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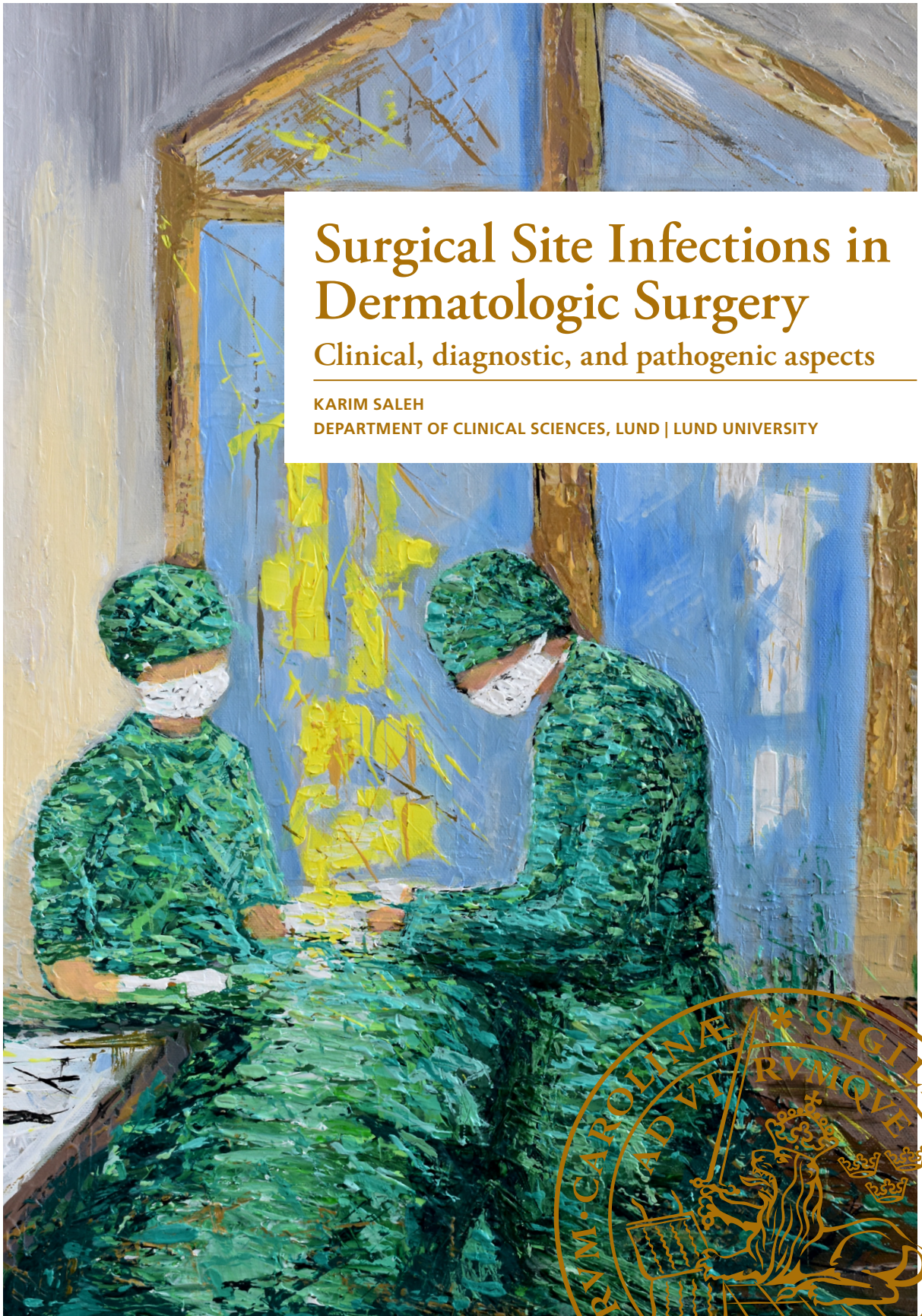
Saleh, Karim

2019

Document Version:
Publisher's PDF, also known as Version of record



Surgical Site Infections in Dermatologic Surgery

Clinical, diagnostic, and pathogenic aspects

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Surgical Site Infections in Dermatologic Surgery

Clinical, diagnostic, and pathogenic aspects

Karim Saleh
M.D.

DOCTORAL DISSERTATION

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To be defended at Segerfalksalen, Lund, 20190201, 13:00.

Faculty opponent
Professor Eduardo Nagore, Instituto Valenciano de Oncologia, Spain

Organization LUND UNIVERSITY Department of Clinical Sciences Lund, Dermatology & Venereology Author: Karim Saleh, MD	Document name: DOCTORAL DISSERTATION 2019-02-01 Sponsoring organization: None

Title and subtitle: Surgical Site Infections in Dermatologic Surgery- Clinical, diagnostic, and pathogenic aspects

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Classification system and/or index terms (if any): None

Supplementary bibliographical information: Lund University, Faculty of Medicine Doctoral Dissertation Series 2019:14		Language: English
ISSN and key title: 1652-8220		ISBN: 978-91-7619-743-1
Recipient's notes	Number of pages: 125	Price
	Security classification	

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To my family for their love and support

Cover: "Dermatologic surgery" Artwork: **Hana Krivska Saleh**

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ISBN 978-91-7619-743-1
ISSN 1652-8220

© Design and layout: **Hassan Hashemian** (m.hassan.hashemian@gmail.com)

Printed in Sweden by Media-Tryck, Lund University
Lund 2018

0341 0903
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Content

Acknowledgments

First and foremost, I would like to extend my gratitude and thanks to my main supervisor, Professor Artur Schmidtchen, for guiding me through this path. I will never forget the first time we met. Never would I have thought I would become a researcher, and now I'm going to be the recipient of a PhD in medicine thanks to his efforts. I am very honoured that you trusted me with this project and left me freedom to discover my own scientific ideas. Thank you for the 100,000 emails you sent me over the years and thank you for always being available when in need. Your motto "Keep it simple" will remain with me forever.

Next, I would like to thank my co-supervisor, Dr. Andreas Sonesson, for all of the support during the course of the work for this thesis. You provided me with all of the tools I needed in the beginning, starting from statistics to microbiology basics, and without you I would not have been able to become a self-sufficient researcher managing to publish my last two studies without even having to bother you.

I want to thank all my co-authors for great team work. Thank you to Bertil Persson for all of the input on my first ever published article. Thank you to Kerstin Persson for all of the work and help in our Prontosan study. Without you we would never have managed to publish in JAAD! A special thanks to Professor Riesbeck and his group for cooperation with three of the studies in this thesis. Thanks to Dr. John Paoli for all of the help and great input for the last study and Johan Palmgren for coping with me as a last author.

Thank you to all of my colleagues in the Department of Dermatology for being such fantastic colleagues and being able to handle my stubbornness from time to time.

Thank you to Bertil Persson, former head of department for giving me a residency within dermatology. I will always be thankful for his help. Thank you for all the hours of surgical wisdom you have given and continue to give me.

Thank you to Gustav Christensen, current head of department, for being so understanding and flexible and the most humble and laid-back boss.

Thank you to all of the patients that participated in the study because without your generosity, this thesis would not have been possible.

Thank you to everybody at the lab at BMC for all of the help and input on my studies. A special thanks to Mina Davoudi for all of the technical assistance at the beginning of my PhD studies and later Ann-Charlotte Strömdahl for sharing all of her expertise during my lab work!

Thank you to all of my fantastic friends for keeping me busy with non-work-related activities. A special thanks to Simon Pålsson for his exceptional proofreading of the Swedish summary in this book.

Thank you to all of my family for their love and support. Without you, I would never have become the man I am today. A special thanks to my mother for all of the sacrifice you have made to our family and thank you for a fantastic painting that covers this book.

Thank you to my in-law family for all of the love and a sincere thanks to my parents-in-law for helping me during this thesis by giving me extra time to work while taking care of Céline. Thank you to my father-in-law, Mansour Jan, for great artwork and a fantastic layout for this book!

Céline Saleh, my beautiful daughter, thank you for coming into this world and letting me be your father. You have given my life a completely new meaning, and I will always do my best to make you happy. I love you so much!

Last, but foremost thanks to my beautiful, loving, and caring wife, Jasmine. Without you beside me in this life NOTHING would have been possible. You are the reason everything in my life is so wonderful, and I am so lucky to be your husband. Thank you for all of your support and for being my consigliere. Thank you for giving us the best gift ever! Thank you for your patience and care of Céline while I worked on this thesis after she was born. From the bottom of my heart, I love you forever!

This thesis was mainly supported by funds from the Swedish Government known as ALF which is a collaboration between Region Skåne and Lund University. Studies included in the thesis were also supported by grants from Swedish Research Council, the Welander-Finsen, Crafoord, Österlund and Kock Foundations, Marianne and Marcus Wallenberg Foundation, the program "Innovation mot Infektion", financed by the VINNOVA- Swedish Governmental Agency for Innovation Systems, the Knut and Alice Wallenberg Foundation, the Swedish Foundation for Strategic Research, and Hudfonden.

List of publications and presentations

Work from this thesis has contributed to the following scientific publications and presentations. Only studies with a Roman numeral are included in this thesis.

International Publications

I. Saleh K, Sonesson A, Persson B, Riesbeck K, Schmidtchen A. A descriptive study of full-thickness surgical wounds in dermatologic surgery. Dermatol Surg. 2011 Jul;37(7):1014-22.

Saleh K, Schmidtchen A. Surgical site infections (SSIs) in Dermatologic Surgery: Preventative guidelines and challenges. Dermatol Surg. 2015 May;41(5):537-49.

II. Saleh K, Sonesson A, Persson K, Riesbeck K, Schmidtchen A. Can dressings soaked with polyhexanide reduce risk for surgical site infections in full-thickness skin grafting? A randomized controlled trial. J Am Acad Dermatol. 2016 Dec;75(6):1221-1228.

III. Saleh K, Riesbeck K, Schmidtchen A. Inflammation biomarkers and correlation to wound healing after full-thickness skin grafting. 2018. Submitted.

IV. Palmgren J, Paoli J, Schmidtchen A, **Saleh K**. Variability in the diagnosis of surgical site infections after full-thickness skin grafting: An international survey. Br J Dermatol. 2018 Dec 10. doi: 10.1111/bjd.17517. E-publication ahead of print.

National Publications

Saleh K, Schmidtchen. Postoperativa sårinfektioner inom hudkirurgi- En evidensöversikt. 2018. Submitted to läkartidningen.

Saleh K. Can dressings soaked with polyhexanide reduce bacterial loads of surgical wounds? Forum for Nordic Dermato-Venereology. 2017;(22):18-18.

Saleh K. Kan Prontosan® minska risken för postoperativa sårinfektioner vid hudkirurgi. Dermatologi&Venereologi. 2016;(8):12.

Saleh K. Dyra och smärtsamma sårinfektioner. Dermatologi&Venereologi. 2015;(2): 5.

Book chapter

Saleh K, Sönnergren H. Control and treatment of infected wounds in: Wound healing biomaterials-Volume 2 (Ågren M), 1st edn. Elsevier, 2016:107-115.

Presentations

November 7th, 2010. "A descriptive study of full-thickness surgical wounds in dermatologic surgery". Presented at **The 1st World Congress on Controversies in Plastic Surgery and Dermatology (CoPLASDY)**, Barcelona, Spain.

March 16th, 2011. " Introduction to dermatological research". Presented for dermatology residents at a regional educational day. Department of Dermatology and Venereology, Lund University Hospital, Lund, Sweden.

November 25th, 2013. " Surgical site infections (SSIs) in dermatologic surgery". Presented at Innovation against infection yearly workshop, SP Technical Research Institute of Sweden.

May 20th, 2016. " Can dressings soaked with polyhexanide reduce risk for surgical site infections in full-thickness skin grafting? A randomized controlled trial". Presented at the annual meeting of the Swedish Society of Dermatology.

March 5th, 2017. " Can dressings soaked with polyhexanide reduce risk for surgical site infections in full-thickness skin grafting? A randomized controlled trial". Presented at the **American Academy of Dermatology annual meeting (AAD)**, Orlando, USA.

