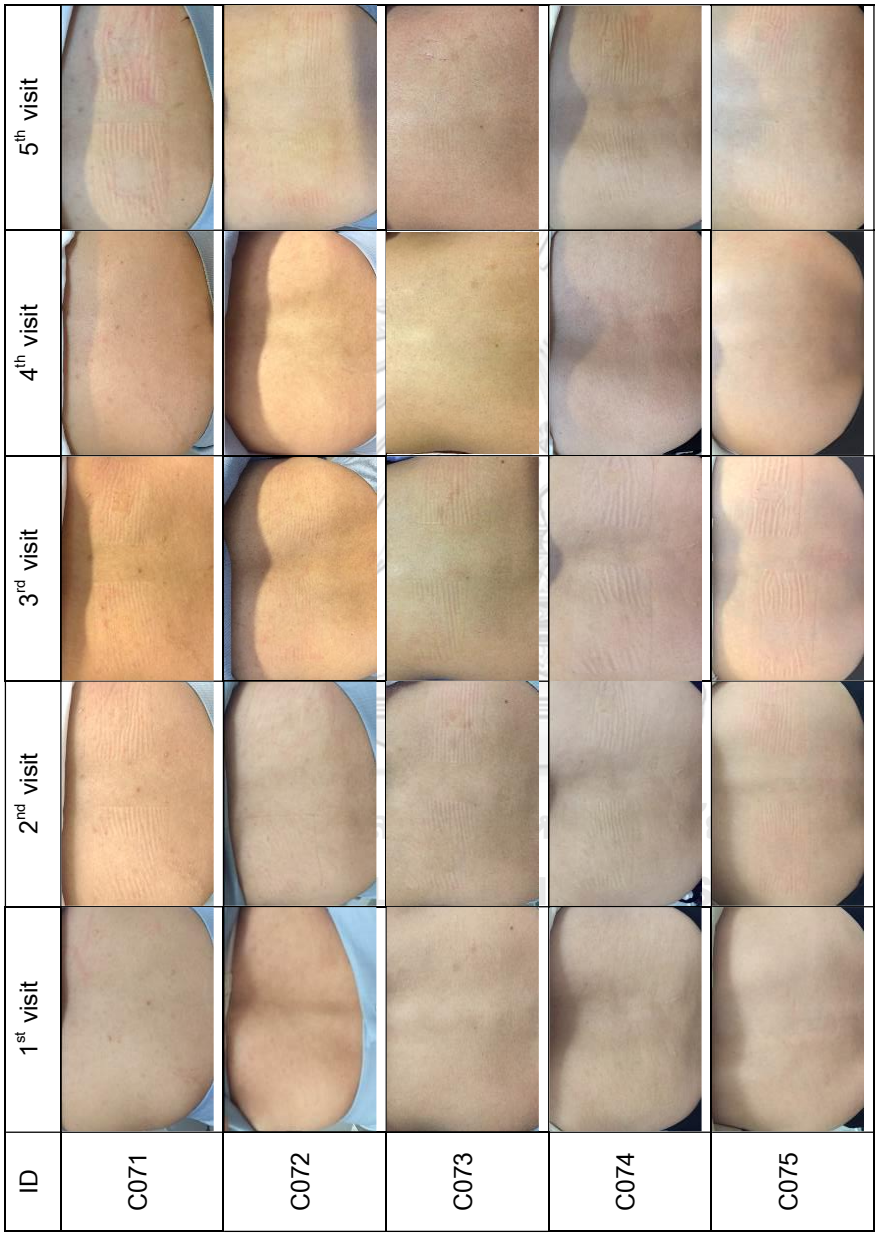
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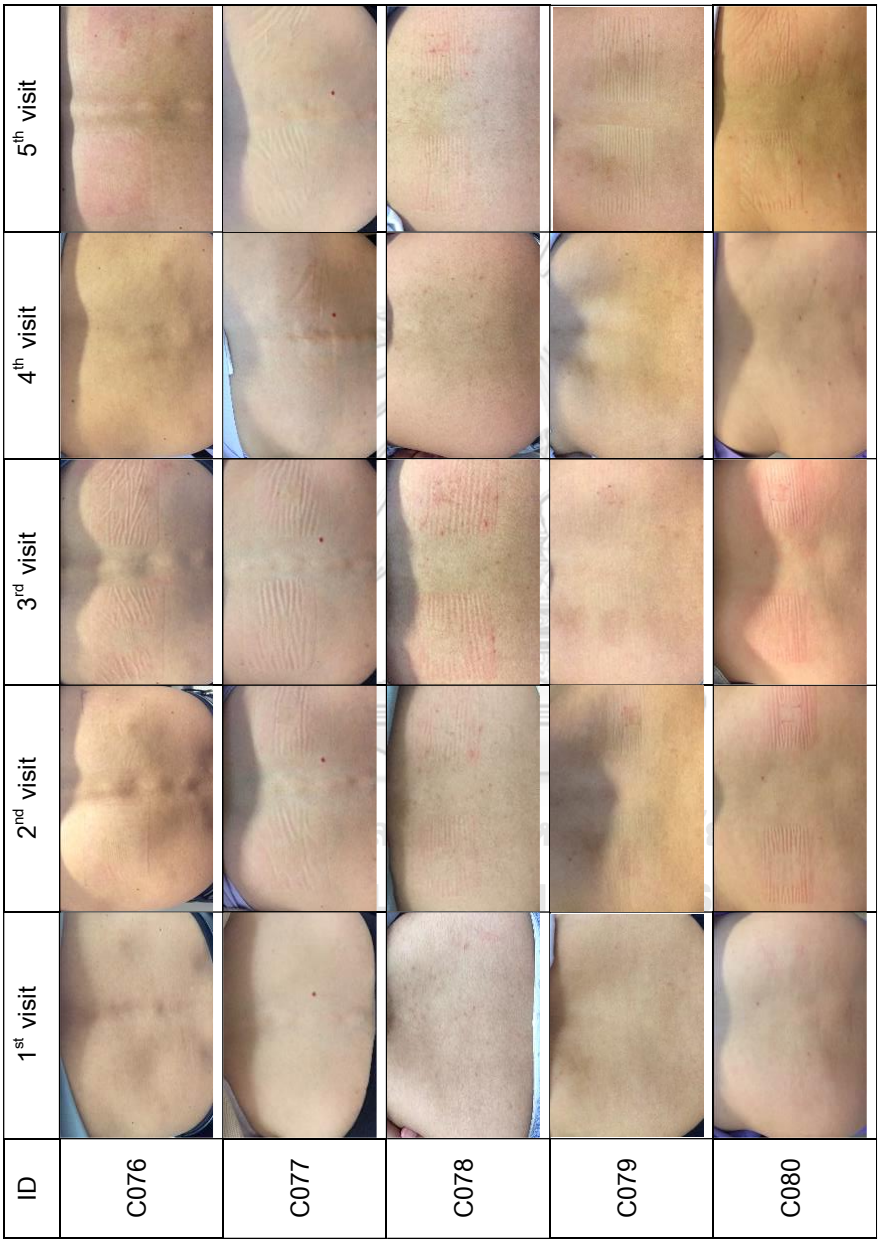
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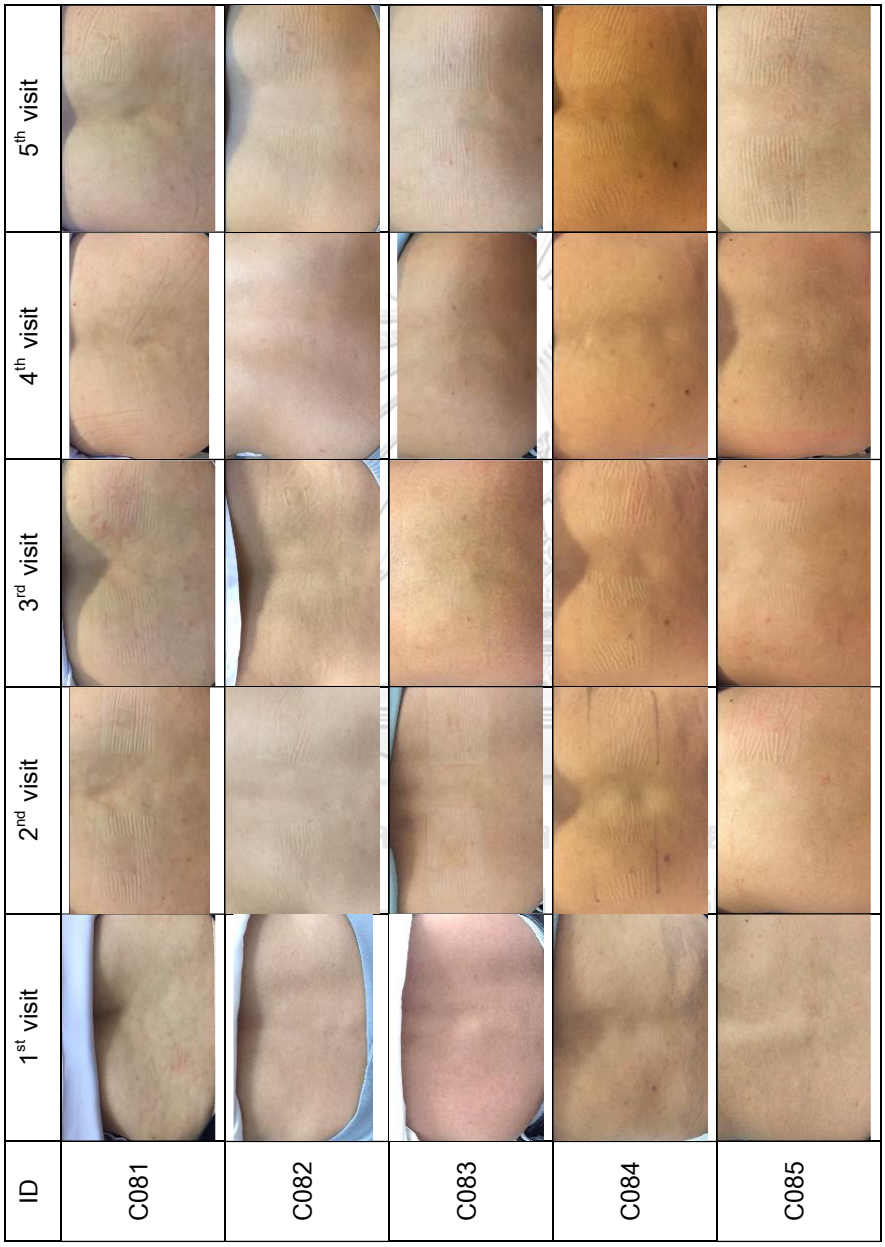
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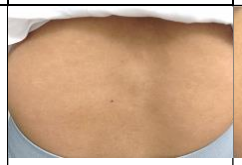
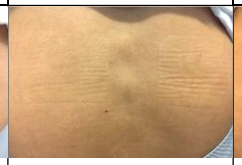
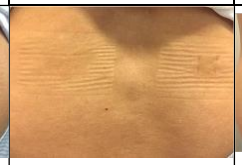
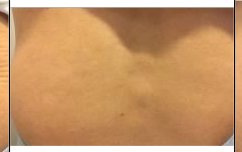
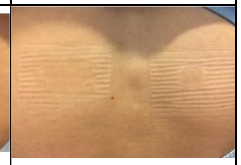
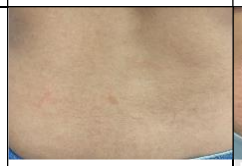
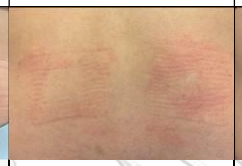
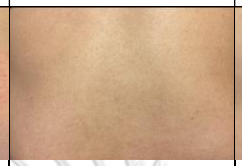
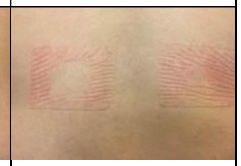
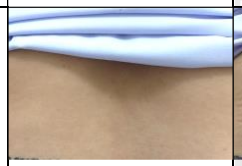
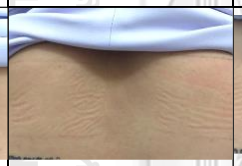
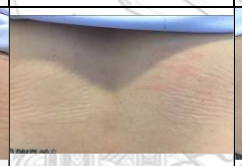
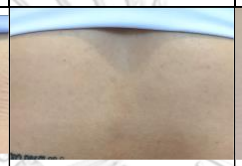
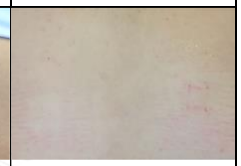
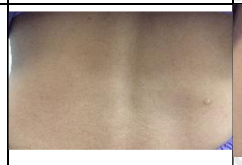
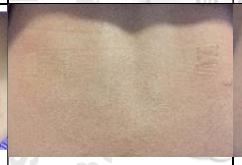
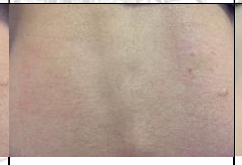
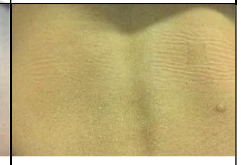
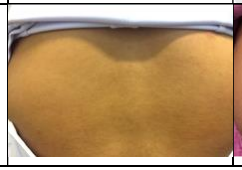
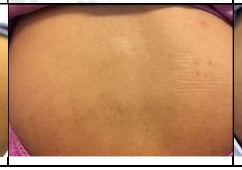
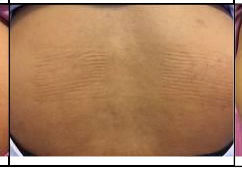
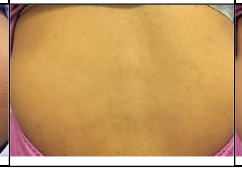
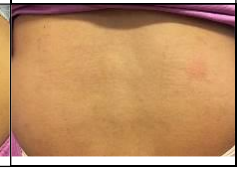
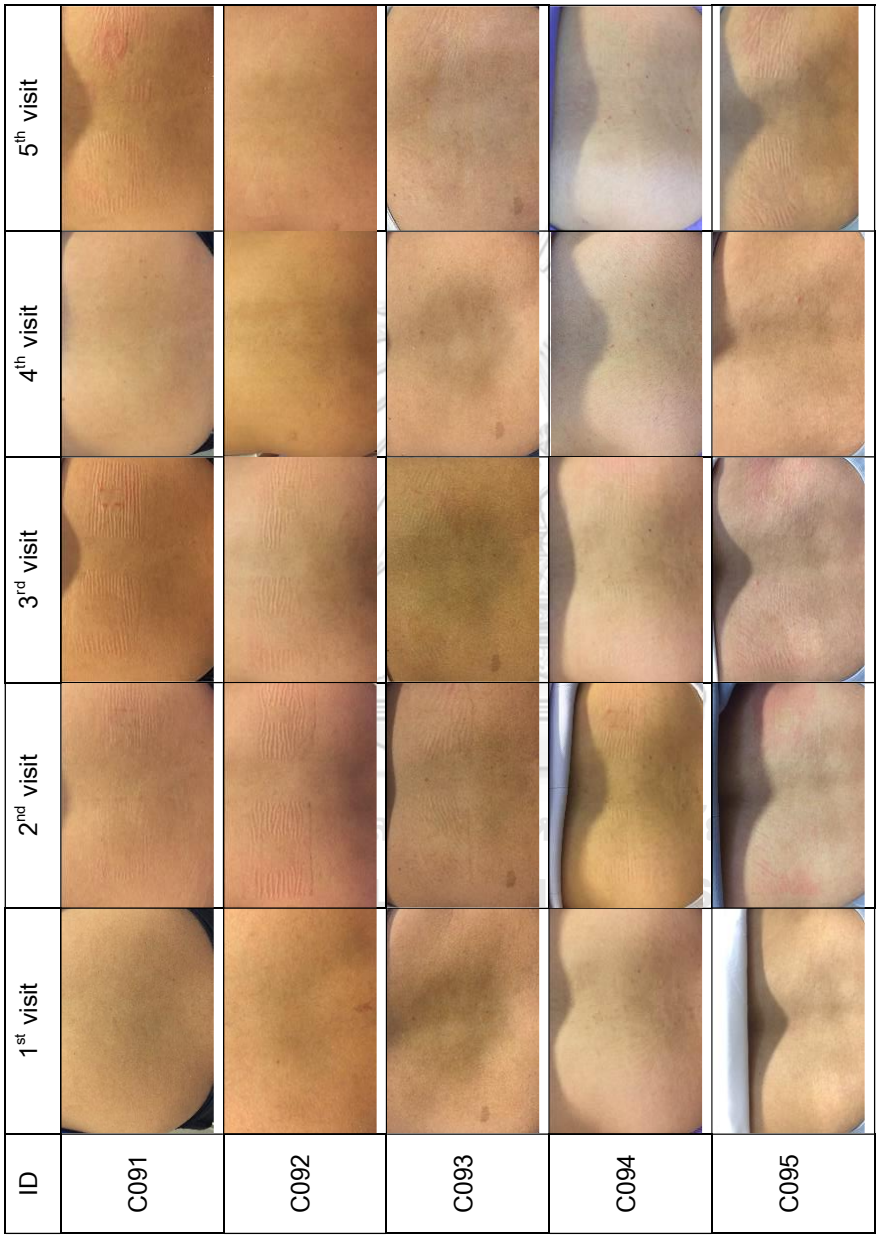
C086					
C087					
C088					
C089					
C090					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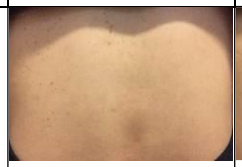
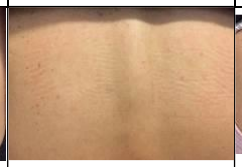
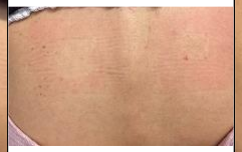
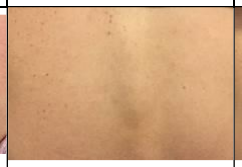
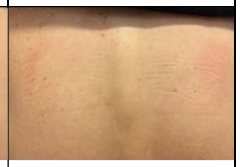
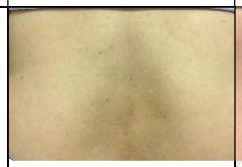
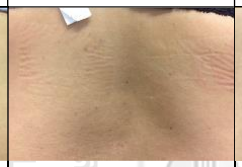
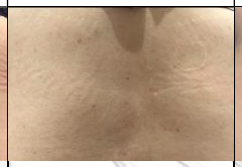
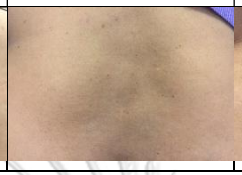
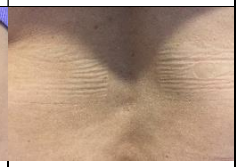
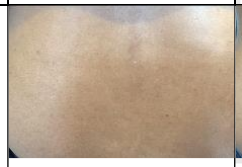
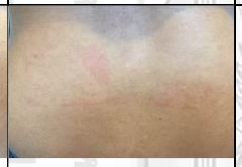
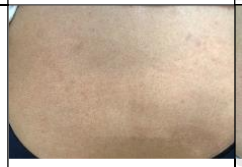
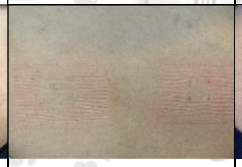
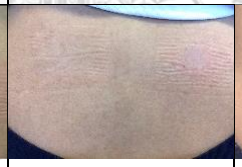
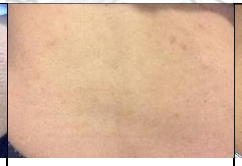
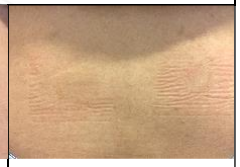
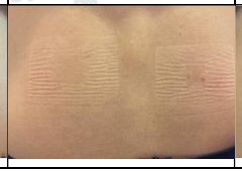
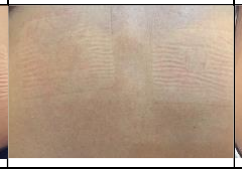
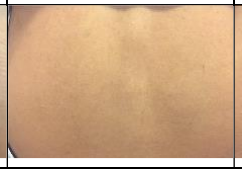
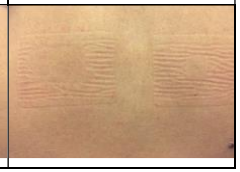
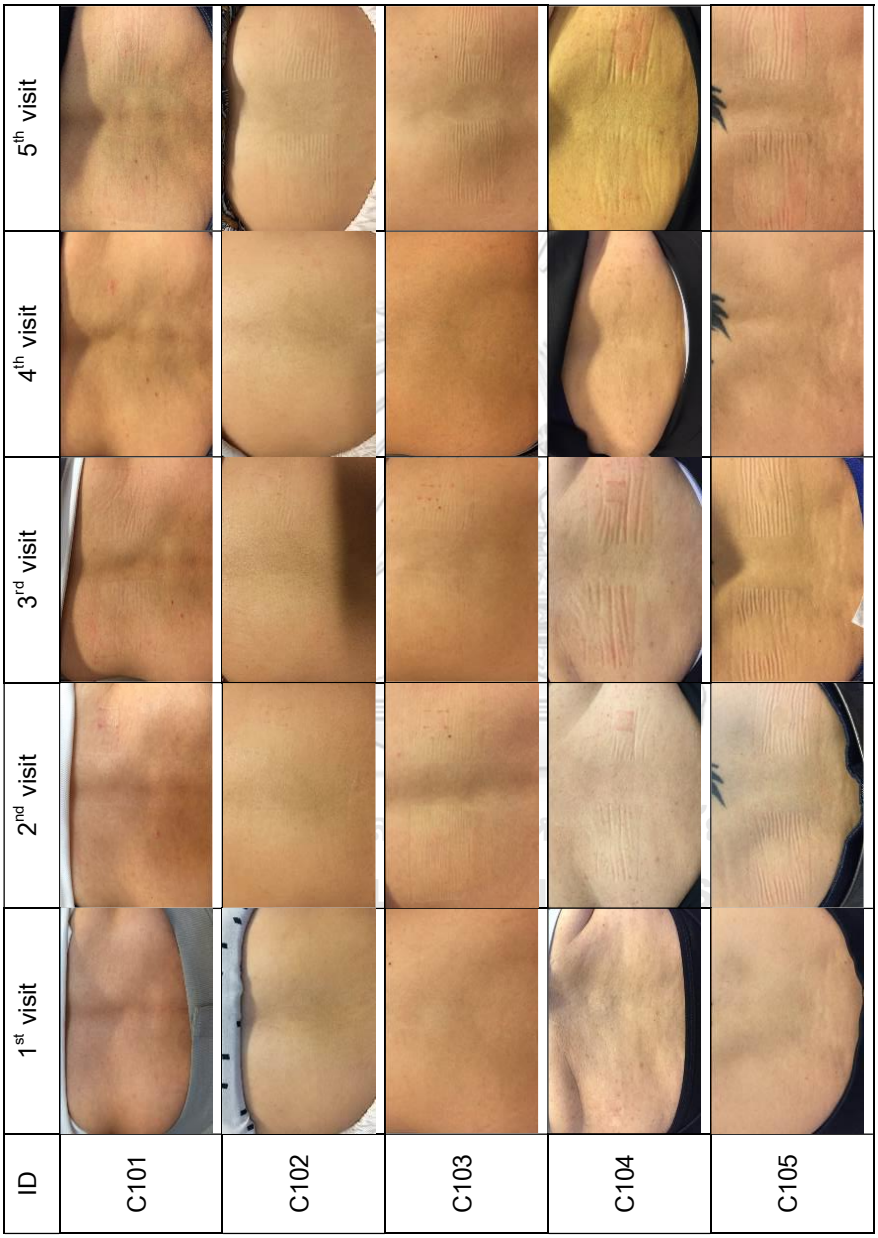
C096					
C097					
C098			Drop out		
C099					
C100					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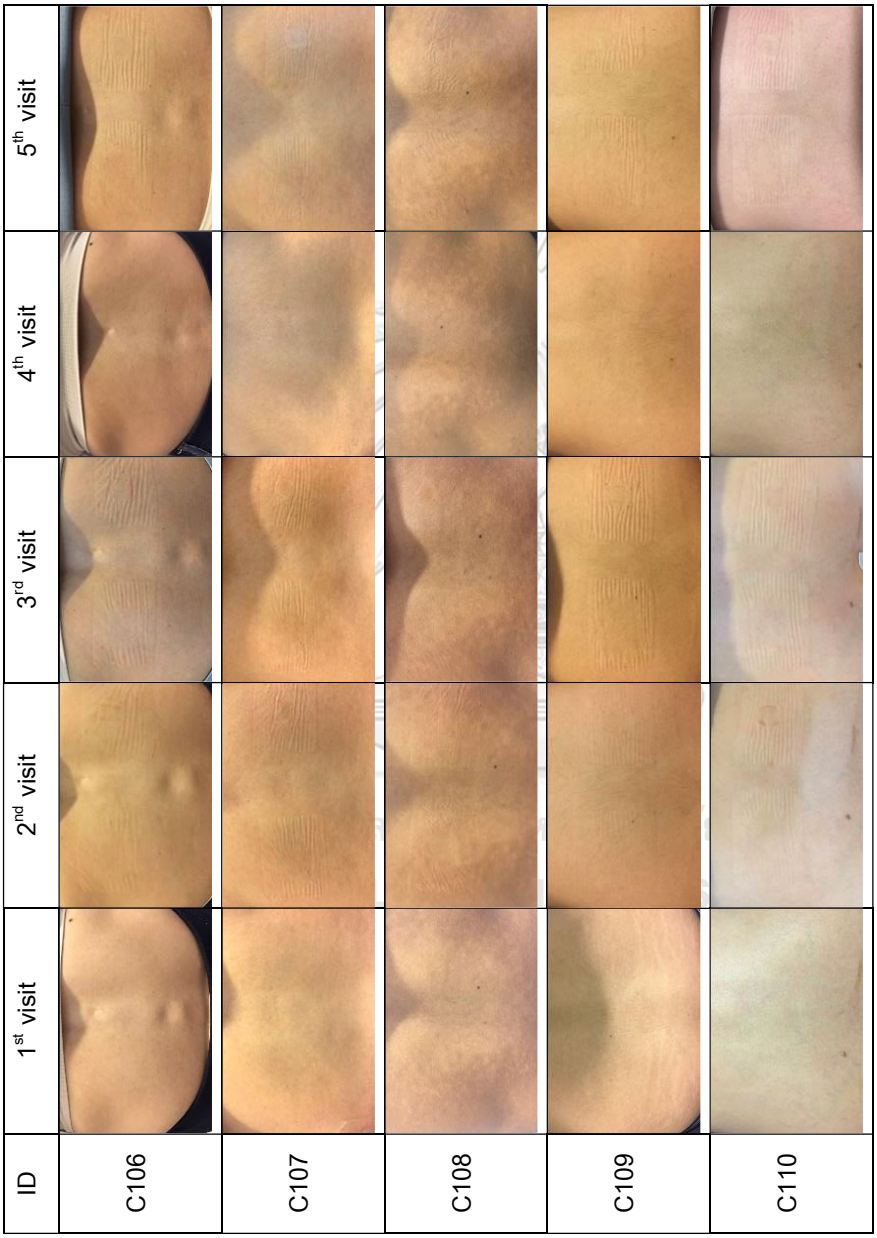
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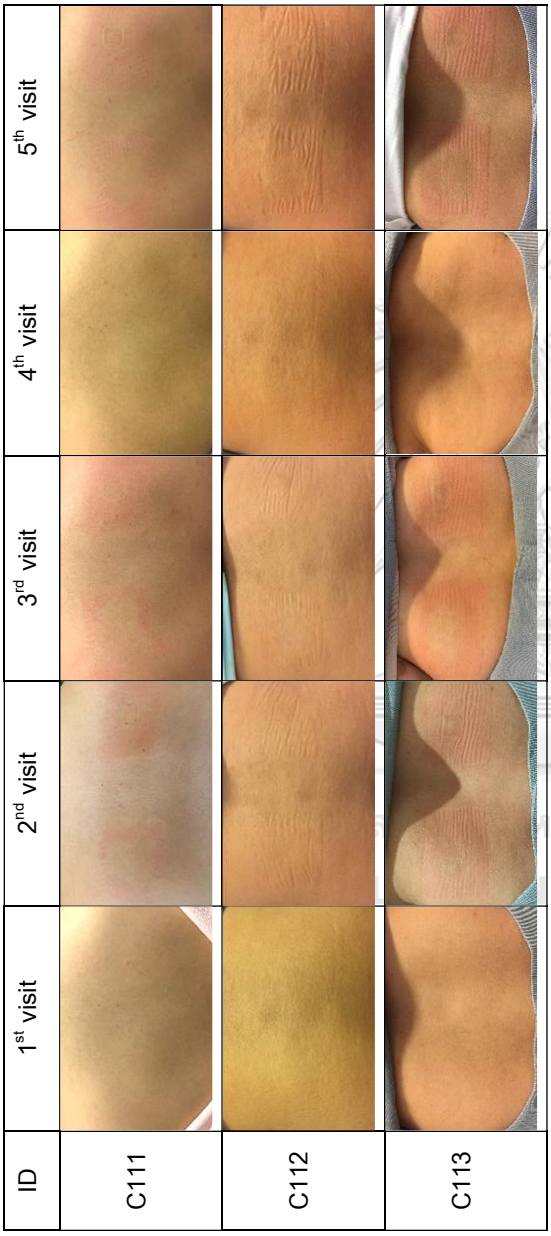
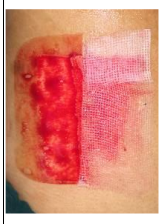
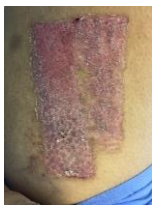
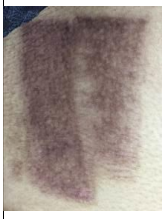
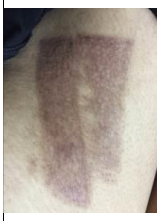
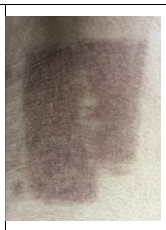
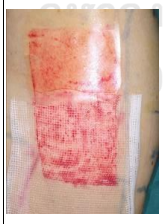
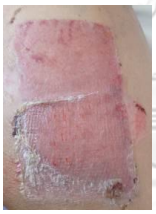
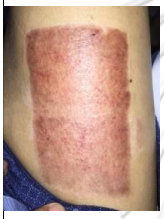
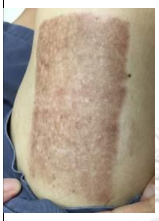
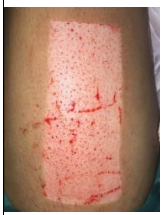
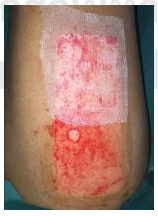
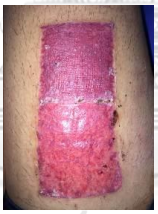
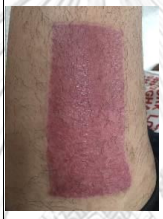
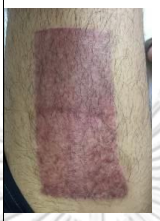
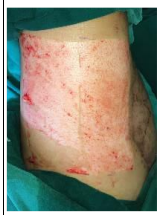
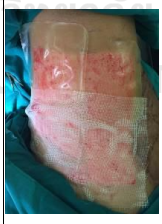
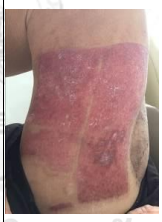
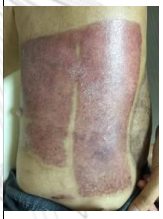
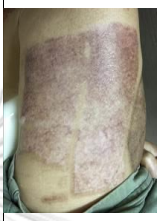
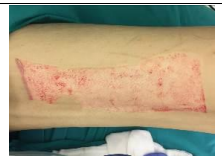
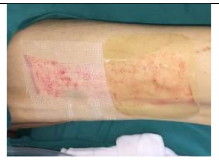
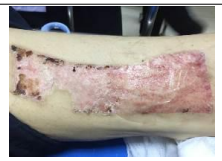
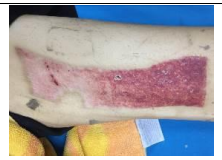
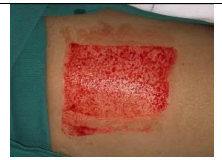
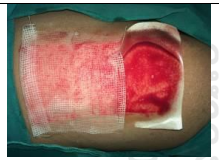
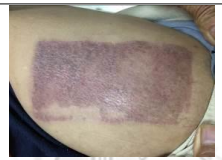
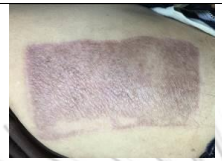
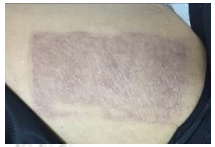
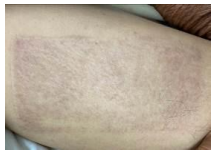
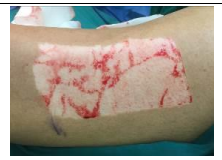
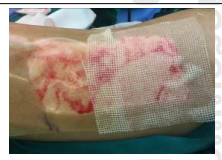
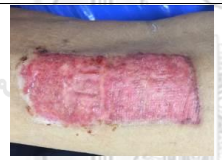
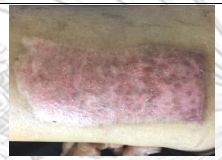
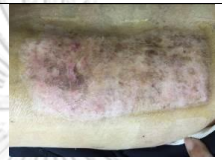
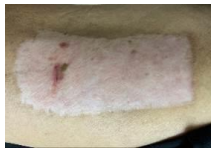
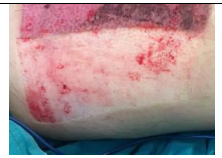
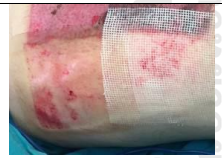
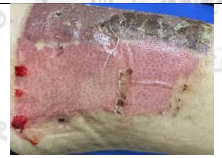
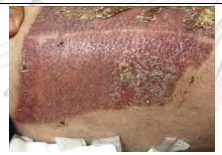
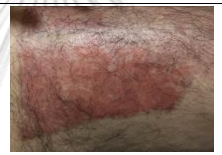
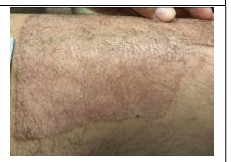
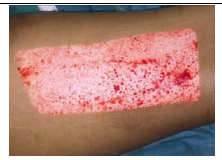
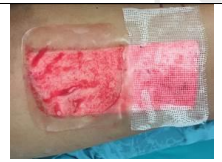
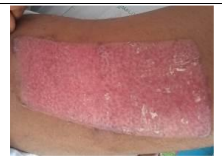
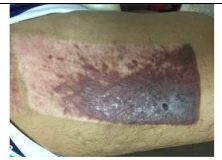
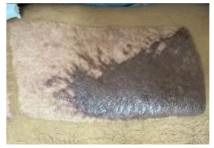


