Studying the healing and long-term outcomes of two partial thickness wound models using different wound dressings

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Till mina barn Selma och Matheo

You should know

anything in this book might be wrong

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ABBREVIATIONS

BMI	Body Mass Index
BUD	Burn Unit Database
CE	Conformité Européenne
CI	Confidence Interval
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CRP	C-Reactive Protein
DAMPs	Damage-Associated Molecular Patterns
DDB	Deep Dermal Burn
ECM	Extra Cellular Matrix
EGF	Epidermal Growth Factor
FGF	Fibroblast Growth Factor
HTS	Hypertrophic Scarring
IGF-1	Insulin-like Growth Factor 1
IL-1	Interleukin-1
ITT	Intention To Treat
LOS	Length Of (hospital) Stay
MMP	Matrix Metalloproteinase
NPWT	Negative Pressure Wound Therapy
OR	Odds Ratio
PDGF	Platelet Derived Growth Factor
POSAS	Patient and Observer Scar Assessment Scale
PPPM	Parents' Postoperative Pain Measure
PX	Porcine Xenograft
RCT	Randomised Controlled Trial
SOC	Standard Of Care
STSG	Split Thickness Skin Graft
TBSA	Total Body Surface Area
TIMP	Tissue Inhibitor of Metalloproteinases
TGF	Transforming Growth Factor
TNF- α	Tumour Necrosis Factor alpha
VEGF	Vascular Endothelial Growth Factor
VIF	Variance Inflation Factor

ABSTRACT

Background: Safe and effective wound dressing treatments are important for proper wound healing. Such procedures therefore need to be evidence-based regarding the most important outcome measures such as healing time, less discomfort for the patient, duration of hospital care and, importantly, less scarring. As the relation between longer healing times and more severe scarring is known, it is important to find dressing treatments that reduces such complications by providing fast and proper wound healing. In this thesis, four established wound dressing treatments (hydrofibre covered with film; porcine xenografts and polyurethane foam, with and without silver), were evaluated for two types of acute, partial thickness wounds: split thickness skin graft (STSG) donor sites and partial thickness burn wounds in two randomised, controlled clinical trials (RCT) with long-term scar follow ups. The relations between factors thought to influence wound healing and scarring as sex, infection, wound extent and depth, healing time and skin grafting were also investigated in these two wound models.

Methods: Data from these trials were collected on sex, infection rates, wound depth and extent, need of skin grafting, healing times and scarring frequency together with demographic data. Scars were evaluated at 8 years in Study II and III and at 6 and 12 months after injury in Study V.

Results: Two dressing treatments; hydrofibre covered with film and porcine xenografts gave significantly faster healing of the STSG donor sites than the standard of care (SOC) dressing, the polyurethane foam. The hydrofibre was thereafter implemented as the new SOC at the department. The long-term scar follow up showed that the hydrofibre group was most satisfied with their donor site scar, providing further evidence for the implementation of this dressing strategy. From the observer's perspective no differences were found between these treatment groups. For partial thickness burns the treatment with a silver-containing foam dressing showed significantly shorter healing time, whereas for the scars, no difference between dressing groups could be detected. A number of factors were identified that affected healing time: for donor sites only male sex was associated with shorter healing time. Sex was also the only factor that influenced donor site scarring, where female patients, both subjectively and objectively, were rated with higher scores (worse outcome). For partial thickness burns a larger extent of the burn wound, presence of deep dermal burns, and the need of

skin grafting, all had a negative impact on both healing time and final scar. The final scar was also significantly affected by longer wound healing times and infection.

Conclusion: The results suggest that the use of hydrofibre dressings covered with film on donor sites resulted in positive short-term and long-term outcomes. Regarding partial thickness burns, silver foam dressing resulted in faster healing but as for the final scar, no difference could be seen. Several factors were associated with longer healing times and more severe scarring such as: female sex, larger burns, deep dermal burns, skin grafting, and infection. Longer healing times were related to more severe scarring.