November 15th, 2017. " Wound infections". Presented for dermatology residents at a regional educational day. Malmö University Hospital.

*Planned: October 12th, 2019. "Surgical site infections (SSIs) in dermatologic surgery". Invited speaker to the **European Academy of Dermatology and Venereology (EADV)** congress, Madrid, Spain.*

Awards

Work from this thesis has resulted into the following awards:

- Travel award from Maggie Stephens to support the participation in the 1st World Congress on Controversies in Plastic Surgery and Dermatology in Barcelona. August 2010.
- Prize award from the Swedish Society for Dermatology for best scientific work. May 2016.
- Travel award from Diagnostiskt Centrum Hud to support the participation in the Annual American Academy of Dermatology meeting in Orlando. January 2017.
- Michael Hornstein scholarship to support the participation in the European Academy of Dermatology and Venereology in Geneva. September 2017.

Abstract

Surgical site infections (SSIs) in dermatologic surgery contribute to unwanted healthcare costs and are complications that cause suffering in patients. The aim of this thesis was to explore clinical, diagnostic, and pathogenic aspects of SSIs in dermatologic surgery.

In study I, we examined bacterial dynamics during normal wound healing and SSIs. We found that quantifying bacteria from wounds was a relevant factor for assessing healing outcomes. Higher bacterial loads in wounds resulted in complicated postoperative healing outcomes.

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Summary in Swedish

Varje gång en patient har genomgått ett kirurgiskt ingrepp löper han/hon en risk för att drabbas av en efterföljande infektion i operationssåret, inom medicin kallat en "postoperativ sårinfektion" (postoperativ = efter operation). Dessa är potentiellt farliga komplikationer som, förutom att de kan leda till allvarliga blodförgiftningar och äventyra patienternas liv, orsakar smärta, lidande, fördröjd sårläkning och slutligen en oestetisk ärrläkning. I tillägg kostar de sjukvården enorma summor, uppskattningsvis 500-1000 miljoner kronor årligen enbart i Sverige. Inom specialiteten dermatologi (läran om hudsjukdomar) opereras det allt mer. Mycket av hudkirurgin inom denna specialitet sker i ansiktet och där kan en postoperativ sårinfektion ge ett missprydande och psykiskt belastande ärr.

Varför vissa råkar ut för postoperativa sårinfektioner och andra inte är ej helt kartlagt.

Syftet med den här avhandlingen bestående av fyra delarbeten var att studera postoperativa sårinfektioner inom dermatologin för att öka kunskapen inom området.

I delarbete I undersöktes sår efter hudtransplantationer i ansiktet. Vi konstaterade att sår med ett komplicerat läkningsförlopp hade högre halter av bakterier en vecka postoperativt jämfört med sår som uppvisade normal infektionsfri läkning. Detta oberoende av vilken bakterieart som växte i såren.

I delarbete II testades ett nytt antiseptiskt (=bakterieavdödande) medel, Prontosan i en dubbelblind studie på 40 patienter som skulle genomgå hudkirurgi i ansiktet. 20 patienter fick medlet på sina sår och 20 patienter fick placebo. I en dubbelblind studie vet vare sig patienten eller läkaren vem som behandlas med aktiv substans och vem som får icke-aktiv substans. Prontosans potential att reducera halten bakterier i operationssåret analyserades, men dessvärre uppvisades ingen minskning av infektionsfrekvensen, utan användningen till och med ökade risken för postoperativa sårinfektioner. Vi har ingen direkt förklaring till detta men spekulerar i om det antiseptiska medlet rubbar balansen mellan skadliga och ofarliga bakterier på huden. Detta skulle kunna bidra till ett minskat skydd från hudens goda bakteriella normalflora, ge de elakartade bakterierna i normalfloran ett övertag och därmed öka infektionsrisken; men fler studier krävs för att kunna undersöka en sådan hypotes.

I delarbete III analyserades sårvätskorna från patienterna i delarbete II. Patienterna med sår som uppvisade en normal läkning hade lägre nivåer av olika inflammationsmarkörer jämfört med sår som uppvisade tecken till infektion. Med hjälp av analys av dessa inflammationsmarkörer kommer vi förhoppningsvis kunna förutsäga vilka sår som kommer att bli infekterade.

I delarbete IV studerades dermatologers förmåga att, oberoende av varandra, göra likvärdiga bedömningar av huruvida fotograferade operationssår var infekterade eller inte. Vi fann en stor oenighet bland svaren. Läkare som inte hade kirurgivana, kvinnliga läkare och läkare under utbildning var mer benägna att bedöma ett sår som infekterat än icke infekterat. Delarbetet belyste vikten av att vi i framtiden får bättre, mer objektiva och precisa metoder som beslutsunderlag i bedömningen av postoperativa sårinfektioner.

Tillsammans har dessa studier gett oss en tydligare bild av förekomst, genes och förutsägelse av postoperativa sårinfektioner. Förhoppningen är en framtida användning av resultaten både kliniskt och som underlag för vidare forskning inom dermatologin och andra kirurgiska specialiteter.

Abbreviations

ACMS: American College of Mohs Surgery
AMP: Antimicrobial peptide
BGC: Bacterial growth change
BSDS: British Society for Dermatological Surgery
CDC: Centers for Disease Control and Prevention
CFU: Colony-forming unit
CoNS: Coagulase-negative staphylococcus
DAMP: Damage-associated molecular pattern
E. coli: Escherichia coli
ESMS: European Society for Micrographic Surgery
IL-1: Interleukin-1
IL-6: Interleukin-6
IL-8: Interleukin-8
IL-12: Interleukin-12
MALDI-TOF: Matrix-assisted laser desorption/ionization time-of-flight
MAPK: Mitogen-activated protein kinase
MHC: Major histocompatibility complex
MMP: Metalloproteinase
MRSA: Methicillin-resistant *Staphylococcus aureus*
NFKβ: Nuclear factor kappa light chain enhancer of activated B cells
PAMP: Pathogen-associated molecular pattern
PHMB: Polyhexanide biguanide
PRR: Pattern recognition receptor
P. aeruginosa: Pseudomonas aeruginosa
RCT: Randomized controlled trial
S. aureus: Staphylococcus aureus
SDS-PAGE: Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEAP: Secreted embryonic alkaline phosphatase
S. epidermidis: Staphyloccus epidermidis
SSDV: Swedish Society for Dermatology and Venereology
SSI: Surgical site infection
TGF-β: Transforming growth factor β
TH: Todd-Hewitt
TLR: Toll-like receptor
TNF-α: Tumor Necrosis Factor α
VEGF: Vascular Endothelial Growth Factor

Introduction

Human skin

Skin is one of the largest organs in the body with a surface area of 1.5 to 2.0 m² and a weight of 4 to 5 kg. [1] The skin is divided into three layers: (1) epidermis; (2) dermis; and (3) subcutis **(Figure 1).** The epidermis is the relatively thin, tough, outer layer of the skin, and its most important function lies in the stratum corneum, a semipermeable laminated surface aggregate of differentiated squamous epithelial cells, which serves as a physiological barrier. [2] The epidermis consists mainly of three different cell types: (1) keratinocytes; (2) melanocytes; and (3) Langerhans' cells. Beneath the epidermis, a vascularized dermis provides structural and nutritional support. It is composed of a polysaccharide gel and a matrix with collagen and elastin fibers, which gives skin its flexibility and strength. Dermis also contains nerve endings, sweat- and sebaceous-glands, hair follicles, and blood vessels, all of which contribute to protecting the body, regulating body temperature, and provide sensation. [1] Subcutis contains fat that helps insulate the body from heat and cold, provides protective padding, and serves as an energy reserve. [1]

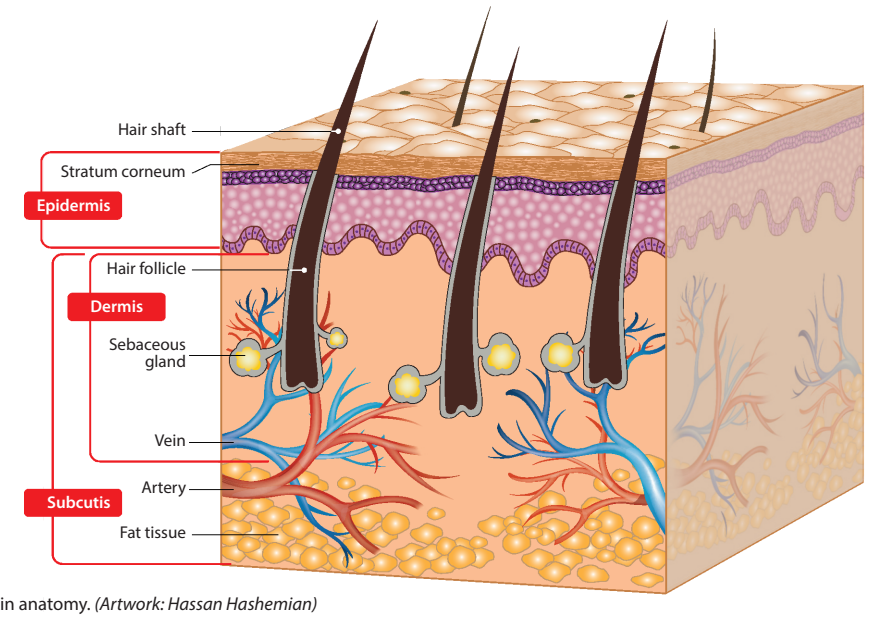

Figure 1. Skin anatomy. *(Artwork: Hassan Hashemian)*

Wounds

One of the skin's main functions is to act a barrier between body tissues and the external environment. The skin with its structures described above protects the human tissues from external potentially dangerous factors such as pathogenic microorganisms, chemical substances, warm or cold conditions, and trauma. When the skin integrity is compromised, a wound is created.

Wounds are classified as either acute or chronic. Acute wounds normally heal spontaneously in an orderly and timely process (see **Wound healing**) in contrast to chronic wounds. Chronic wounds have a tendency to heal slowly and require significant interventions. Acute wounds can be further divided into traumatic or surgical wounds. Traumatic wounds include abrasions, crush wounds, puncture wounds, lacerations, and cuts. Surgical wounds are incisions made purposefully by a health care professional. [3]

Surgical wounds

Surgical wounds are usually classified into four different categories depending on their anatomic location and preoperative skin condition **(Table 1).** [4, 5] As noticed, this classification system is based on general surgical procedures and is not easily applied to wounds deriving from dermatologic surgery. [6]

Table 1. Surgical Wound Classification [4, 5]

Classification	Wound description
Class I – Clean	Uninfected operative wound in which no inflammation is encountered and the respiratory, alimentary, genital, or uninfected urinary tract is not entered. In addition, clean wounds are primarily closed and, if necessary, drained with closed drainage. Operative incisional wounds that follow nonpenetrating (blunt) trauma should be included in this category if they meet the criteria.
Class II – Clean-contaminated	An operative wound in which the respiratory, alimentary, genital, or urinary tracts are entered under controlled conditions and without unusual contamination. Specifically, operations involving the biliary tract, appendix, vagina, and oropharynx are included in this category, provided no evidence of infection or major break in technique is encountered.
Class III – Contaminated	Open, fresh, accidental wounds. In addition, operations with major breaks in sterile technique (such as open cardiac massage) or gross spillage from the gastrointestinal tract, and incisions in which acute, nonpurulent inflammation is encountered are included in this category.
Class IV – Dirty-infected	Old traumatic wounds with retained devitalized tissue and those that involve existing clinical infection or perforated viscera. This definition suggests that the organisms causing postoperative infection were present in the operative field before the operation.