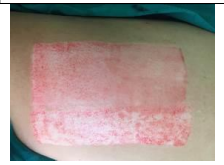
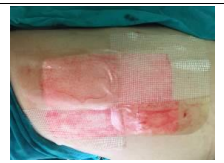
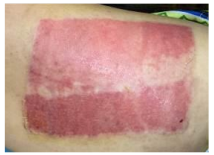
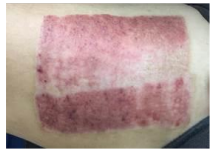
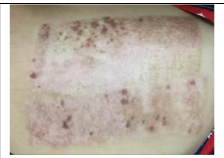
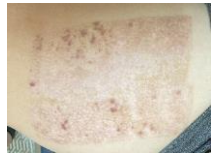
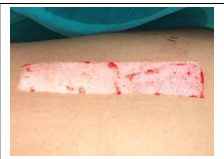
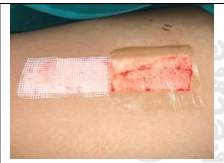
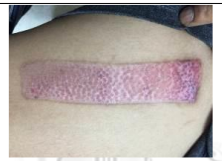
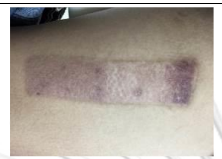
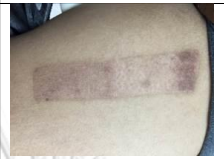
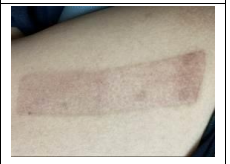
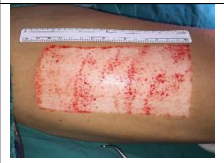
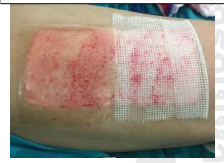
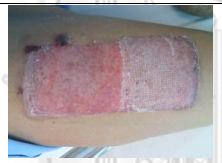
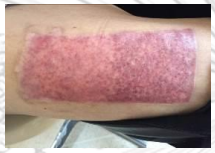
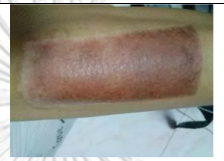
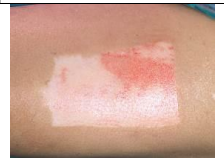
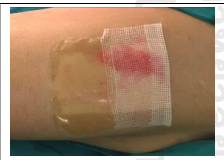
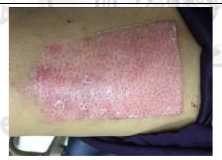
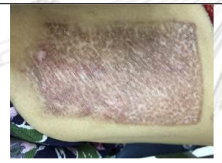
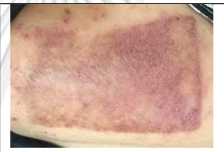
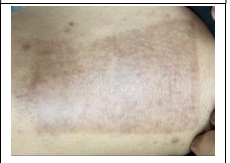
จุฬาลงกรณ์มหาวิทยาลัย
Chulalongkorn University