SVENSK SAMMANFATTNING

Bakgrund: Säkra och effektiva förbandsbehandlingar är av stor klinisk betydelse. Därför behövs evidensbaserade sårbehandlingar med fokus på de viktigaste utfallen som läkningstid, minskning av patientens lidande, vårdtid och mindre ärrbildning. Eftersom förhållandet mellan längre läkningstid och mer allvarlig ärrbildning är känt är det viktigt att hitta sårbehandlingar som minskar komplikationer, ger snabb läkning av såret och acceptabla ärr. I denna avhandling utvärderades etablerade sårbehandlingar (hydrofiber täckt med film, xenotransplantat från gris och polyurethanskumsförband, med och utan silver), för två typer av akuta delhudskador; tagställen för delhudstransplantatet och dermala brännskador, i två randomiserade kontrollerade kliniska studier. Förhållandet mellan påverkansfaktorer såsom kön, infektion, sår djup, såromfattning, läkningstid och hudtransplantation och utfall för läknings- och ärrresultat har också undersökts.

Metoder: Data för kön, infektionsfrekvens, sår djup och omfattning, behov av hudtransplantat, läkningstid och ärrbildning från två randomiserade kontrollerade studier samlades in tillsammans med demografisk data. Ärren utvärderades efter 8 år i studie II och III samt vid 6 och 12 månader efter skada i studie V.

Resultat: Två sårbehandlingar; hydrofiber täckt med film och xenotransplantat från gris visade signifikant snabbare reepitalisering av tagställen än standardförbandet (polyuretanskum). Hydrofibern implementerades som den nya standardbehandlingen på kliniken. Den långsiktiga ärruppföljningen avslöjade att gruppen med hydrofiber var den mest nöjda med sina tagställes-ärr, vilket gav ytterligare styrka till genomförandet av behandlingen. Ur observatörspektivet hittades inga skillnader mellan dessa grupper. För dermala brännskador gav behandlingen med silverinnehållande skumförband signifikant bättre läkningsresultat, för ärrbildning upptäcktes inte någon skillnad mellan grupperna. Ett antal faktorer som påverkade läkningstider identifierades; för tagställen gav endast manligt kön kortare läkningstider. Kön var också den enda faktorn som påverkade tagställets ärrresultat där kvinnor, både subjektivt och objektivt, bedömdes med högre poäng (sämre utfall). För dermala brännskador var det faktorer såsom en större omfattning av brännskadorna, närvaron av djupa dermala brännskador och behovet av hudtransplantation som hade negativ påverkan på både läkningstider och slutliga ärrresultat. Det slutliga ärrutfallet påverkades också signifikant av längre läkningstider och infektion.

Konklusion: Data tyder på att användningen av hydrofiber täckt med film på tagställen gav positiva resultat både på kort- och lång sikt. När det gäller förband för dermala brännskador resulterade silverskumförband i snabbare läkning men vad gäller ärr kunde ingen skillnad hittas. Flera faktorer var relaterade till längre läkningstider och mer ärrbildning som kvinnligt kön, större omfattning av brännskador, djupa dermala brännskador, hudtransplantation och infektion. Längre läkningstider var relaterat till mer allvarlig ärrbildning.

LIST OF ORIGINAL PAPERS

This thesis is based on the following studies, which will be referred to in the text by their roman numerals

- I. Karlsson M, Lindgren M, Jarnhed-Andersson I, Tarpila E. Dressing the split-thickness skin graft donor site: a randomized clinical trial. *Adv. Skin Wound Care*. 2014 Jan; 27(1):20-5.
- II. Karlsson M, Elmasry M, Steinvall I, Sjöberg F, Olofsson P, Thorfinn J. Scarring at donor sites after split-thickness skin graft: a prospective, longitudinal, randomized trial. *Adv. Skin Wound Care*. 2018 Apr; 31(4):183-188.
- III. Karlsson M, Elmasry M, Steinvall I, Sjöberg F, Olofsson P, Scarring at donor sites after split-thickness skin graft: a prospective, longitudinal, randomized trial; the Observer view. [Submitted]
- IV. Karlsson M, Elmasry M, Steinvall I, Sjöberg F, Olofsson P, Thorfinn J. Superiority of silver-foam over porcine xenograft dressings for treatment of scalds in children: A prospective randomised controlled trial. *Burns*. 2019 Sep;45(6):1401-1409
- V. Karlsson M, Steinvall I, Sjöberg F, Olofsson P, Elmasry M. Burn scar outcome at 6 and 12 months after injury in children with partial thickness scalds: did the dressing treatment matter? [Submitted]