Dermatologic surgery

Dermatologic surgery is on the rise due to an increase in skin cancer incidence and increases in the number of excisions performed by dermatologists. [7] It is estimated that 40% of all visits to a dermatologist result in a surgical procedure. [7] In the United States (US), 42.2 million dermatological procedures were performed in 2007 according to a study by Ahn *et al.* [7] The most common type of procedure was local excision of a lesion. Studies show that the number of dermatological procedures performed annually is increasing. [7, 8]

Wound healing

Under normal conditions, acute wounds heal in a sequenced and timely manner, characterized by four major overlapping phases (namely, hemostasis, inflammation, proliferation, and remodelling) **(Figure 2)**. This is a complex process involving chemokines, cytokines, proteases, and their respective counterregulatory molecules through the healing process. [9] The primary goal of wound healing is to re-establish a functional skin barrier as quickly as possible. [1] Ideally, wound healing would be a *regenerative* process, with complete restoration of original skin function and morphology as is the case in scarless fetal skin wound healing, [10] but this type of healing does not actually occur. Wound healing in children and adults is a *reparative* process resulting in a loss of normal skin function and a certain degree of impaired morphology. [1] Each of the wound healing phases **(Figure 3)** beneath are critical to successful wound closure, and any deviations from the norm may be associated with an abnormal or delayed wound healing. [11]

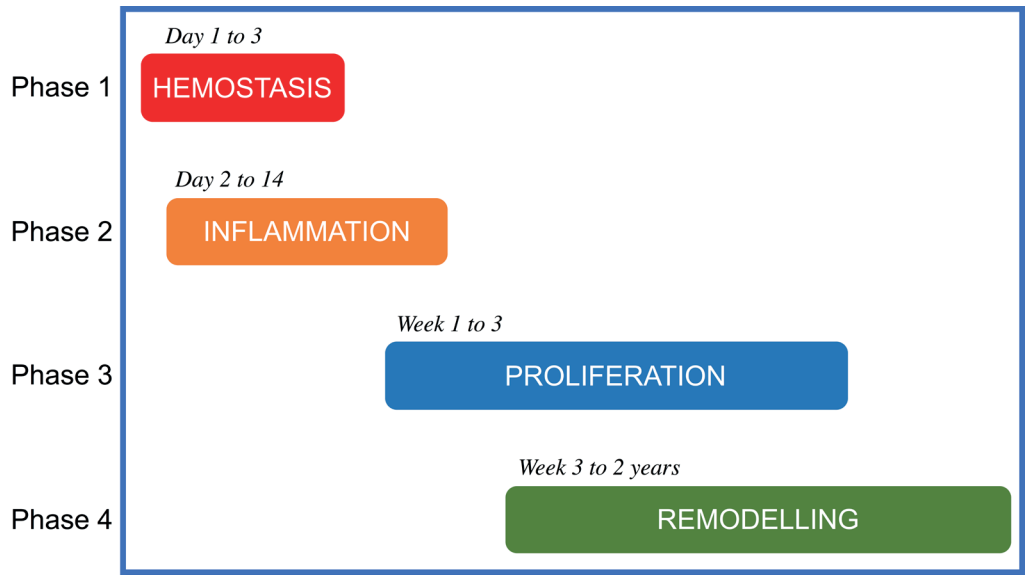

Figure 2. Phases of wound healing. *(Artwork: Karim Saleh)*

Hemostasis

Skin injury causes the disruption of blood vessels and exposure of the basal lamina, which result in extravasation of blood constituents and concurrent platelet activation. [11, 12] The formed blood clot composed of fibrin re-establishes hemostatis and provides a provisional extracellular matrix for cell migration. [12] This stage also results in the release of growth factors involved in the deposition of extracellular matrix (transforming growth factor β), chemotaxis (platelet-derived growth factor), epithelialization (both fibroblast growth and epidermal growth factors), and angiogenesis (vascular endothelial growth factor). [11]

Inflammation

Hemostatis is followed by inflammatory cell influx. Neutrophils are the predominant cell type in this phase (48 h after injury) and begin to decline in numbers via apoptosis at the same time monocytes start arriving at the wound and mature into macrophages. [13] Neutrophils also produce tumor necrosis factor α (TNF-α) and interleukins (ILs) such as IL-1 and -6, which recruit fibroblasts and epithelial cells. [14, 15]

Macrophages are responsible for phagocytosing debris and bacteria, secreting additional cytokines such as TNF-α, transforming growth factor β (TGF-β), vascular endothelial growth factor (VEGF), IL-1 and -6. IL-1 promotes angiogenesis, and TNF-α is a mitogen for fibroblasts. [12, 13] It is also believed that macrophages are responsible for clearing neutrophils that may otherwise result in a prolonged inflammatory phase. [16] Macrophages also have a role in clearing fibrin from the wound. [17] Lymphocytes are the last to infiltrate wounds. [18] Normally, inflammation goes on for two weeks, and it is this stage that results in the traditional signs of inflammation: (1) dolor (pain); (2) rubor (redness); (3) calor (warmth); and (4) tumor (swelling). [18]

Proliferation

Proliferation is composed of several steps occurring during this phase, namely epithelialization, angiogenesis, granulation tissue formation, and collage deposition and normally occurs 2–10 days after injury. [11, 19]

In this phase, epidermal cells migrate and proliferate after being stimulated by growth factors, metalloproteinases (MMP), and integrins. [20] Within 24 h after injury, keratinocytes at the wound edge are activated and undergo phenotypic and functional changes in order to adhere to the newly formed provisional wound matrix and to be able to migrate laterally towards wound edges. [12] The provisional wound matrix is synthesized by activated fibroblasts and is composed of fibronectin, vitronectin, and type 1 collagen interwoven in the fibrin clot. [12] The provisional wound matrix serves as the basis for granulation tissue formation, which is gradually replaced with a collagenous matrix produced by fibroblasts. [12] MMPs such as MMP-2 and -9 have important functions in accelerating cell migration and promoting reepithelization. [21] Angiogenesis, a crucial part of wound healing, is initiated and promoted by secretion of fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and VDGF. [11]

Remodelling

In this final stage, which starts after the formation of granulation tissue, granulation tissue is converted to scar tissue by remodelling of the extracellular matrix. [12] Remodelling begins 2–3 weeks after injury and lasts for a year or more. [19] This phase is characterized by a balance between the synthesis of new scar matrix components and their degradation by proteases. [1] Myofibroblasts play the main part in wound contraction, resulting in decreased wound size. [22] Wounds can reach 80% of the tensile strength of the original tissue. [19]

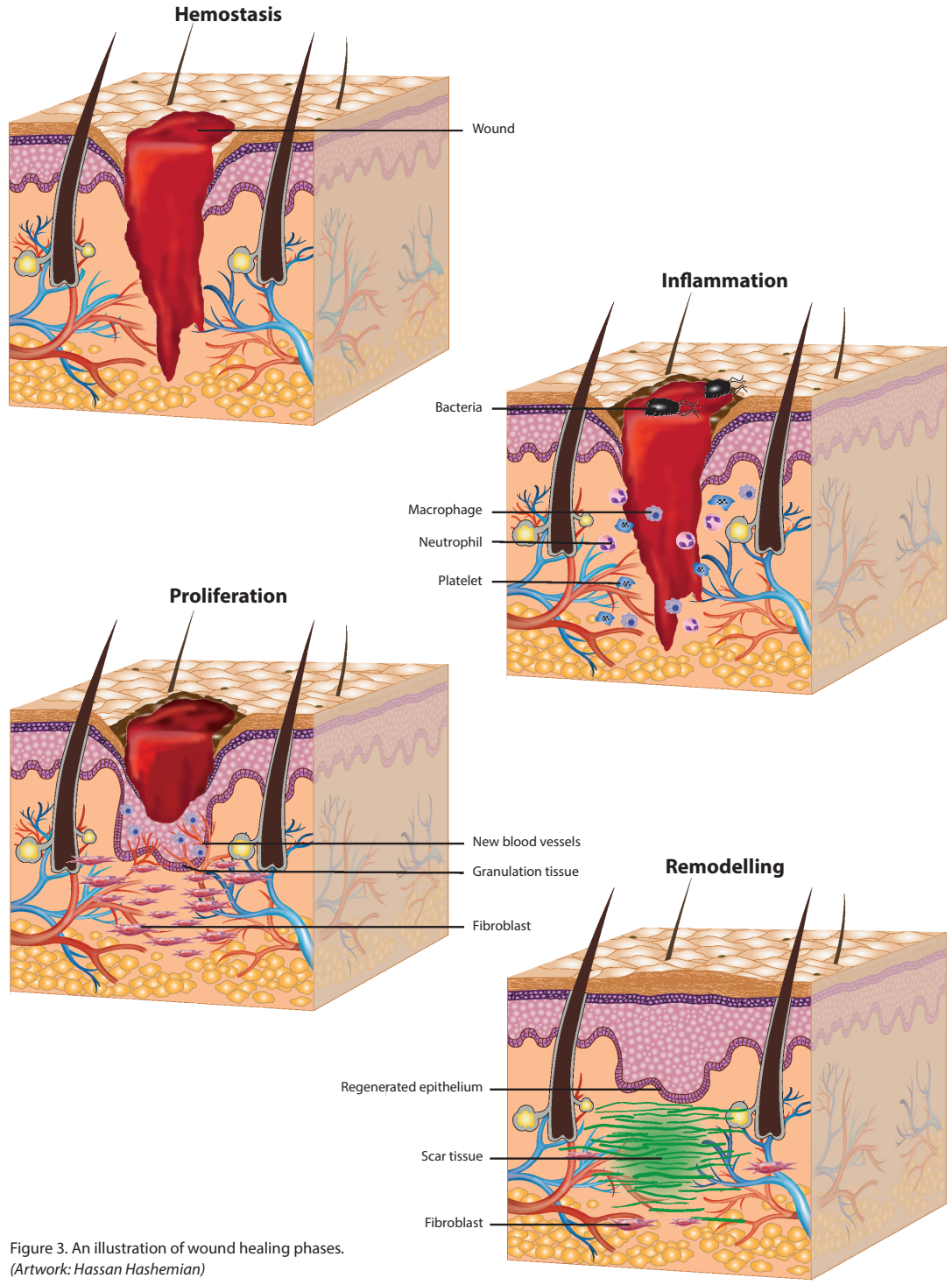

Figure 3. An illustration of wound healing phases.
(Artwork: Hassan Hashemian)

Skin's immunity in wound healing

The skin not only functions as a mechanical barrier to microbial and physical insults but can also generate an immune response for protection. The skin's immune system has elements of both the innate (nonspecific) and adaptive (specific) immune systems and both contribute to regulating the wound healing process. [1] Innate immunity provides the first defence mechanism during wounding while adaptive immunity responds later.

Innate immunity relies on recognizing structural patterns of microorganisms called pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) by a class of receptors known as pattern recognition receptors (PRRs). [23] Known PAMPs include bacterial liposaccharides and bacterial DNA, while known DAMPs are cytosolic and nuclear proteins. [23] PAMPs and DAMPs are recognized by PRRs, known as Toll-like receptors (TLRs), which, once activated, ultimately lead to activation of the nuclear factor kappa (NF-κB) light chain enhancer of activated B cells and mitogen-activated protein kinase (MAPK) pathways. [24] This process then results in the production of ILs-1, -6, -8, and -12 and TNF-α that leads to chemokine release and subsequent inflammatory cell migration to the site of wound injury. [24] The secretion of inflammatory mediators via TLR-dependent activation also promotes dendritic cell maturation that is able to induce an adaptive immune response. [25] Keratinocytes' role in innate immunity, other than expressing TLR, is to produce antimicrobial peptides (AMPs). The best characterised AMPs are cathelicidins and defensins.

Another important part of innate immunity is the complement system that helps destroy invading microorganisms by attracting neutrophils to the wound. [1]

The adaptive immune system provides a more delayed and specific response. [26] It is unique in its capability to generate and retain memory. It consists of B and T cells that carry out humoral and cell-mediated responses. [26] These effector cells are activated upon recognizing either free or bound antigen via professional antigen presenting cells. [26] For T cells, the type of effector response depends on the major histocompatibility complex (MHC) molecule that is employed for antigen presentation. MHC class I complexed with endogenously produced antigens is recognized by CD8 cytotoxic T cells, while MHC class II expresses ingested exogenous antigen and is recognized by CD4 helper T lymphocytes. [26] CD4 activation results in cytokine production, whereas CD8 cells are much more specific in cell targeting. [26] Activated B cells produce antibodies, which serve to inactivate toxins, opsonize bacteria, flag pathogens for destruction, and activate complement among other functions. [26]

Surgical site infections (SSIs)

Research in SSIs is mainly based on studies of general surgery. Results from these studies have then been extrapolated to dermatologic surgery.