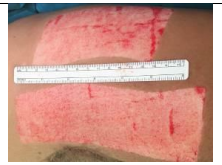
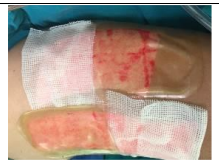
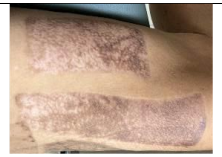
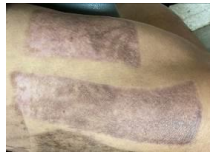
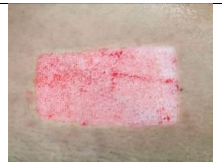
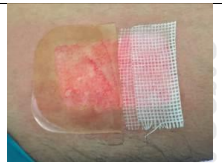
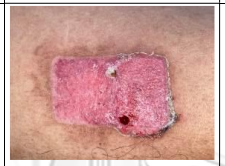
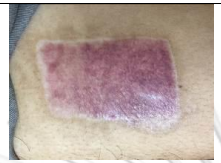
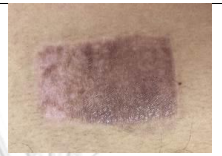
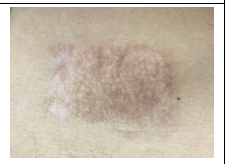
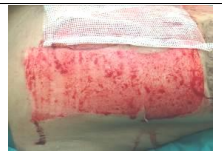
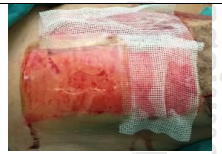
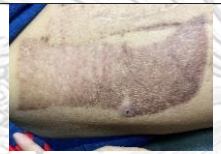
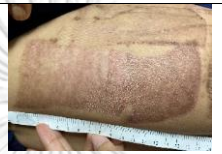
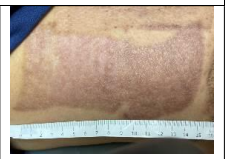
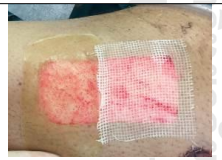
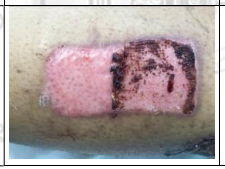
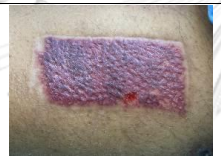
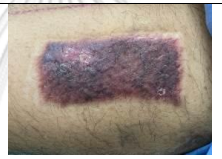
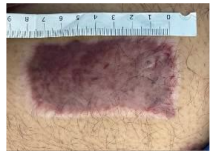
Appendix N

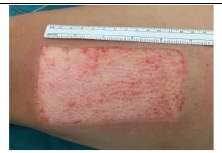
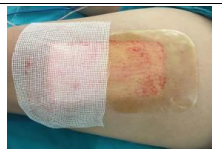
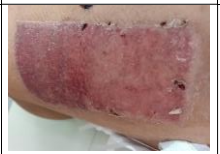
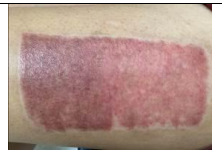
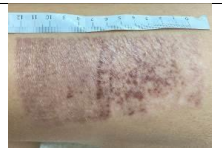
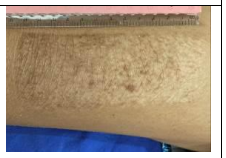
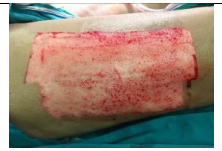
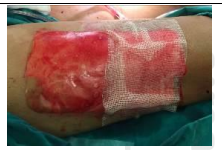
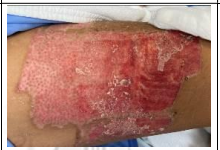
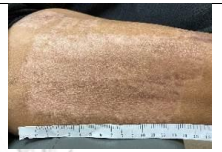
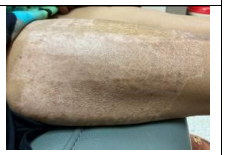
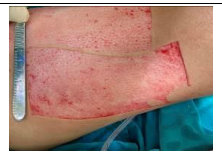
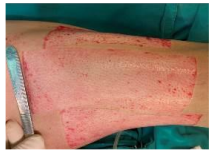
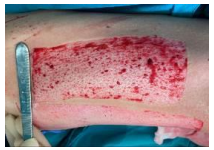
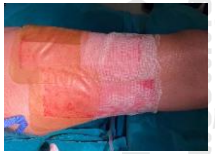
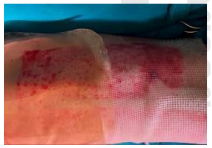
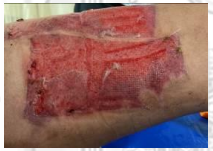
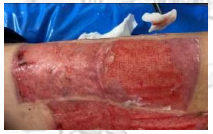
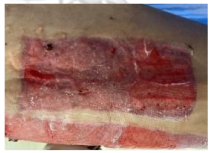
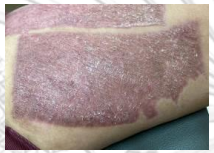
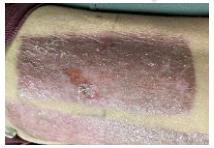
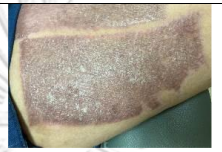
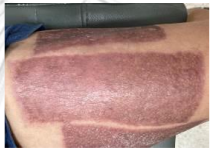
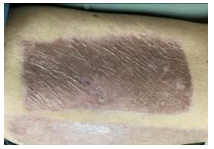
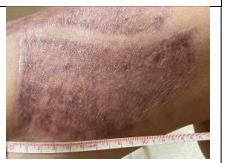
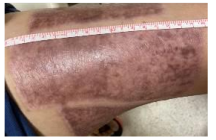
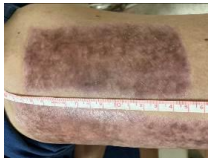
Pictures of STSG donor sites

Code	Operative day after harvesting STSG	Operative day after covering with dressings	Complete healing day	1 month after complete healing	3 months after complete healing	6 months after complete healing
P001	N/A					
P002	N/A					
P003						Loss to follow-up
P004, P005						Loss to follow-up

Code	Operative day after harvesting STSG	Operative day after covering with dressings	Complete healing day	1 month after complete healing	3 months after complete healing	6 months after complete healing
P006					Loss to follow-up	Loss to follow-up
P007						
P008						
P009						
P010						