Epidemiology

Surgical site infections (SSIs) are known to be the leading healthcare-related infection in developing countries and the second most common healthcare-related infection in developed countries. [27, 28] It is estimated that 230 million surgical procedures are carried out globally on an annual basis, which result in more than seven million complications, of which SSIs represent the most common complication. [29] In the US alone, SSIs incur $10 billion in annual costs. [30, 31] These include direct costs such as prolonged hospitalizations and readmission to hospitals, outpatient visits, visits to emergency departments, additional surgeries ranging from incision and drainage to staged reimplantation, prolonged antibiotic therapy, more use of ancillary services such as laboratory tests, drugs, and durable medical equipment, and professional fees. Indirect costs, harder to quantify, include lost productivity, decreased patient satisfaction, reduced referrals, and possibly litigation. [32] SSIs are associated with a doubled increased relative risk for hospital mortality, and over a third of all postoperative deaths are due to an SSI. [33, 34]

Half of all antibiotics used in a hospital have been attributed to prophylaxis prior to surgery. [35] This can lead to antibiotic resistance. [36] Approximately each fifth visit to an emergency department is due to an antibiotic-induced side-effect. [37]

Within the field of dermatologic surgery, the risk for an SSI occurrence is assumed to be low, varying from 5% to 10%, [38] but depending on the procedure type and anatomical location, it can be as high as 28.5%. [39] Skin grafting and complex reconstructions are procedures normally associated with a higher risk of SSI. [38-41]

Regardless of the generally low SSI rates, it is of paramount concern to achieve acceptable cosmetic results and normal wound healing within dermatologic surgery, owing to the fact that many procedures involve critically aesthetic facial units.

Definition

SSIs are infections arising in wounds within 30 days of surgery according to the Centers for Disease Control and Prevention (CDC). [42]

These infections are classified as either incisional or organ/space **(Figure 4)**. Incisional SSIs are further divided into those involving only skin and subcutaneous tissue (superficial incisional SSI) and those involving deeper soft tissues (deep incisional SSI). [42]

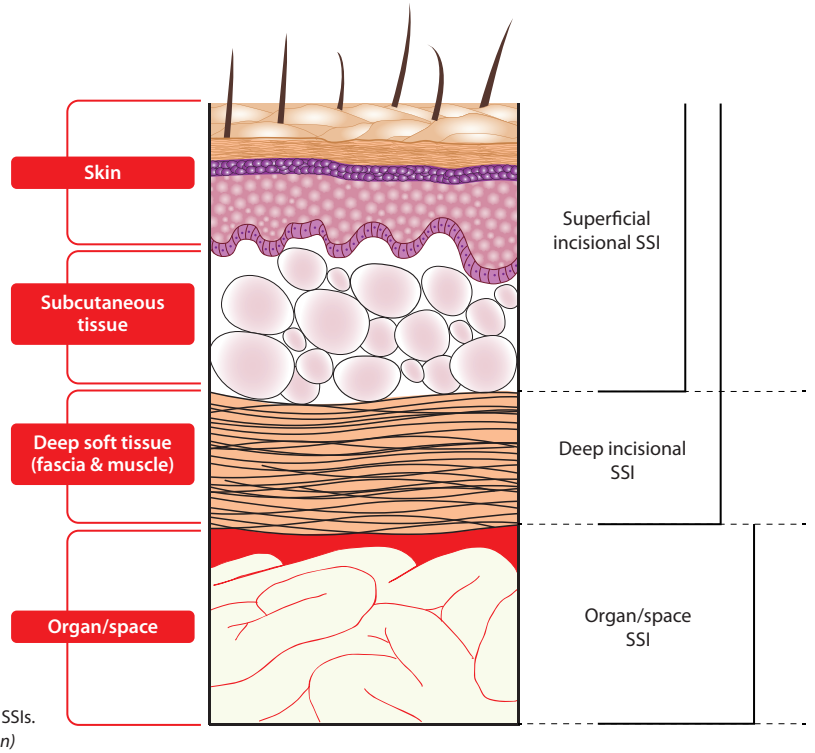

Figure 4. Different types of SSIs. *(Artwork: Hassan Hashemian)*

Superficial incisional SSI is the most common type of SSI, accounting for 2/3 of all SSIs, and the most relevant for dermatologic surgery. [43]

According to the CDC, a superficial incisional SSI is defined as an infection involving skin and subcutaneous tissue occurring within 30 days after surgery and fulfilling one of the several criteria: [42]

1. Purulent drainage, with or without laboratory confirmation from the superficial incision;

2. Organisms isolated from an aseptically obtained fluid or tissue culture from the superficial incision;

3. At least one of the following signs or symptoms of infection: (a) pain or tenderness; (b) localized swelling; (c) redness; (c) or heat and superficial incision is deliberately opened by surgeon unless incision is culture-negative;

4. Diagnosis of superficial incisional SSI by the surgeon or attending physician.

It should be noted that more than 30 different definitions of SSI have been published, but the definitions based on the CDC guidelines are the most commonly used. [44] This illustrates the limitations of the way in which SSIs are defined. The same wound can be defined as infected according to one definition but non-infected when using another one. [45]

Even when using the CDC's definition alone, there is a wide variation in wound assessments. [45, 46] Studies have shown both inter-and intra-observer variations. [47] Another limitation to the definition of SSIs according to CDC includes a 30-day postoperative evaluation timeframe, which imposes practical difficulties in monitoring all surgical wounds and is not likely to occur. CDC's fourth criteria for an SSI was updated in 2018, allowing a physician's designee to diagnose an SSI thus making SSI assessments perhaps even less accurate. [48] Clearly more objective definitions of SSIs are needed.

Pathogenesis of SSIs

In healthy skin, an equilibrium between skin microbiota and innate immunity exists. As soon as the skin is damaged due to surgery, this equilibrium is under the risk of being disrupted. When a surgical wound is created, a primed host immune-inflammatory process, as explained earlier, starts. This is beneficial to a certain degree and has to be balanced by homeostatic anti-inflammatory mechanisms. The increased non-specific inflammatory response at this stage limits the hosts' ability to defend itself from microbes entering the wound. If a certain degree of microbial invasion occurs, causing an additional inflammatory response, the surgical wound is now too inflamed and an SSI has developed. [49-51] Distinguishing an inflamed wound from an infected one *in vitro* and clinically is a major challenge taking into consideration that measurable parameters are lacking.
According to CDC, the risk of an SSI to occur can be visualized by the following equation: [42]

$$\frac{\text{Dose of bacterial contamination} \times \text{virulence}}{\text{Resistance of the host patient}} = \text{Risk of SSI}$$

Earlier studies felt that an SSI occurred when the wound's microbial load exceeded 10^5 colony-forming units (CFU) per gram of tissue. [52] According to the CDC, the number of bacteria present in wounds is the most important factor associated with the development of SSIs. [42] On the other hand, growing evidence suggests that the pathogenesis for an SSI is far more complex than just being dependent on the bacterial load of a wound, and specific bacterial interactions and virulence factors need to be taken into account. [53]

Skin microbiota

Skin microbiota contains bacteria, virus, and fungi and is classified as either "resident flora", which is constitutes the most abundant microbes that are always present, or "transient flora" which are microbes not always present or are present for only a few days, weeks, or months before disappearing under various environmental and host conditions. Bacteria are the most-studied flora isolated from skin. These include those from the genera *Staphylococcus, Corynebacterium, Propionibacterium, Streptococcus, and Pseudomonas.* [54]

The way in which these bacteria interact among themselves, with each other, and with skin in both a normal and pathological skin states has yet to be explored.

S. epidermidis, a coagulase-negative *Staphylococcus* (CoNS), is the most common clinical isolate from skin and is believed to represent greater than 90% of the aerobic resident flora. It has long been regarded as only a commensal bacterium, yet research has shown it has both a pathogenic role in nosocomial infections and acts a mutualistic organism that can enhance skin innate immunity by eliciting responses through TLRs. A microbe can therefore simply not be labelled as either a commensal or a pathogen in the context of SSIs. [55]

S. aureus is the microbe that has received most attention in studies of SSIs, perhaps due to its high virulence. In fact, it was the most commonly isolated organism in surgical site infections between 1986 and 1996 according to the CDC. [56]

In breast and gynaecological surgery, the most frequently isolated organisms included *S. aureus* (20%), CoNS (14%), *Enterococci* (12%), *E. coli* (8%), and *P. aeruginosa* (8%). [57] Similar studies examining dermatologic surgery are few with small sample sizes. However, it has been shown that reducing the nasal carriage of *S. aureus* reduces the risk for SSIs. A randomized controlled trial (RCT) published in 2018 showed that patients that received preoperative intranasal mupirocin had a reduced risk for SSI. [58] It appears that SSIs in dermatologic surgery are due to an endogenous origin. [58-60] This is also the case within other types of surgery, in which specific DNA fingerprint analysis confirmed that the source of SSIs was endogenous. [61] The way in which bacteria end up in dermatosurgical wounds is not fully understood but is believed to depend on nasal outflow and skin-to-skin contact and not through respiratory droplets, which mainly contain *Streptococcus,* an organism that rarely causes SSIs. [62] Studies on airborne transmission have shown conflicting results on its role in SSI development. [63, 64]

Risk factors

SSI development is multifactorial and related factors can generally be divided into either endogenous (patient-related) and exogenous. Most research within SSIs has focused on exogenous factors, but in recent years more focus has been placed on endogenous factors that could perhaps have a larger impact on SSIs. [65]

Examples of endogenous factors include age, concomitant diseases such as diabetes, immunosuppression, malnutrition, obesity, pulmonary disease, renal failure, smoking, and wound type. The latter has been a source of controversy within dermatologic surgery. As explained earlier, the surgical wound classification system (class I to IV) is based on general surgical procedures. An attempt to adapt it to dermatologic surgery was made in 1995 by adding examples of dermatologic surgery procedures to each wound category. [66] Most dermatological procedures were defined as "clean-contaminated". This was later changed in 2003 after classifying dermatologic surgery procedures as "clean". [67] Some authors [6] agree with this while others do not. [68]

Examples of exogenous factors are preoperative antisepsis, hair removal, intraoperative technique, type of surgical procedure, duration of surgery, use of antibiotic prophylaxis, use of surgical gloves, skin antisepsis, hand antisepsis, surgical face masks, and wound dressings. [69]

Available evidence-based guidelines for preventing SSIs examining endogenous and exogenous factors

are mainly based on general surgical procedures. [70, 71] The way in which these factors impact dermatologic surgery has not been fully explored since not so many RCTs exist. **Table 2** lists all of the published RCTs involving dermatologic surgery to date.

Antibiotic prophylaxis has received the most attention as an SSI preventative method, yet it was only this year that the very first RCT supporting its use in dermatologic surgery was published. Previous antibiotic prophylaxis recommendations such as the advisory statement published in 2008 by Wright *et al.* [72] or the guidelines proposed by Maragh *et al.* [73] relied solely on retrospective and prospective observational studies. More RCTs are needed in order to evaluate if antibiotics should be used as prophylaxis in dermatologic surgery. Up to this date, only three meta-analyses examining SSIs in dermatologic surgery procedures have been published. [74-76] In 2014, Nast *et al.* [74] published a meta-analysis examining complications in dermatologic surgery in patients receiving anticoagulative medications. However, the primary outcome of this systematic review was the risk of bleeding. The risk for SSI was only part of a secondary outcome of the study. It concluded that there was an insufficient number of studies to draw any conclusions on the effects of anticoagulative therapy on SSIs.

In 2015, Saco *et al.* [75] published a meta-analysis in which it was concluded that topical antibiotics should not be used to prevent SSIs in dermatologic surgery.