Code	Operative day after harvesting STSG	Operative day after covering with dressings	Complete healing day	1 month after complete healing	3 months after complete healing	6 months after complete healing
P011, P012						
P013						
P014						Loss to follow-up
P015						

Code	On operative day after harvesting STSG	On operative day covering with dressings	Complete healing day	1 month after complete healing	3 months after complete healing	6 months after complete healing
P018, P019						
P020						
P021						
P022						

Code	Operative day after harvesting STSG	Operative day after covering with dressings	Complete healing day	1 month after complete healing	3 months after complete healing	6 months after complete healing
P024						
P026, P027						
P028, P029, P030	  	 	  	  	  	  

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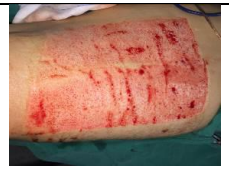
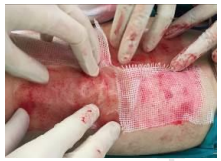
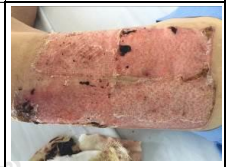
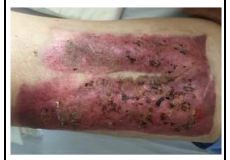
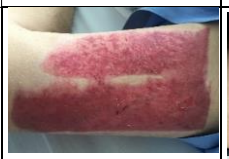
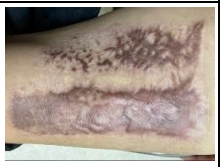
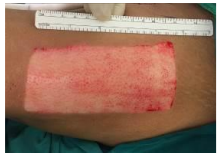
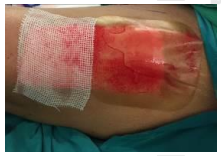
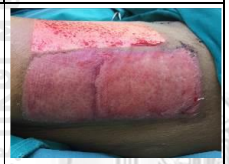
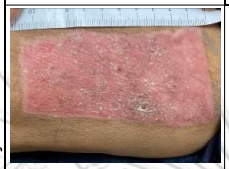
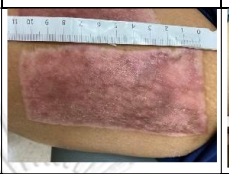
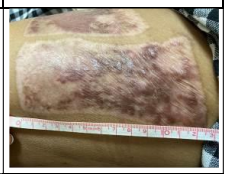
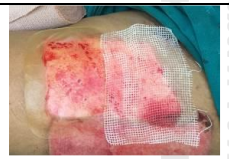
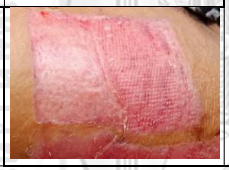
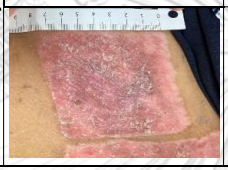
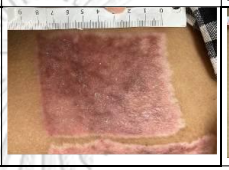
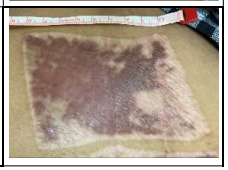
P016, P017							
P023							
P025							

Appendix O

Summary report of silk sericin dressing with collagen

Silk sericin dressing with collagen (SCD) in this study consists of polyvinyl alcohol (PVA), silk sericin, collagen hydrolysate, and glycerin. The fabrication used in this dressing is cast-drying method, which is the physical cross-linking technique without the addition of chemical crosslinking. The physicochemical, mechanical, and biological properties SCD dressing were evaluated and summarized in this report.

Experimental Section

Materials

PVA (degree of polymerization 1,700-1,900; %hydrolysis 98-99 mol%; M.W. approx. 1, 15,000) was purchased from Loba Chemie Pvt., Ltd. (Mumbai, India). *Bombyx mori* silk cocoons were kindly supplied by The Thai Silk Co., Ltd. (Nakhon Ratchasima, Thailand). Briefly, the silk sericin was extracted from small pieces of silk cocoons using a high temperature and pressure degumming technique as the previously reported (1). Analytical grade glycerin was used without further purification. Fish collagen hydrolysate (CH) and the commercial dressing, Bactigras® were purchased from Nippi, Incorporated (Tokyo, Japan) and Smith & Nephew, London, UK, respectively.

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Hydrogel Preparation

PVA powder was dissolved in deionized (DI) water at 80°C for 4 hours. Silk sericin extract was concentrated by heating until obtaining the required concentration (measured by BCA Protein Assay Reagent, Pierce, Rockford, IL, USA). CH was dissolved in DI until a clear solution was formed. The ingredients were mixed according to the formulations to obtain the final concentration of 10%w/w PVA, 3%w/w sericin, 5%w/w CH, and 1%w/w glycerin for SDC (10P3S5C1G), and 10%w/w PVA and 1%w/w glycerin for 10P1G. Then, the mixtures were homogenized and stirred until the homogeneous mixture was obtained. The mixture was poured into a Teflon plate and left at controlled temperature (15-20°C) and humidity (70-75%RH) until the stable weight was obtained. Cast-dried hydrogel samples were peeled off and sterilized by gamma radiation (25 kGy) by the Thailand Institute of Nuclear Technology (Nakornnayok, Thailand).

Measurement of Physicochemical Properties

Fourier-Transform Infrared (FT-IR) Spectroscopy

The sample was freeze-dried to remove water before examining the functional groups and their changes through Fourier transform infrared (FT-IR) spectroscopy (PerkinElmer, Waltham, MA, USA) analysis. All samples were scanned in the wavenumber ranging from 4000 to 400 cm^{-1}.

Determination of non-crosslinking fraction and water content

The samples (1x1 cm^2) were dried in an oven at 50°C for 24 hours before determining their initial weight (W_0). Each sample was submerged in deionized water (DI) at room temperature for 24 hours. The swollen weight (W_s) was taken from samples before placing them in an oven at 50°C for 24 hours. The remaining weight of the sample (W_t) after drying was collected. The concentration of released protein in DI water was measured by a BCA protein assay kit and calculated by comparing it with a standard curve prepared from bovine serum albumin (BSA). The percentage of non-crosslinked protein ($NC_{Protein}$) was calculated from the protein release compared with the initial dry weight. Non-crosslinked PVA/glycerin ($NC_{PVA/Gly}$) and water content of samples were calculated using equations 1 and 2:

$$NC_{\mathrm{PVA/Gly}}\ (\%) = 100 - \frac{W_t}{W_0} \times 100\% - NC_{\mathrm{Protein}}\ (\%) \quad (1)$$

$$\text{Water content } (\%) = \frac{W_s - W_t}{W_s} \times 100\% \quad (2)$$

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Protein Release Profiles

The samples (1x1 cm^2) were dried in an oven 50°C for 24 hours and their initial weight (W_0) was determined before immersing them into phosphate buffer saline (5 ml), PBS (pH = 7.4) in a tight container and placing them in a shaking incubator (100 r/min) at 37°C. All PBS samples (5 ml) were taken out for protein assay at 0.5, 2, 4, 6, 8, 12, 16, 24, 48, 72, 120, and 168 hours before substituting with fresh PBS (5 ml). The released protein concentration was measured using a BCA protein assay kit and calculated using a standard curve prepared from bovine serum albumin (BSA). The percentage of accumulative released protein was calculated by comparing it with the initial weight of samples.

Measurements of Mechanical Properties

Samples were fully hydrated and kept in a sufficient amount of deionized water ?? overnight before being cut into a dumbbell shape and measured for thickness and width. Tensile strength, %elongation, and Young's modulus were determined using a Universal Testing Machine (SHIMADZU EZ-S, Japan) equipped with a 500 N load cell at a 50 mm/min constant rate at room temperature. The elastic modulus (Young's modulus) was calculated in the linear stress and strain portion between 0-10 % strain.

Measurements of Biological Properties

Sterile samples (1x1 cm^2) were extracted in 5 ml of Dulbecco's modified Eagle medium (DMEM) for 8 hours at 37°C. L929 mouse fibroblast cells (Chinese Academy of Preventive Medical Sciences, Beijing, China) were cultured in DMEM containing 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% antibiotic under 5 %CO_2 at 37°C. The media were changed every two days. The cells were collected by adding phosphate buffer (pH 7.4), 0.25% trypsin, and 0.02% EDTA before being cultured under 5 %CO_2 at 37°C for 5 minutes.

For the cytotoxicity test, 1x10^4 cells/ 100 µl L929 were seeded in a 96-well plate and allowed to attach for 24 hours. The media were removed before replacing with the extracts of samples. The extract of Bactigras® and DMEM were used as the control. After incubating for 24 hours, the cell viability (%) was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay before comparing with DMEM.

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For the proliferation test, 1x10^4 cells/ 100 µl L929 were seeded in a 48-well plate for 24 hours before replacing the media with the extracts from the samples. DMEM was used as a control. The cells were continually cultured for 24 and 48 hours. Then, an MTT assay was used to determine the increase of the number of cells.

For the scratch test (migration test), L929 (1x10^5 cells/ 100 µl) were seeded in a 24-well plate for up to 48 hours until a confluent monolayer was formed. A linear scratch was created by using a sterile pipette tip. The cellular debris was removed by washing with PBS. After that, the extracts of samples were added to the cells. DMEM was used as a control. The photograph of each well was taken in 3 positions (top, middle, and bottom) on day 0, day 1 (24 hours), and day 2 (48

hours) using an optical microscope (10X, Olympus CK2, Japan) before incubating at 37°C under a humidified atmosphere of 5% CO_2. ImageJ 1.42q/Java 1.6.0.10 was used for determining the remaining area uncovered by the cells. The remaining area (%) were calculated following equation 3:

$$\%\ Remaining\ area = \frac{A_0 - A_t}{A_0} \times 100\% \quad (3)$$

where A_0 and A_t represent the average remaining area at day 0 and day t, respectively

Statistical Analysis

The data were analyzed using SPSS version 17.0 (SPSS Co., Ltd., Bangkok, Thailand). All experiments were done in triplicate unless otherwise specified. Independent t-test or Analysis of variance was used to compare groups, and post hoc analysis were Bonferroni or Dunnett's T3.

Results

Physiochemical Properties

FTIR Spectra

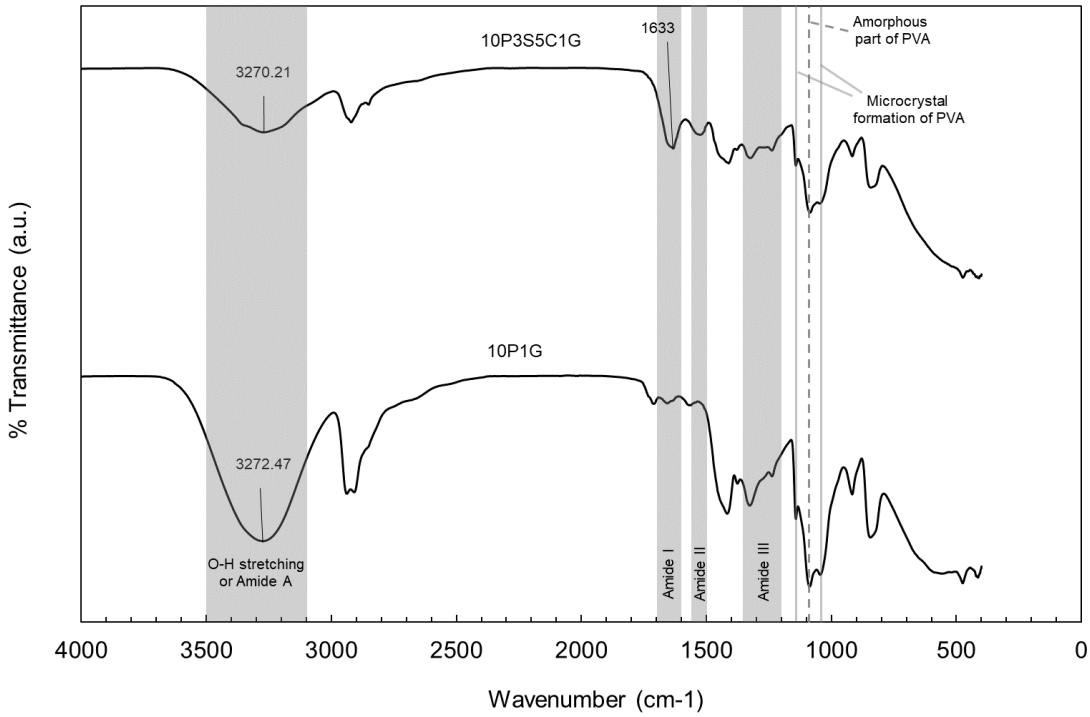

Fig. 1 FTIR spectra of samples

The FTIR spectra of the pure PVA and blended hydrogel samples are displayed in Figure 1. The absorption peaks with different intensities at 3200-3500 cm^{-1} (O-H stretching or Amide A), 1086-1088 cm^{-1} (C=O stretching and O-H bending), and both 1142 cm^{-1} and 1050 cm^{-1} (C-O stretching) were found in all samples. The slight shifts of O-H stretching or amide A peak were found in the sample containing protein, compared to 10P1G. The absorption of amide I (1700-1600 cm^{-1}), amide II (1500-1560 cm^{-1}), and amide III (1200-1350 cm^{-1}) were found in 10P3S5C1G. The location of amide I peaks in 10P3S5C1G (1633 cm^{-1}) suggests the secondary structure of the protein.

Non-Crosslinked Fraction and Water Content

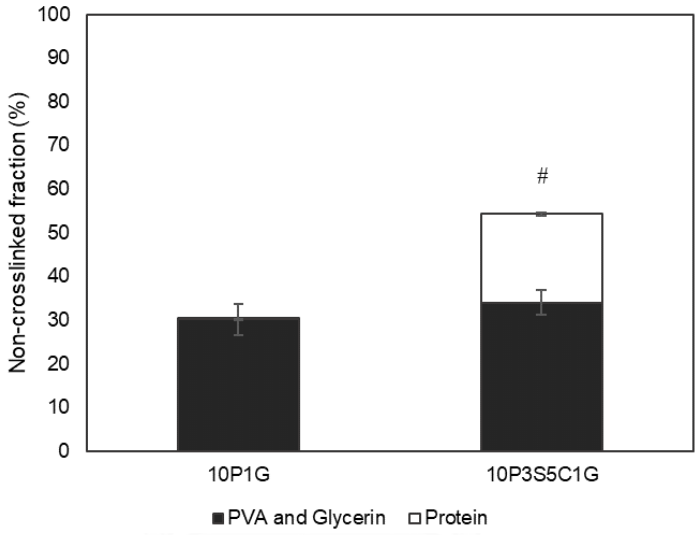

Fig. 2 Non-crosslinked fraction of samples

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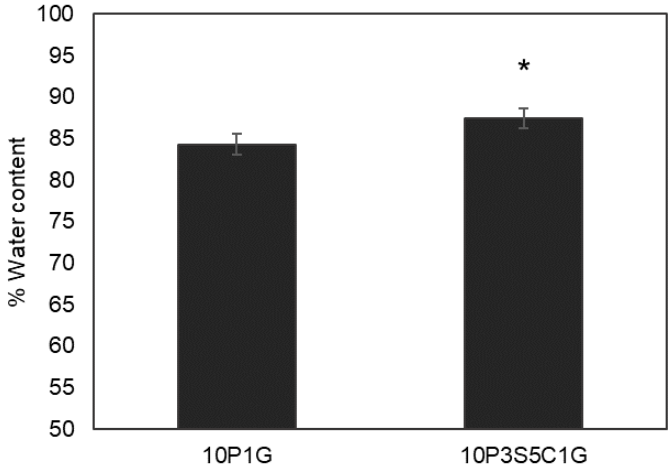

Fig. 3 The water content of the samples

Figure 2 shows the non-crosslinked fraction of the hydrogels. There was no significant difference in non-crosslinked PVA between 10P3S5C1G and 10P1G, while the non-crosslinked protein also significantly increased in 10P3S5C1G, compared with 10P1G. Moreover, the water content of 10P3S5C1G was significantly higher than 10P1G, Figure 3.

Protein Release Profile

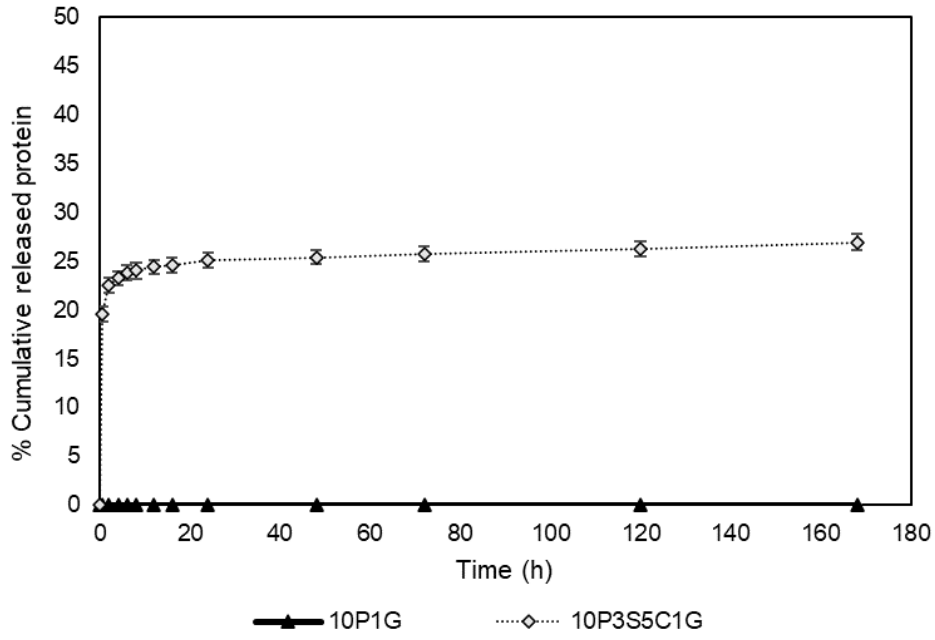

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Fig. 4 Protein release profile for the pure PVA and blend hydrogels

The protein release profile was presented as the percentages of accumulative released protein (Figure 4). The burst releases of protein from 10P3S5C1G was found in the first 12 hours, and then the release became slow after 12 hours, continued showing the sustained release of protein until 168 hours. At 168 hours, the accumulative released protein of 10P3S5C1G was around 27% of total initial weight.

Mechanical Properties

The mechanical properties of the various hydrogel samples are presented in Figure 5. The tensile strength of 10P3S5C1G were significantly lower than 10P1G, and there was no significant

difference in the percentage of elongation between samples. The statistical analysis showed no significant differences in the Young's modulus among samples.

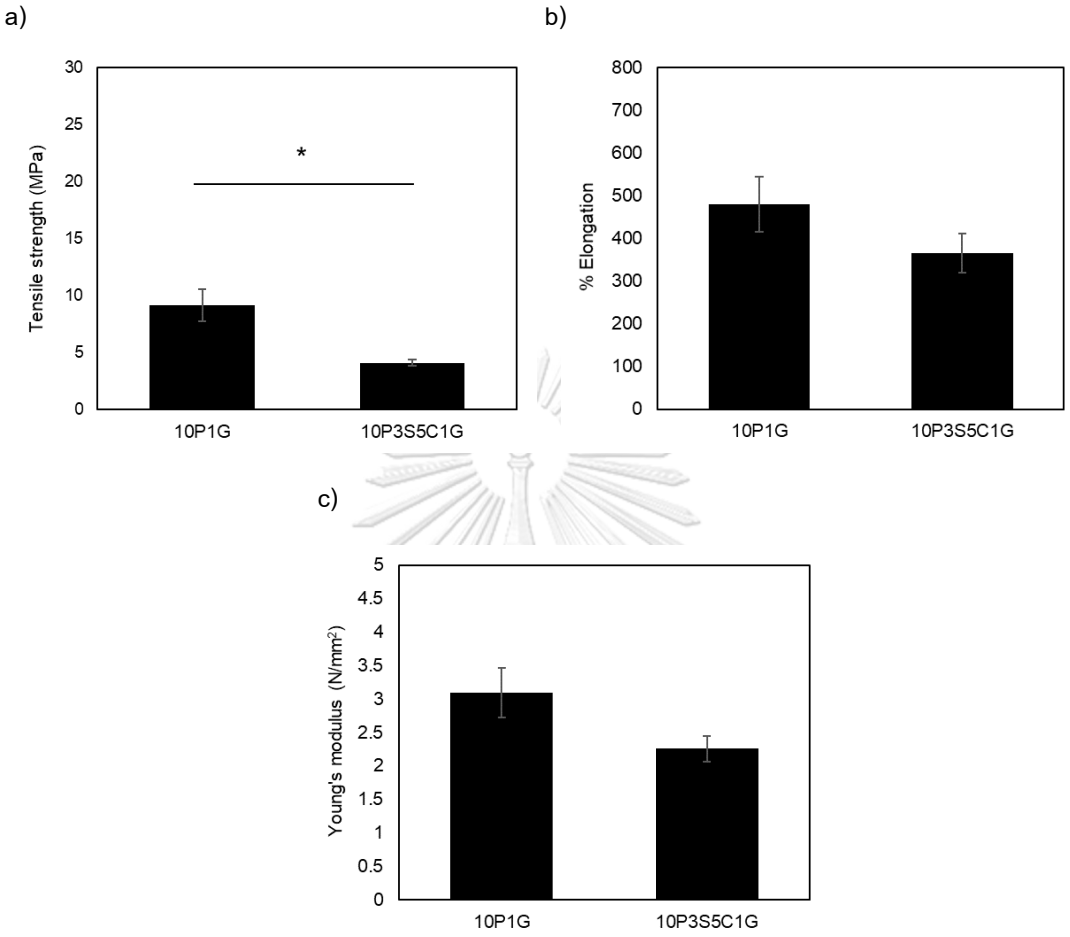

Fig. 5 Mechanical properties a) Tensile strength, b) %Elongation, and c) Elasticity

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Biological Properties

Figure 6 shows the results from cytotoxicity. Compared to DMEM, there was no significant difference in %cell viability after exposing 10P3S5C1G. In contrast, %cell viabilities in the extract of 10P1G and Bactigras® were significantly lower than %cell viability in DMEM. The extract of Bactigras® showed cytotoxic effects (%cell viability <30%); therefore, the extract from Bactigras® was not used in the proliferation and scratch test.