The last available meta-analysis, published in 2016 by Brewer *et al.* [76], showed that use of sterile gloves did not lower the risk for SSI in dermatologic surgery when compared to use of non-sterile gloves. However, only two out of the eight included studies [77, 78] were based on dermatologic surgery. Five were based on dental surgery, and the sixth on emergency surgery. [76]

Studies with lower quality evidence examining various factors are summarized in **Table 3**, and a noticeable point indicates that these provide conflicting results, making it impossible to yield any conclusions. In summary, more RCTs specific to dermatologic surgery are needed in which each factor believed to contribute to the development of SSI is examined.

Table 2. A list of all available RCTs examining SSI development in dermatologic surgery

First author	Year	Patients	Intervention	Authors conclusion (SSI rate in intervention group versus placebo)
Smith[58]	2018	1350	Preoperative intranasal mupirocin treatment versus placebo	Intranasal mupirocin reduced SSI incidence significantly (2% versus 4%)
Rosengren[79]	2018	154	2g cephalexin before flap and graft surgery versus placebo	Cephalexin reduced SSI incidence significantly (1.4% versus 11.6%)
Saleh[80]	2016	40	Soaking tie-over dressings with polyhexanide biguanide versus water	Polyhexanide biguanide increased SSI incidence significantly (40% versus 10%)
Heal[77]	2015	478	Use of sterile gloves versus non-sterile gloves	No significant difference in SSI incidence (8.7% versus 9.3%)
Cherian[81]	2013	693	Intranasal mupirocin preoperatively versus pre-and postoperative per oral cephalexin	Intranasal mupirocin reduced SSI incidence significantly (0% versus 9%)
Xia[78]	2011	60	Sterile gloves versus non-sterile gloves	No significant difference in SSI incidence (3.3% versus 1.6%)
Heal[82]	2009	972	Topical use of chloramphenicol versus paraffin	Chloramphenicol use reduced SSI incidence significantly (6.6 % versus 11%)
Dixon[83]	2006	778	Mupirocin ointment versus petrolatum versus no ointment applied to wounds postoperatively	No significant difference in SSI incidence (2.3% vs 1.6% versus 1.4%)
Campbell[84]	2005	142	Topical gentamicin versus petrolatum	No significant difference in SSI incidence (4.76% versus 6.67%)
Huether[85]	2002	1030	Intraincisional local anesthetic with clindamycin versus local anesthetic without clindamycin	Intraincisional clindamycin reduced SSI incidence significantly (0.5% versus 2%)
Griego[86]	1998	790	Intraincisional local anesthetic with nafcillin versus local anesthetic	Intraincisional nafcillin reduced SSI incidence significantly (0.2% versus 2.5%)
Smack[87]	1996	922	White petrolatum versus bacitracin applied to wounds postoperatively	No significant difference in SSI incidence (2% versus 0.9%)

Table 3. Other studies published examining SSIs in dermatologic surgery

First author	Year	Patients	Study design	Authors conclusion
Belakirski[88]	2018	177	Retrospective	Immunosuppression didn't increase SSI risk
Bari[89]	2018	271	Retrospective	SSI rates were lower in patients that underwent Mohs compared to wide local excision below knee. Subcuticular or vertical mattress suturing reduced SSI rate when compared to other sutures. Antibiotic prophylaxis did not reduce SSI. Doxycycline prophylaxis did not reduce SSI rate when compared to cephalexin prophylaxis
Liu[40]	2018	1977	Retrospective	Higher risk for SSI for location on ear, larger defects, closure with flaps, and secondary intention healing
Nuzzi[90]	2016	700	Retrospective	Surgery performed in a strictly sterile operation room did not lower risk for SSI. Incidence of SSI did not vary according to antibiotic usage, surgeon, age, lesion size, type, or location
Nasseri[91]	2015	338	Prospective observational	Using a single set of instruments for both tumor extirpation and repair stages of Mohs did not increase risk for SSI
Lee[92]	2015	414	Retrospective	Fusidic acid applied topically to wounds postoperatively had no effect on SSI rates when compared to petrolatum
Mehta[93]	2014	942	Retrospective	No difference in SSI rates between using sterile or non-sterile gloves
Liu[94]	2014	1415	Retrospective	Implementation of stricter sterilization guidelines did not lower risk for SSI
Tai[60]	2013	738	Prospective randomized	Decolonization with intranasal mupirocin reduced risk for SSI
Alam[95]	2013	20821	Prospective cohort	Mean patient age among registered SSIs was 70.8 years
Kulichova[96]	2013	1088	Retrospective	Contaminated preoperative skin or ulcerated tumor and advanced age increased risk of SSI development
Heal[97]	2012	972	Prospective observational	Incidence of SSI was in direct proportion to the patient's age
Bordeaux[98]	2011	1911	Prospective observational	No association between anticoagulative therapy and SSIs
Rogers[68]	2010	1000	Prospective observational	Patients that didn't receive antibiotic prophylaxis prior to Mohs surgery had an extremely low SSI rate
Cordova[59]	2010	963	Prospective observational	Preoperative methicillin-resistant Staphylococcus aureus (MRSA) screening and subsequent decolonization in Mohs surgery reduced SSI in MRSA carriers
Dixon[99]	2009	4197	Prospective observational	Diabetes was a risk factor for SSI
Dixon[100]	2009	4197	Prospective observational	Smoking was not a risk factor for SSI
Rogues[101]	2007	3491	Prospective observational	SSI incidence was higher in patients that underwent reconstruction vs excision, male patients, patients on immunosuppressive therapy, and when surgeons didn't use sterile gloves
Dixon[38]	2006	2424	Prospective observational	Lips, ears, perineum, inguinal area, and below knee were sites associated with a higher risk of SSIs. Flaps and graft reconstruction surgery were associated with a higher SSI risk compared with other types of surgery
Wahie[102]	2006	100	Prospective observational	SSIs occurred more frequently in smokers, when biopsies were taken below the waist, in patients taking corticosteroids, and when procedures were performed in the ward instead of operating room
Amici[41]	2005	3788	Prospective observational	Prolonged operation duration was a risk factor for SSI
Futoryan[39]	1995	1047	Retrospective	Large defects and ear surgery were associated with higher risk for SSI

Aims

The overall aim of this thesis was to enhance our understanding of SSIs in dermatologic surgery by examining clinical, diagnostic, and pathogenic aspects.

Focus was on four main questions:

- How does bacteria behave in wounds during normal and pathological healing after dermatologic surgery?
- Can we prevent SSIs within one type of dermatologic surgery?
- Can we predict SSIs using biomarkers extracted from wound dressings?
- How well are SSIs defined in dermatologic surgery?

Materials and Methods

Study I

Patients

This study was designed as a prospective descriptive study. Eighteen patients scheduled for facial full-thickness skin grafting **(Figure 5)** at the Department of Dermatology at Skåne University Hospital were recruited. We limited inclusion to surgery localized to the face because bacterial loads are known to vary from one anatomical site to another. [103]

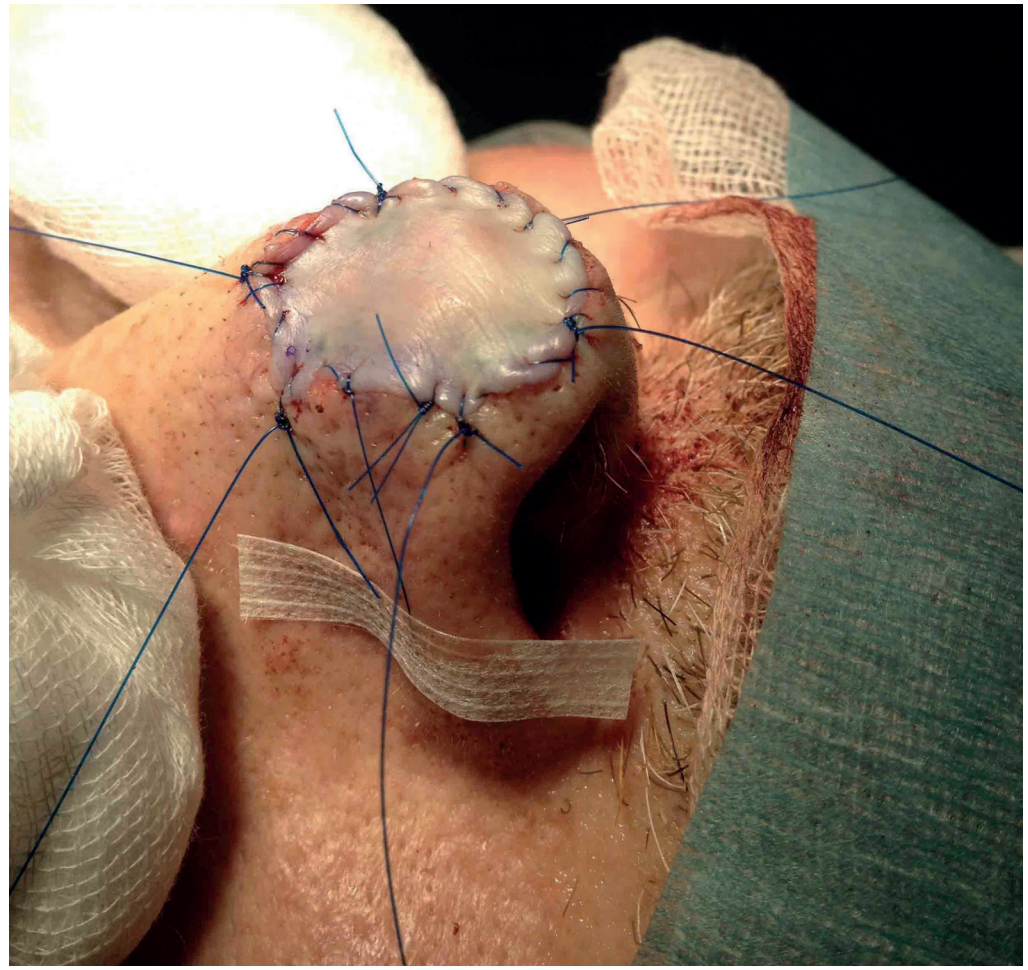

Figure 5. Photograph of a full-thickness skin graft sutured to a nasal wound. Published with patient's permission.
(Photo: Department of Dermatology and Venereology, Skåne University Hospital, Lund)

Sampling method

Levine *et al.* established one of the most accurate techniques for swabbing believed to correlate well with quantitative biopsies. [104, 105] It involves swabbing a wound area of 1 cm^2. In order to better conform to the wound's anatomy in this study we modified Levine's technique by swabbing a standard circular area. Copan® swabs were used **(Figure 6)**. Bacterial samples were collected from wounds at three intervals: (1) Before surgery (BS) prior to scrubbing with antiseptics; (2) End of surgery (ES) directly after suturing the graft; and (3) One week after surgery (1W) after removing the tie-over dressing.

Serial dilutions of the swab fluids were then plated onto blood agar, and the CFUs were counted. Qualitative analyses of all swabs were carried out according to standard methods and performed at the Clinical Microbiology Laboratory at Skåne University Hospital in Malmö.

Clinical assessment of postoperative course

All wounds were monitored by a single investigator one week after surgery. A wound was considered infected if three or four of the following features were present: (1) discharge; (2) pain; (3) induration; and (4) erythema.

The complete postoperative course until complete healing had been achieved was followed for all patients by reviewing their medical journals.

Figure 6. Photograph of a Copan® swab used for collecting bacteria in study I. *(Photo: Karim Saleh)*

Study II

Patients

This was a prospective, double-blinded, randomized, placebo-controlled trial registered with ClinicalTrials.gov (NCT02253069). Patients over age 18 scheduled for facial full-thickness skin grafting were asked to participate in this trial. Exclusion criteria included diabetes, treatment with antibiotics within the last four weeks prior to surgery, and planned antibiotic therapy. The latter criterion was important due to the findings in study I.

Power analysis

In a previous *in vitro* study, a reduction of >5 $\log_{10}$ was achieved with a concentration of 0.02% polyhexanide biguanide (PHMB) against *S. aureus*. [106] We hypothesized that application of 0.1% PHMB as found in the commercially available Prontosan® Wound irrigation solution (B. Braun Medical, Switzerland) would at least reduce bacterial load in wounds by half versus placebo. In order to obtain 80% power with an α-value of 0.05, it was calculated that 16 patients were required in each group. By including 20 patients in each group in this trial to allow for dropouts, noticeable differences in bacterial reduction would be detected.