In the proliferation test, all groups significantly increased the number of cells between time points. Compared to DMEM, there was no significant difference in the number of cells between 10P3S5C1G and DMEM at both time points, Figure 7.

In the migration test, the remaining area of each group significantly decreased compared to its previous time point, and most of the wound areas were closed after 48 hours (<3% of the remaining area). There was no significant difference between 10P3S5C1G and DMEM at each time point (Figure 8).

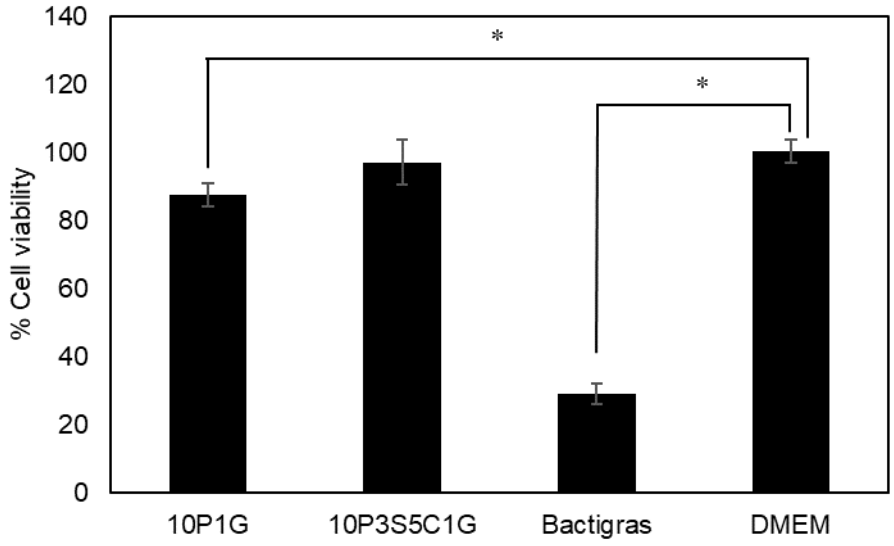


Fig. 6 Percentages of cell viability at 24 h

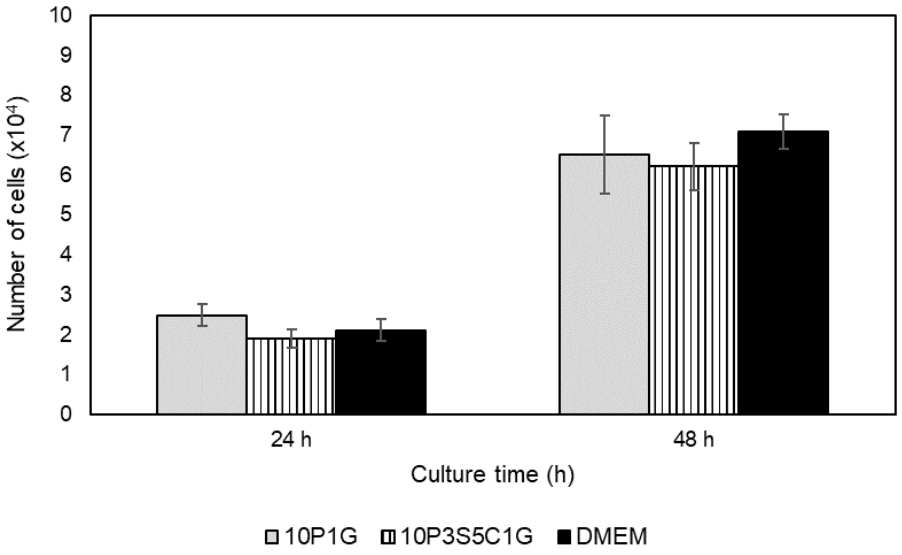
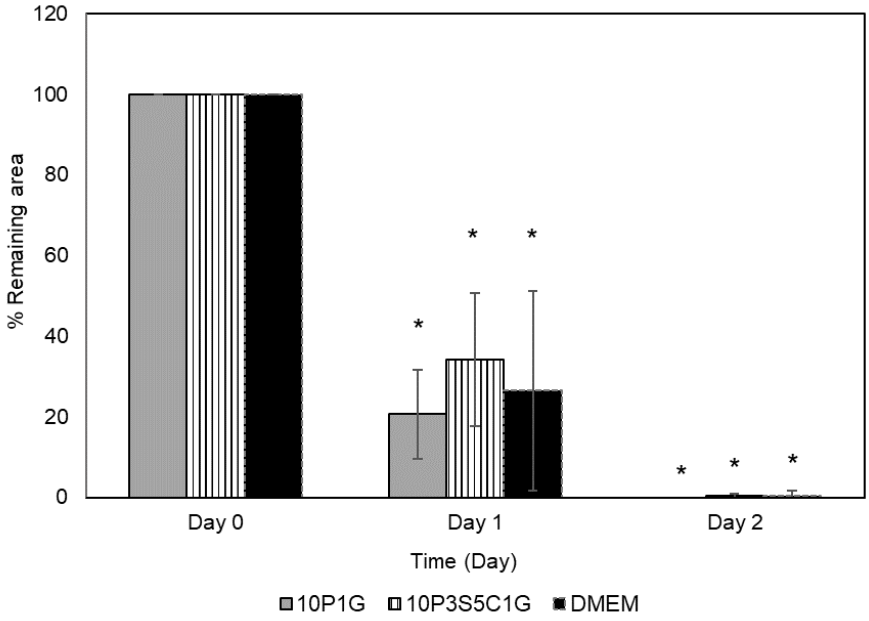


Fig. 7 The number of cells in each extract at 24 and 48 hours (Proliferation test)

a)



b)

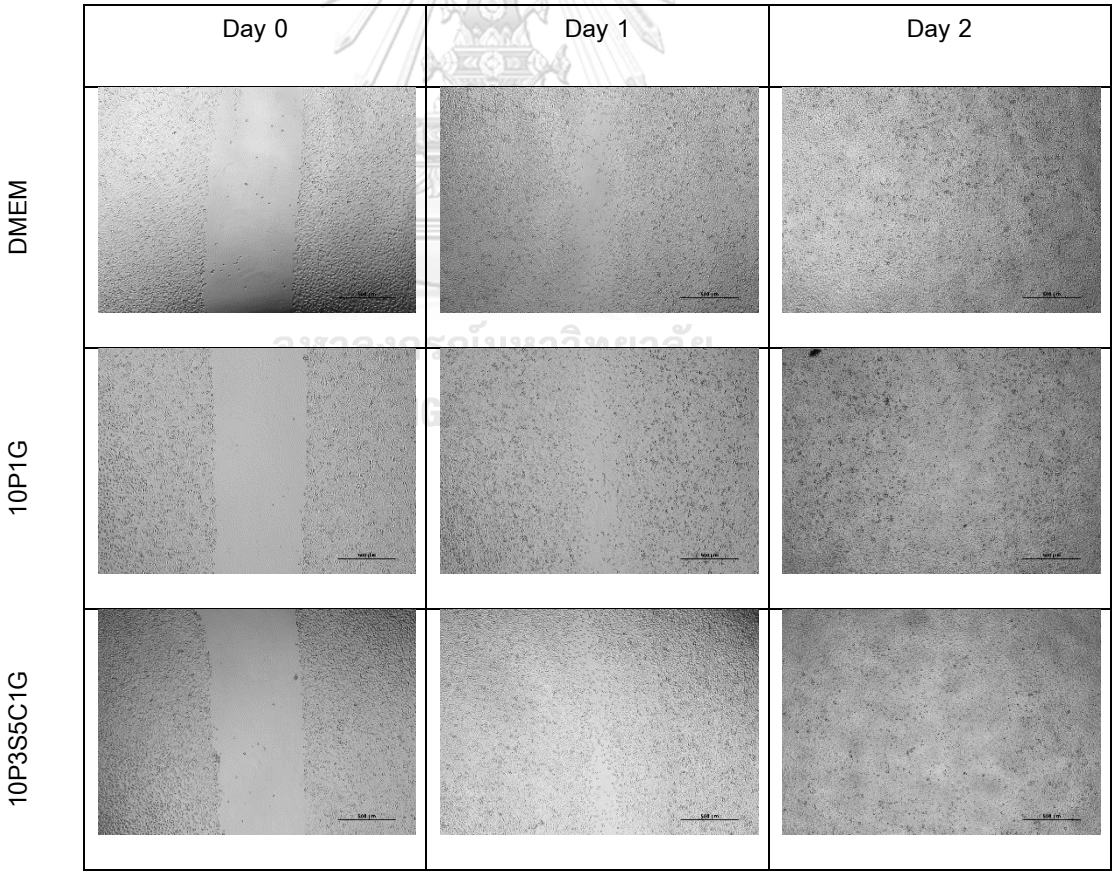


Fig. 8 a) Remaining area of each group b) The migration of L929 cells in the scratch test

* Significant differences at $p < 0.05$ compared to its previous time point

Discussions

In the present study, physically crosslinked PVA hydrogels containing bioactive proteins have been successfully obtained through the cast drying method without the need for additional crosslinking agents. Transparent hydrogels are formed due to the uniform distribution of microcrystallites and the network structure of PVA (2). The transparent hydrogels assist in inspecting the wounds, without the removal of dressing (3), which was not possible with the hydrogels prepared using other biopolymers/processes.

The strong absorption at 3200-3500 cm^{-1} found in 10P1G indicates hydrogen bond formation between PVA and sericin/collagen. The slight shift of peaks at lower intensities at 3200-3500 cm^{-1} in 10P3S5C1G are due to the N-H stretching of Amide A. The amorphous part of PVA (C=O stretching and O-H bending at 1086-1088 cm^{-1}) (4) and microcrystal formation of PVA (C-O stretching at 1142 and 1050 cm^{-1})(2) could be seen in the 10P1G spectrum, while the absorption intensities seem to decrease in 10P3S5C1G spectrum. The absorption bands of amide I, II, and III were found in 10P3S5C1G spectrum, suggesting the existence of proteins in the hydrogels. Location of amide I could imply that the proteins exist in their secondary because of different C=O stretching frequencies (5). The amide I peak of 10P3S5C1G occurred at 1633 cm^{-1} suggesting the beta-sheet structure, which is the stable structure of sericin (5-7).

The addition of both sericin and CH seems to preserve the crosslinking of the hydrogel. The small molecular weight and high solubility of CH possibly increases the solubility of the PVA hydrogel (8, 9). In contrast, sericin has a higher molecular weight and longer molecular chain than CH. Therefore, sericin may interact with both PVA and CH, decreasing the interfering effect of CH on PVA crosslinking. After releasing some of the non-crosslinked fraction, the hydrogels containing protein could hold a higher amount of water than 10P1G. The prepared hydrogels showed the ability to deliver protein to the wound bed before absorbing the excess exudate, substituting the released protein. The high-water content of hydrogel possibly provides advantages for wound healing application, which ensures the biocompatibility and appropriate environment for quicker wound healing.

The burst releases of proteins were found at the early stage (first 12 hours) are the non-crosslinked proteins trapped in hydrogel as a matrix, which were released after swelling. The

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remaining proteins seem to be continually released from 10P3S5C1G until 168 hours. The sustained release of protein possibly brings about the benefits in clinical practice as it could reduce the need for dressing change, decreasing the pain of patients and the workload of healthcare providers. Moreover, the free radicals released during the inflammatory phase might diminish the bioactivity of proteins. Therefore, the sustained release of protein could enhance the healing process continuously. Moreover, the percentage of accumulative protein released at 168 hours may indicate that the sericin could be released concurrently with CH. The concurrent release of CH and sericin may synergize wound healing, as reported in the literature (10-12).

The tensile strength of hydrogel decreased because of the protein addition. Both sericin and CH have lower mechanical strength than PVA. Moreover, crystallization can also affect hydrogel's mechanical strength (13). From FTIR spectra, it appears that sample containing protein had lower absorption intensities of microcrystal formation. Therefore, the tensile strength of the hydrogel decreased. It was also mentioned earlier that the tensile strength of PVA hydrogels prepared by freeze-thaw and chemical crosslinking decreased due to protein addition (14, 15). In contrast, the elongation seems to be unchanged. The elongation is more challenging to explain because it can be influenced by many factors (13), including crystallization, PVA content, and swelling of the sample. Young's modulus or elastic modulus can indicate the elasticity of the hydrogel (3, 16). There was no significant difference in the Young's modulus between samples. Moreover, the mechanical properties of prepared hydrogels are probably in an appropriate range required for wound healing applications. Regardless of the concentrations, the prepared hydrogels seem to have higher flexibility than those prepared by chemical crosslinking and have higher mechanical strength than those hydrogels fabricated by gamma-radiation, freeze-thawing, and other physical hydrogels (14, 15, 17, 18). Although the 10P3S5C1G seems to have the decreased tensile strength in the present study, the strength was higher than the commercial polyurethane (PU) foam-film dressing, and the % elongation of 10P3S5C1G was comparable with those of commercial products (19). The elastic moduli of 10P3S5C1G were similar and matched with Young's modulus of human skin (20-24).

The cytotoxicity was evaluated after 24 hours of exposure to the extracts from the hydrogels and Bactigras®. Although the %cell viability in 10P1G was significantly lower than DMEM, the %cell viability was around 88% compared to DMEM. It supposes that all samples showed their safety on

L929 mouse fibroblast cells. In contrast, the cytotoxicity effect was found from Bactigras® because the %cell viability decreased to less than 30%. Therefore, Bactigras® was not evaluated for the effects on cell proliferation and migration. After exposure for 48 hours, the extracts could accelerate the proliferation of fibroblast at the comparable level with DMEM. The combination of CH and sericin may enhance the beneficial effects on cell proliferation. All extracts could also decrease the remaining area of the created wound, and the wound area was mostly healed after 48 hours of exposure to all groups. The % remaining area at each time point was not significantly different compared to DMEM. It might suggest that exposure to the extracts could comparably facilitate the migration of L929 fibroblasts compared to DMEM.

According to the findings, both CH and sericin could interact with PVA, leading to the change of hydrogel properties. While the PVA crosslinking could be decreased by CH addition, the addition of sericin seems to help maintain the PVA crosslinking. Moreover, both CH and sericin could be released from the hydrogel in a sustained manner. After releasing protein, the hydrogel could absorb water to a higher amount. The water content of hydrogel previously containing protein was increased compared to the neat PVA hydrogel. Although the mechanical strength of hydrogels containing protein decreased, their mechanical properties were still in the suitable range. According to the biological test, the hydrogel containing both CH and sericin (10P3S5C1G) seems superior to 10P1G in cell cytotoxicity, cell proliferation, and cell migration. The findings seem to concur with the previous studies (10-12). Therefore, the 10P3S5C1G could be used for further evaluation in the healthy volunteers.

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Conclusions

PVA hydrogels containing bioactive proteins with acceptable wound healing properties could be prepared by cast drying in an eco-friendly and straightforward process. The addition of CH and sericin enhances the advantages of PVA hydrogel. Both CH and sericin proteins could be concurrently released in a sustained manner from the hydrogel promoting wound healing. The hydrogel had excellent flexibility and durability to protect the wound from the outer environment. The biological properties of 10P3S5C1G also seem preferable over the neat PVA hydrogel. Accordingly,

10P3S5C1G could become a promising hydrogel for utilizing as wound dressing, and the further investigation for the clinical efficacy and safety in human may be conducted.

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Appendix P

Antibiotics administration record

Patient ID	Antibiotics	Operative day	Post-operative day							
		0	1	2	3	4	5	6	7	14
P001	Augmentin	1.2 g q 8 h								
	Tazocin								4.5 g IV q 8 h	
P002	Cefazolin		1 g IV q 6 h							
P003	Cefazolin	1 g IV q 6 h								
P004 P005	Cefazolin	1 g IV q 6 h								
P006	Cefazolin	1 g IV q 6 h								
P007	Cefazolin	1 g IV q 6 h								
P008	Cefazolin	1 g IV q 6 h								
P009	Cefazolin	1 g IV q 6 h								
P010	Augmentin	1.2 g q 8 h								
P011 P012	Ciprofloxacin		400 mg IV q 12 h							
	Clindamycin		600 mg IV q 8 h							
	Tazocin					4.5 g IV q 6 h				
P013	Cefazolin	1 g IV q 6 h								
P014	Augmentin	1.2 g q 8 h								
P015	Cefazolin	1 g IV q 6 h								
P016 P017	Augmentin	1.2 g q 8 h								
	Ciprofloxacin					400 mg IV q 12 h				
	Clindamycin					600 mg IV q 8 h				
P018 P019	Augmentin	1.2 g q 8 h								
P020	Cefazolin	1 g IV q 6 h								
P021	Ceftazdime	2 g IV q 8 h								
P022	Augmentin	1.2 g q 8 h								
	Ciprofloxacin			400 mg IV q 12 h						
	Clindamycin			600 mg IV q 8 h						
P023	Augmentin	1.2 g q 8 h								
P024	Cefazolin	1 g IV q 6 h								
P025	Augmentin	1.2 g q 8 h								
P026 P027	Augmentin	1.2 g q 8 h								
P028-30	N/A									

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Appendix Q

Analgesics administration record

Patient ID	Analgesics	Operative day	Post-operative day							
		0	1	2	3	4	5	6	7	14
P001	Morphine		1 mg/ml IV drip 3 ml/h							PCA
			5 mg IV prn q 4 h					3 mg IV	3 mg IV	
	Paracetamol		1,000 mg IV stat	1,000 mg IV q 6 h						
			1,000 mg oral			500 mg prn q 4 h			500 mg prn q 6 h	500 mg prn q 8 h
P002	Morphine		4 mg or 3 mg IV prn q 4 h							3 mg IV
	Paracetamol				1,000 mg oral					
P003	Paracetamol	1,000 mg oral								
P004 P005	Morphine			3 mg IV prn 4 h						
	Fentanyl	500 mcg/50 ml IV drip								
	Paracetamol			500 mg				500 mg q 6-8 h		
P006	Morphine	3 mg IV prn 4 h								
P007	Morphine	4 mg IV prn q 4 h	3 mg IV prn q 6 h							
	Paracetamol		500 mg							
P008	Morphine		3 mg IV				3 mg IV			
P009	Morphine		3 mg IV							
P010	Morphine		3 mg IV q 4 h						3 mg IV q 4 h	
	Paracetamol		1,000 mg oral					500 mg oral		
P011 P012	Morphine		PCA (1 mg/ml)					4 mg IV prn q 4 h		
	Fentanyl		PCEA							
	Tramadol		50 mg oral q 6 h		50-100 mg oral			50 mg oral q 6 h		
	Paracetamol		500 mg oral q 4 h					500 mg oral q 8 h		
	Arcoxia		90 mg							
P013	Morphine	4 mg IV prn q 4 h								
	Paracetamol	500 mg oral q 6 h								
P014	Morphine	PCA				4 mg IV prn q 4-6 h				
	Paracetamol		1,000 mg oral q 6 h				500 mg oral q 8 h			500 mg q 6 h
P015	Morphine		Epidural morphine 3 mg			3 mg IV q 4h			3 mg IV q 4h	
	Tramadol		1x2					50 mg oral		
	Paracetamol		500 mg oral q 4 h		500-1000 mg oral					
	Gabapentin								300 mg	
P016 P017	Morphine									
	Fentanyl	500 mcg/50 ml IV drip						500 mcg/50 ml IV drip		
			30 mcg IV			30 mcg IV 3 times				
	Pethidine	20 mg IV, 30 mg IV								
	Paracetamol				1,000 mg IV drip					
						500 mg			500 mg q 4 h	
P018 P019	Morphine	0.6 mg/ml IV drip 3ml/h				3 mg IV q 4h				3 mg IV
	Fentanyl	500 mcg/50 ml IV drip								
			50 mcg IV							
	Tramadol								50 mg oral	
	Paracetamol		1,000 mg IV drip							
				500 mg q 4 h	500 mg q 8 h				500 mg	
P020	Tramadol		50 mg oral						50 mg oral	
	Paracetamol						500 mg			
P021	Morphine	4 mg IV					4 mg IV			
	Paracetamol						500 mg			
P022	Morphine	4 mg IV	4 mg IV q 4h	4 mg IV q 6 h	4 mg IV 2 times				3 mg IV 2 times	3 mg IV q 4 h
	Fentanyl		50 mcg IV before D/S							
	Tramadol				50 mg oral					
	Paracetamol		500 mg oral q 4 h			500 mg oral			500 mg oral	
	Celecoxib					200 mg 1x2				
	Gabapentin	300 mg q 6h								
P023	Morphine		3 mg IV q 4 h							3 mg IV
	Ultracet	1 tab oral								
	Paracetamol		500-1000 mg oral				500 mg oral		500 mg oral	
P024	Paracetamol	500 mg oral q 6 h								
	Arcoxia	60 mg								
P025	Morphine	3 mg IV								
	Paracetamol	500 mg oral								
P026	Morphine	30 mg IV drip	PCA 1 mg/ml						3 mg IV	4 mg IV q 4-6 h
	Pethidine	25 mg IV stat								
	Paracetamol					1,000 mg IV drip q 8 h				
		500 mg oral q 6 h						500 mg q 12 h		500 mg oral
	Arcoxia								90 mg	
P028-30	Morphine	4 mg IV q 6 h	4 mg IV q 4 h		4 mg IV q 6 h	3 mg IV q 6 h	3 mg IV q 4 h		3 mg IV q 8 h	
	Tramadol	50 mg 1x4								

VITA

NAME	Apirujee Punjataewakupt
DATE OF BIRTH	26 July 1988
PLACE OF BIRTH	Bangkok
INSTITUTIONS ATTENDED	Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand
HOME ADDRESS	36/1 Moo 3, Erawan, Poojaosamingprai Road, Samrong-Tai, Phrapradaeng, Samutprakarn 10130 Thailand
PUBLICATION	Punjataewakupt A, Napavichayanun S, Aramwit P. The downside of antimicrobial agents for wound healing. Eur J Clin Microbiol Infect Dis. 2019;38(1):39-54. Punjataewakupt A. and Aramwit P. Wound dressing adherence. J Wound Care. (In process).
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