In vitro antibacterial assay

In vitro assays were carried out prior to the main RCT to verify the bactericidal effect of Prontosan® solution. Todd-Hewitt (TH) agar plates were streaked with *S. aureus* ATCC 29213 and *S. epidermidis* ATCC 14909. Polyurethane dressings soaked with Prontosan® solution or sterile water were applied on top to simulate an *in vivo* situation in which the dressing is applied to a wound. The zone of inhibition around the discs was measured.

Preparation of Mepilex® dressings

Prior to surgery, seven circular dressing templates with varying diameters ranging from 10 to 34 mm were cut from Mepilex®. The liquid volume required to achieve 70% wetting was calculated. Seventy percent wetting was chosen to avoid leakage after surgery. For each dressing template, 20 test tubes were prepared containing sterile water and 20 contained Prontosan® solution. These were marked with either A or B by an external investigator not involved in this trial and blinded to the nurse, surgeon, and principal investigator. Prontosan® solution is similar to water in that it is both colorless and odor-free. The dressing templates were used for proper determination of the volume of Prontosan® or sterile water required for wetting tie-over dressings used during surgery.

Intervention

At the end of each operation, once the skin graft had been sutured to the wound, a tie-over dressing **(Figure 7)** was cut from Mepilex®. It was then soaked with either Prontosan® solution or sterile water according to the randomization protocol.

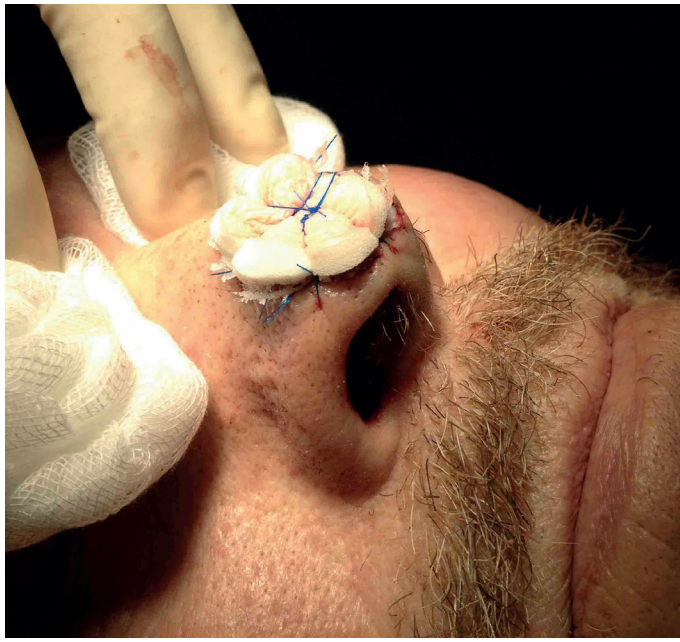

Figure 7. Shows a tie-over dressing sutured on top of a graft on a nose. Published with patient's permission. *(Photo: Department of Dermatology and Venereology, Skåne University Hospital, Lund)*

Bacterial analysis

Bacterial samples were blindly collected from each patient using Eswabs (Copan, Brescia, Italy) by one principal investigator. This procedure was done during the same three different phases examined in study I. Before surgery (BS), at the end of surgery (ES), and 1W after removal of the tie-over dressing. An additional swab was rotated in the patient's naris that was closest to the neoplasm planned for excision.

All swabs were analysed quantitatively by calculating CFU per cm² of area swabbed using the same methods as in study I. Bacterial species were determined via matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.

Clinical assessment

All patients were scheduled for a single follow-up seven days after surgery. Skin grafts were assessed in terms of redness, edema, discharge, graft take, and pain resulting in an overall assessment by the blinded principal investigator classifying a wound as infected or non-infected. No scoring system was used for this purpose. Digital photographs were taken of all wounds pre- and postoperatively.

Study III

Patients and biological samples

This was a descriptive *in vitro* study. Wound fluids in this study were extracted from tie-over dressings belonging to 20 patients that constituted the control group in study II. [80]

In vitro assays

Several assays were used in this study. First, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli (1970) using a Novex® pre-cast gel system (Invitrogen, Carlsbad, CA) on Tricine gels using wound fluid samples containing 20 µg protein/sample **(Figure 8)**.

Next zymography was carried out by mixing 5 µg protein from each sample with sample buffer and separating it on 10% polyacrylamide gels.

Total protease activity from each wound fluid was then determined by the azocasein method as described by Tomarelli. [107] One-hundred micrograms from each sample were added to 50 µl azocasein substrate.

Thereafter, NF-κB was assessed in THP1-Xblue™-CD14 reporter cells (InvivoGen, San Diego, CA) according to the manufacturer´s instructions.

Finally, IL-6 and TNF-α concentrations were assessed using a human IL-6 and TNF-α Kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions.

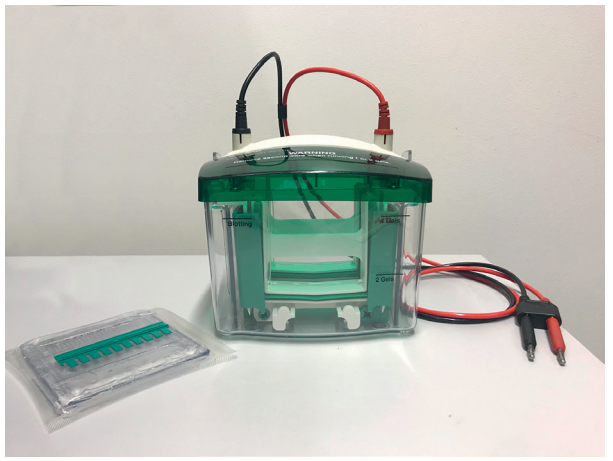

Figure 8. Photograph of the electrophoresis chamber used for SDS-PAGE. *(Photo: Karim Saleh)*

Clinical assessments of wounds

Each wound photograph was blindly assessed by two independent reviewers using a three-step scale (low, moderate, high) for degree of inflammation.

Microbiota

Quantitative and qualitative data concerning microbiota was retrieved from study II.

Study IV

Study design

An anonymous electronic survey was conducted using the REDCap software, [108] and distributed randomly by email to physicians from the British Society for Dermatological Surgery (BSDS), the American College of Mohs Surgery (ACMS), the Swedish Society for Dermatology and Venereology (SSDV), the European Society for Micrographic Surgery (ESMS) and to personal contacts of the authors. Only board-certified dermatologists and dermatology residents that fully completed the survey were included in the data analysis.

The questionnaire consisted of two sections. The first section included demographics and basic characteristics of the respondents. In the second section, the respondents were presented with eight clinical photographs of as many patients taken one week after facial full-thickness skin grafting **(Figure 9).**

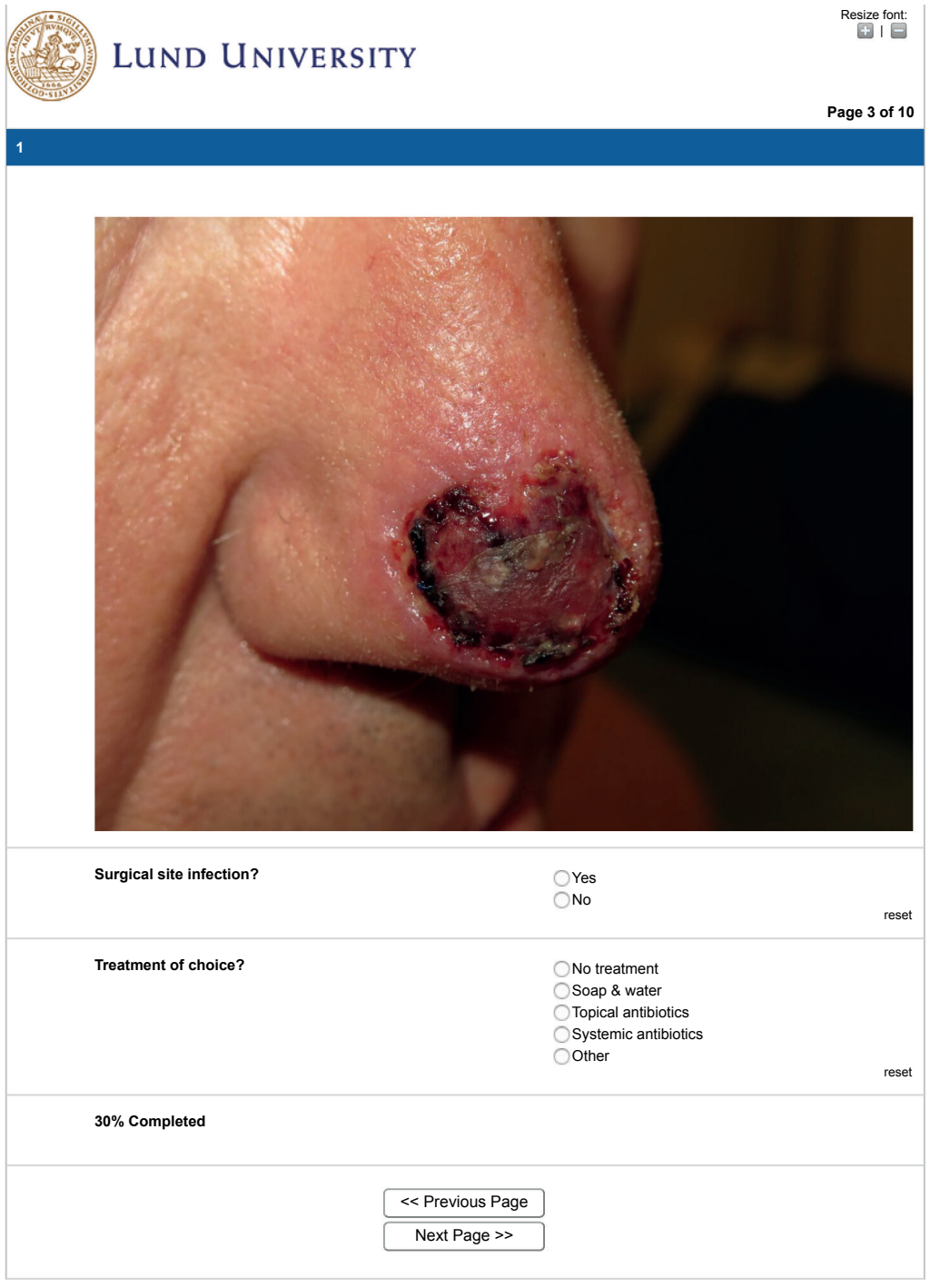

Figure 9. illustrating one of the cases from the electronic survey.

The cases were randomly selected from study II. [80] In order to aid the respondents' SSI assessments, all photographed wounds in this study were from patients who exhibited no pain/tenderness, heat, or purulent discharge during palpation at the time they were photographed. This information was provided to all respondents. For each photograph, the respondents had to classify the wound as infected or not. Subsequently, respondents were asked to suggest the most appropriate treatment through a multiple-choice questionnaire with several options: (1) no treatment; (2) soap and water; (3) topical antibiotics: (4) systemic antibiotics; or (5) other. Prior to sending the survey, three investigators blindly assessed the photographs in terms of visual criteria. A 4-step scale (none, mild, moderate, or severe) was used for assessing erythema, presence of black and yellow crusts, and epidermolysis. The graft take was described as 0%–25%, 26%–50%, 51%–75% or 76%–100%. Subsequently, an average of these assessments was agreed upon.

Statistics

Study I

Statistical analyses were carried out using SigmaStat (Systat Software, Point Richmond, CA). Median CFU/swab sampled BS and ES were compared using a Mann-Whitney test. Bacterial growth change (BGC) from BS to 1W was calculated using the equation:

$$BGC = \frac{\text{CFU sampled at 1W}}{\text{CFU sampled BS}}$$

BGC values were compared using a Mann-Whitney test. A P value ≤ 0.05 was considered significant.

Study II

Statistical analyses were performed with SPSS v.22 software (SPSS Inc., Chicago, IL). Bacterial load reduction was calculated by using the following formulas:

CFU(1W)-CFU(BS), CFU(1W)-CFU(ES), CFU(1W)/CFU(BS), and CFU(1W)/CFU(ES). All median values obtained were compared using a Mann-Whitney U test in order to examine whether differences existed between the groups. Differences in categorical variables were determined using the chi-squared test. Differences in continuous variables were estimated using Student´s t test. Statistical significance was set at $P < 0.05$.

Study III

All statistical evaluations were performed using GraphPad Prism software 7.0 with ns not significant, $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, and $****P \leq 0.0001$. In order to compare more than two groups, a one-way analysis of variance (ANOVA) with the Kruskal-Wallis test was used. Differences in categorical variables were determined using chi-squared test.

Study IV

For each respondent, an SSI score was calculated. This was measured as the percentage of wounds assessed as infected. Similarly, topical antibiotic and systemic antibiotic scores were also calculated for each respondent measured as the percentage of wounds treated by topical and systemic antibiotics, respectively. All scores were analysed using SPSS v.22 software (IBM Corp, Armonk, NY). Linear regression models adjusted for other characteristics were used to determine differences. Statistical significance was set at $P < 0.05$. Respondent characteristics were presented using frequencies and descriptive statistics. Inter-observer agreement for all assessments was measured using Fleiss kappa. [109]

Ethics

Study I: Ethical approval for this study was granted by the ethical committee in Malmö/Lund, registration number (2008/646). Written consent was obtained from all patients.

Studies II, III, and IV: These studies were approved by the ethical committee in Malmö/Lund, registration number (2013/762). Written consent was obtained from all patients.

Results

Study I

Saleh K, *Sonesson A, Persson B, Riesbeck K, Schmidtchen A. A descriptive study of full-thickness surgical wounds in dermatologic surgery. Dermatol Surg. 2011 Jul;37(7):1014-22***.**

Sixteen out of 18 patients were colonized with bacteria intraoperatively. Bacterial loads measured at the end of surgery were significantly lower than preoperative levels in all patients. ($P < 0.001$). **Figure 10** illustrates bacterial loads from one patient.

There was a statistically significant difference when comparing bacterial growth changes between patients that received antibiotic treatment and patients that did not ($P = 0.02$). Patients that received antibiotics had lower postoperative bacterial loads compared to preoperative loads, and patients that received no antibiotics had higher bacterial loads postoperatively compared to preoperative ones.

Anticoagulative therapy, diabetes, smoking, tumor ulceration, and gender were not associated with any statistical difference in bacterial growth change. Postoperative bacterial loads correlated well with the occurrence of a complicated postoperative outcome ($P < 0.001$).

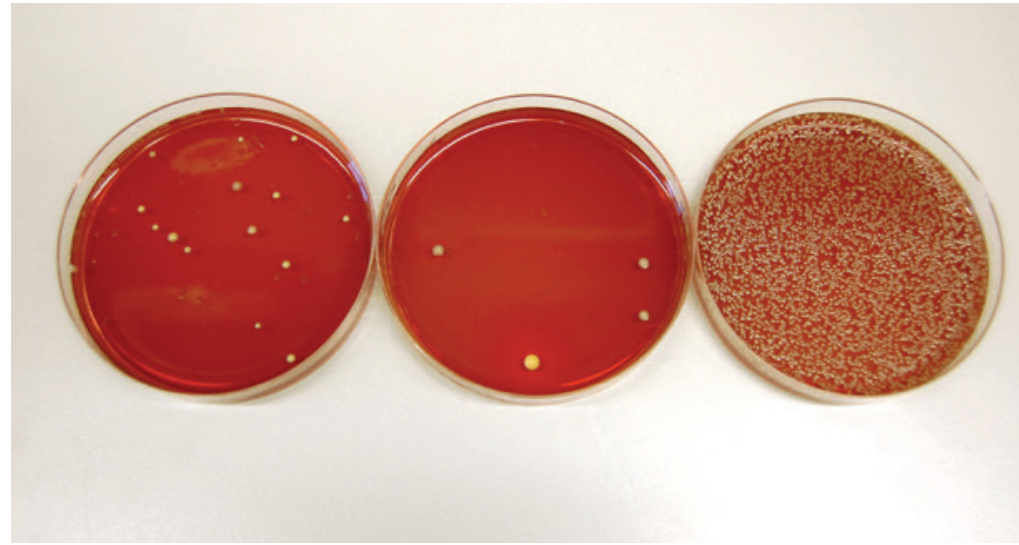

Figure 10. Bacteria growing on blood agar plates from swabs taken from a patient's wound at three different intervals. From left to right: Before surgery, at the end of surgery, and one week after surgery. *(Photo: Karim Saleh)*

Study II

Saleh K, *Sonesson A, Persson K, Riesbeck K, Schmidtchen A. Can dressings soaked with polyhexanide reduce risk for surgical site infections in full-thickness skin grafting? A randomized controlled trial. J Am Acad Dermatol. 2016 Dec;75(6):1221-1228.*

In vitro trials

Our preliminary in vitro trials showed *S. aureus* and *S. epidermidis* growth inhibition when testing Mepilex® dressings containing PHMB. **Figure 11** illustrates an example of one of the trials. The primary aim of this RCT was to determine if adding PHMB to tie-over dressings reduced postoperative bacterial loads.

Intervention

Patients belonging to the intervention group had a higher incidence of SSIs compared to the placebo group (chi-squared 4.8, $P = 0.028$).

Bacterial dynamics

Bacterial loads measured one week after surgery were significantly higher in patients with an SSI ($P = 0.1$). The presence of *S. aureus* in wounds after one postoperative week also resulted in higher bacterial loads ($P = 0.03$). The absence of intranasal *S. aureus* before surgery resulted in significantly lower postoperative bacterial loads ($P = 0.1$).

CoNS and *S. aureus* were the predominant species in all swabs in this study. Six out of 10 infected wounds contained species other than *S. aureus*.

Figure 11. Six 8 mm dressings placed on TH plates growing *S. aureus*. Upper and middle row dressings were saturated with different concentrations of PHMB. Bottom row dressings were saturated with distilled water and served as controls. *(Photo: Karim Saleh)*

Study III

Saleh K, *Riesbeck K, Schmidtchen A. Inflammation biomarkers and correlation to wound healing after full-thickness skin grafting. 2018. Submitted.*

Wound fluids

Wound fluids were successfully extracted from wound dressings collected from study II. These fluids were then analysed by SDS-PAGE and showed bands ranging from 10 kDa up to higher molecular weight proteins of 100 to 200 kDa. Proteases from all wound fluids were visualized using zymography.

Wound inflammation

Wounds exhibited variable degrees of inflammation **(Figure 12)**. Wounds with a clinically higher degree of inflammation had statistically higher levels of NF-κB activation and IL-6 and TNF-α concentrations ($P < 0.05$). Wounds with a higher degree of inflammation also had higher levels of MMP activity and total protease activity ($P < 0.05$).

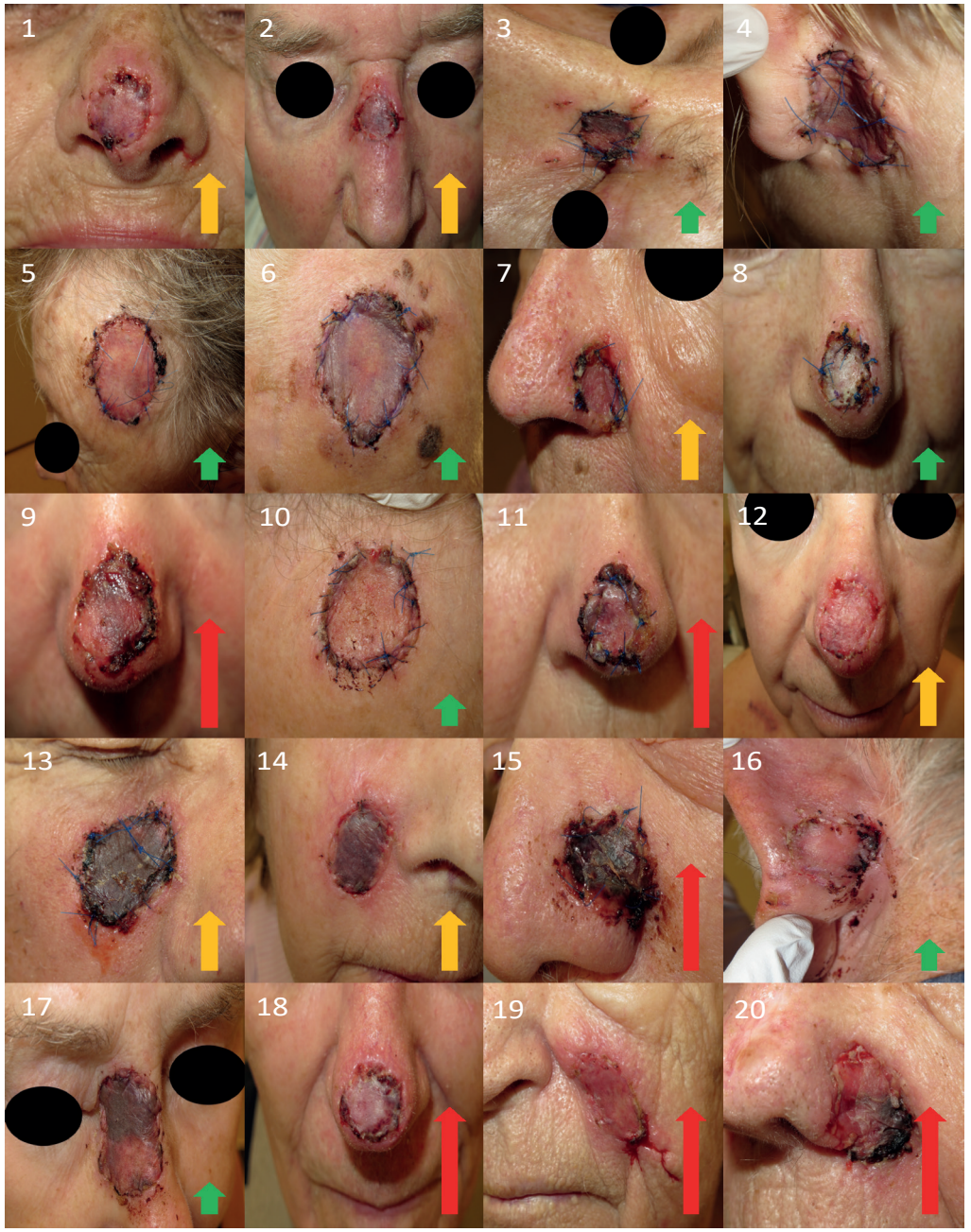

Figure 12. Photographs of all wounds. Wound inflammation was assessed as mild (green arrow), moderate (orange arrow), or high (red arrow). Published with the patients permissions. *(Photo: Karim Saleh)*

Study IV

Palmgren J, Paoli J, Schmidtchen ***A, Saleh K.*** *Variability in the diagnosis of surgical site infections after full-thickness skin grafting: An international survey.* Br J Dermatol. 2018 Dec 10. doi: 10.1111/bjd.17517. E-publication ahead of print.

Diagnostic agreement

Three-hundred ninety-three physicians that completed the survey had only a slight inter-observer agreement when all wounds were assessed in terms of SSI presence or absence (Fleiss kappa coefficient = 0.19).

The majority of respondents were board certified dermatologists, involved in assessment of surgical wounds with a surgical experience involving full-thickness skin grafting.

SSI scores

Male physicians had lower SSI scores than female physicians ($P = 0.03$).

Board-certified dermatologists had lower SSI scores than residents ($P = 0.001$). Significantly lower SSI scores were also observed for physicians who regularly assessed SSIs ($P = 0.03$) and physicians performing full-thickness skin grafting ($P < 0.001$).

Wound treatment

All wounds were treated differently by all respondents **(Figure 13)**.

Teledermatology

A third of all respondents were already involved in teledermatology, and more than half believed that they would be involved in teledermatology within the next five years. There was no difference in SSI scores between physicians involved in teledermatology and those who were not ($P = 0.8$).

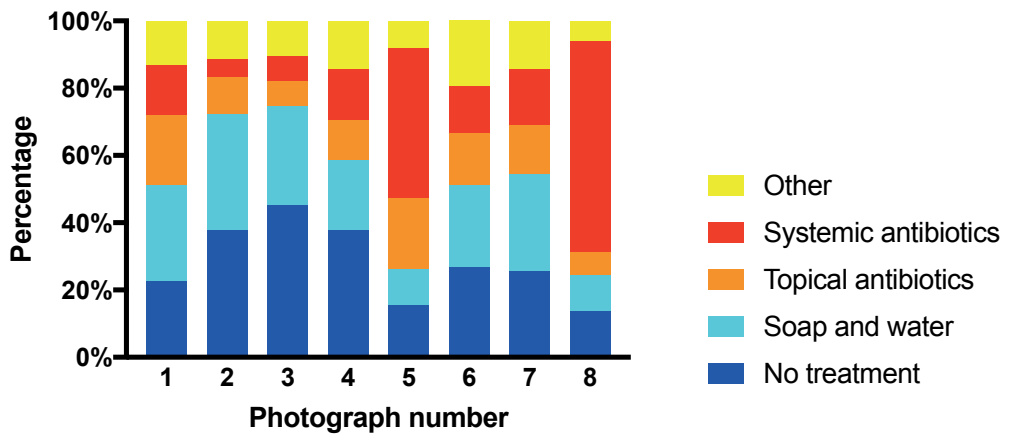

Figure 13. illustrates the way in which each wound was treated by all respondents. *(Artwork: Karim Saleh)*

Discussion

Study I

Study I was a descriptive study in which no prior power analysis had been performed. No similar studies within dermatologic surgery had been published. We aimed to study 20 patients but ended after 18 patients when we had interesting results. Our aim was to investigate bacterial quantities and species during different time intervals in one type of dermatologic surgery and to see if these variables could be diagnostic of an SSI. The reasons for choosing full-thickness skin grafting was because this type of surgery has higher SSI rates compared to other types of surgery. [72] We also wanted to analyse different patient-related factors. A major limitation of this study was the presence of several confounding factors that could have affected SSI development. These factors included age, gender, diabetes, smoking, anticoagulative therapy, immunosuppression, and antibiotic prophylaxis. Regardless, we were able to establish a trend in the bacterial dynamics of a graft wound and noticed that postoperative bacterial loads were higher than starting preoperative levels in patients not treated with antibiotics and suppressed postoperative bacterial loads in patients given antibiotics. This trend was not seen when other patient-related factors were examined. A larger study with a multivariate analysis would have been able to confirm or reject our observed trend. The most significant finding in this study was the correlation between high postoperative bacterial loads and clinical outcome. This is in line with the earliest studies of SSIs illustrating the importance of wound bacterial load. [52, 110, 111] This has, on the other hand, been questioned recently as it is hypothesized that the pathogenesis of SSIs is far more complicated than bacterial quantities. Nevertheless, results from study II confirmed the positive correlation between bacterial quantities and SSI development.

Analysis of intraoperative bacterial species was not predictive of SSI development. In some patients, the bacterial species causative of postsurgical complications differed from the species isolated intraoperatively. Previous studies trying to predict SSIs by analysis of intraoperative bacterial species have shown conflicting results. [112-117] It should be noted that these studies were not based on dermatologic surgery.

In this study, we collected bacteria using swabs. A limitation of this study was the use of a predetermined area of swabbing that was set to an area of

4.9 cm^2 corresponding to the area within a plastic circle that we had used at our labs when applying Kligman's technique for quantifying bacteria. [118] This area covered graft wound areas in most of the cases, but in a few cases the graft wound area was larger than 4.9 cm^2 and resulted in swabbing only part of the wound. Another limitation in this study was the lack of donor transplant skin swabbing. The skin microbiota from the donor skin was probably different from the graft wound. The implication of this requires exploration in further studies swabbing pre, intra, and postoperative samples from both donor skin and graft wounds in order to observe any differences among these groups.

Study II

The main aim of this study was to assess if PHMB, a novel antiseptic, [119-124] could reduce postoperative bacterial loads in a full-thickness skin grafting wound, and thus reduce SSI occurrence. Our preliminary *in vitro* trials showed PHMB inhibited growth of both *S. aureus* and *S. epidermidis* in different assays. We chose to use PHMB because another study had shown numerous advantages of its use, including broad antibacterial activity, good cell and tissue tolerability, low risk of contact sensitization, promotion of wound healing, and no development of bacterial resistance. [120]

In this RCT, we used the same method of bacteria sampling as in study I, except that each graft wound was fully swabbed regardless of its area. The area was roughly measured using a prepared size template in order to calculate bacterial load per cm^2. This provided a more standardized bacterial load measurement when comparing all wounds. In this study, we excluded diabetic patients and those that were recently treated or scheduled for antibiotic therapy since these factors have shown a strong association with SSIs. [99, 125] We were not able to exclude factors such as immunosuppression and anticoagulative therapy due to a limited timeframe for patient collection. On the other hand, studies available have not been able to establish a correlation between immunosuppression or anticoagulative therapy and SSIs in dermatologic surgery. [74, 126, 127]

The main finding of this study was that PHMB surprisingly caused an increase in the risk of SSI. We hypothesized, in concordance with previous studies, [87] that PHMB might have supressed growth of commensals that might have caused an overgrowth of pathogenic species, which might have contributed to SSI development. Due to the limited number of patients in this study, we were not able to draw any conclusions on the protective role of commensals and how PHMB affects them.

The most frequently isolated species from wounds in this study were *S. aureus* and coagulase negative *Staphylococci*, which reinforced our findings in study I. An interesting observation in this study was the extensive variety of preoperative bacterial species. A recent study showed that neoplasms had a high bacterial variety, which could explain our findings since preoperative swabbing always involved a neoplasm. [128]

Study III

In this study, our aim was to extract wound fluids from tie-over dressings obtained from study II and analyse these *in vitro* in terms of protein composition, proteinase activity, levels of pro-inflammatory factors, and how this correlated to clinical degree of inflammation. Ultimately, we wanted to see if analysed biomarkers could aid diagnosis of SSIs. To the best of our knowledge, no similar studies on full-thickness skin grafting wounds exist.

Protein composition was similar to previous results involving collected wound fluids from chronic wounds, [129, 130] thus validating our methodology in using SDS-PAGE. We chose to use zymography to analyse proteinase activity since this is a widely used method in studies of acute and non-healing ulcers. [131-133]

Since this study was an observational descriptive study, we analysed total activity of the wound fluids semi-quantitatively by examining total band intensity on our zymograms.

Our results illustrating elevated proteinase activity in highly inflamed wounds was in agreement with studies demonstrating that high levels of proteases are a marker of poor wound healing. [132, 134, 135]

Study IV

Study IV was a reliability study in which an electronic survey of eight surgical wounds was sent to dermatologists from different countries. The aim was to assess the variability in the subjective diagnosis of SSIs using the most common SSI definition. No previous studies examining photographic SSI assessments in dermatologic surgery have been published. We found a broad inter-rater variability in SSI diagnosis. Assessments varied based on country of practice, gender, and clinical and surgical experience. However, no multivariate analysis was possible due to the small respondent sample. Another interesting finding was how many of the respondents were already involved in teledermatology. Our results also suggested a degree of correlation between in-person clinical evaluations and photography assessments. The wounds with the highest suspicion of SSI as determined by the respondents were wounds that were clinically assessed as infected. However, further studies are needed to evaluate this correlation. Assessing wounds after full-thickness skin grafting proved to be hard not only clinically but using only photographs. This led to different treatment choices for each case. When respondents agreed in terms of an infected or non-infected wound, their treatment still differed. **Figure 14** below illustrates a selection of cases and the way in which these were treated based on a wound being assessed as an SSI in cases 1, 5, and 8 and a wound being assessed as non-infected in case 2.

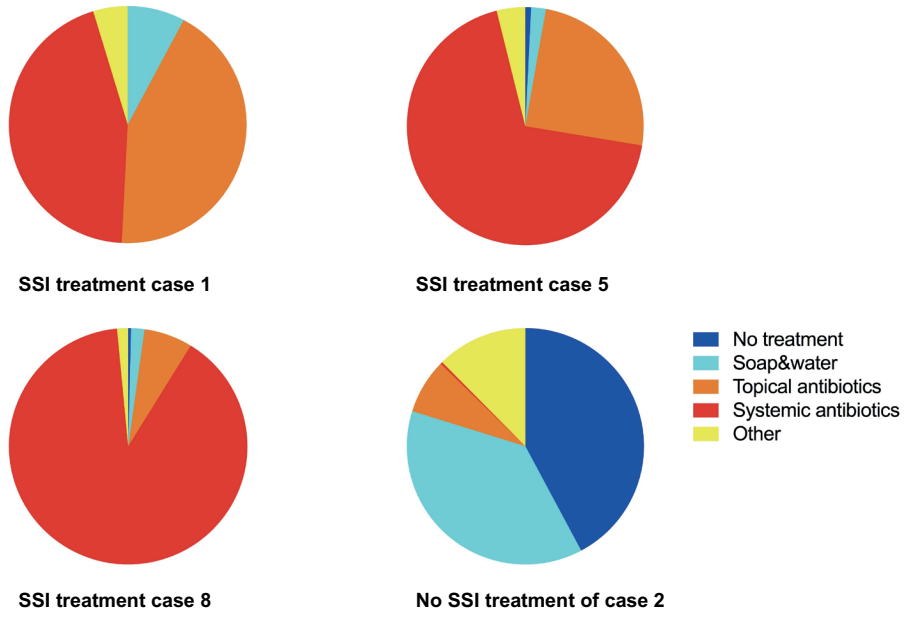

Figure 14. Treatment of different cases depending on wound assessment. *(Artwork: Karim Saleh)*

Conclusions

Study I

Quantifying bacteria from wounds following full-thickness skin grafting can be used as an important parameter in assessing wound healing.

Study II

Soaking tie-over dressings with PHMB in full-thickness skin grafting had no effect on postoperative bacterial loads and increased the risk of SSI development.

Study III

Biomarkers obtained from tie-over dressings can serve as diagnostics for assessment of wound healing.

Study IV

There was a broad inter-rater variability in the diagnosis of SSI among dermatologists when assessing photographs of full-thickness skin grafting wounds.

Future remarks

RCT studies of SSIs within dermatologic surgery are few and more should be initiated. Before this process, we need a better and more objective definition of an SSI in dermatologic surgery. Perhaps biomarkers similar to the ones we have examined could prove to be beneficial. Once we have a clear definition of an SSI, future studies testing different preventative measures believed to lower risk of an SSI will be more accurate. Many preventative guidelines used within our field of surgery are extrapolated from studies involving other surgery types. The vast majority lack strong evidence and are yet still used routinely in the belief that they lower the risk for SSI, accounting for unnecessary costs for our healthcare systems. We need to start questioning every practice in a surgical room in addition to all routines applied once we discharge our patients after surgery. Novel drugs are needed to combat SSIs prophylactically since antibiotics have not been so promising so far. Excess wound inflammation needs to be reduced. Microbiota during wound healing deserves further studies, especially the dynamics at different time intervals and the way in which all species interact. In the future, we hope that we can predict which wounds will become infected and have an effective prophylaxis that will eradicate the risk for a wound to become infected because that imposes a risk for the patient and causes pain and unnecessary costs to the healthcare systems, resulting in poor wound cosmesis.

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Surgical Site Infections in Dermatologic Surgery

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Printed by Media-Tryck, Lund 2019 NORDIC SWAN ECOLABEL 3041 0903

LUND UNIVERSITY

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Dermatology & Venereology
Department of Clinical Sciences, Lund

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2019:14
ISBN 978-91-7619-743-1
ISSN 1652-8220