INTRODUCTION

Wound history

As long as humans have existed, we have experienced and feared wounds. A wound, by its definition, is a disruption of the integrity of skin [1]. It is known to not only cause pain, but also threaten humans with infections, and in the worst cases sepsis, amputation, and death. It is then not surprising that the treatment of wounds is one of the first medical interventions that we know of that has been described in historical records.

The history of wound treatment dates back more than 5000 years. And as documentation of these early activities cannot be found, we have to rely on more recent publications.

By the year 2200 BC, the process of wound treatment was described in ancient Egypt. Procedures such as washing the injury, making dressings, and bandaging the wound are pictured in historical documents such as the Edwin Smith papyrus (dated at approximately 1700 BC) [2]. Modern treatment is based on the same basic ideas: cleaning and debridement of the wound, followed by the application of dressings; the only step excluded these days is the part where we make the dressing ourselves. In ancient times, dressings were not produced by multinational companies, but at home using nature's own materials such as mud, plants, herbs, or animal parts. Many of these "natural treatments" such as honey, plant cellulose, maggots, alginates, and animal skin are still in use and some of them more popular than ever [3-5]. Others that are often referred to historically, such as the application of fresh meat, milk, wine, and the licking of wounds by dogs, are less popular now.

More than 2000 years before bacteria were discovered; members of early civilisations such as Mesopotamia, Arabia, Egypt, and Greece described the connections between a clean environment and the proper healing of wounds. The first description of bacteria is thought to have taken place around 1676, by the Dutch textile merchant Antonie Van Leeuwenhoek, who observed them in water through a magnifying glass. Van Leeuwenhoek wrote a letter to the Royal Society of London describing his observation of thousands of living creatures in the water. He called them "animalcules"[6]. Today, knowledge of bacteria and how they spread and affect the healing process has evolved considerably, although

many questions still remain. The medical breakthrough of the discovery of antibiotics by Alexander Fleming in 1929 and the introduction of penicillin in therapy in 1941 has had a significant impact on the course of infections. Today, few would doubt its favourable effects on infections, but unfortunately, widespread use has led to the development of antibiotic resistance, a problem that is anticipated to significantly affect health care outcome in times to come if a solution has not been reached. The role of antiseptic agents in wound care is therefore more important than ever [7, 8].

Antiseptics are agents that inhibit the growth and development of microorganisms, but unlike antibiotics, antiseptics are non-specific and are thought to be of a lower risk for the development of bacterial resistance. Antiseptic substances applied to wounds have a far longer history than that of antibiotics, but with the introduction of antibiotics, clinicians started to rely on them to prevent and treat infections [9].

One of the oldest antiseptics in wound care is probably honey [10]. Other antiseptics such as iodine and silver have been used for hundreds of years, and similarly to honey, they are still in use. Several Cochrane reports have been published in the last years regarding the use of topical antimicrobials (antibiotics and antiseptics) in acute wounds [10-12], surprisingly often with a focus on healing time rather than rate of infection. To summarise current knowledge (that is mostly based on scientific studies of lower quality and therefore provides low levels of evidence) it is suggested that topical antiseptics may result in shorter healing times than topical antibiotics. Evidence on whether antiseptic treatment is actually better than non-antiseptic techniques is still lacking [12].

The history of wound surgery, surprisingly, also dates back a long way in history. Covering wounds with skin grafts¹ was first described thousands of years ago, but most advances have been achieved over the past 200 years, particularly since the 1940s.

In 1869, a Swiss surgeon, Jacques-Louis Reverdin, reported his technique of “epidermic grafting,” which involved implanting “islands” of epidermis into the wound to serve as centres of re-epithelialisation and growth. The “islands” were harvested from the arm, by using a sharp lancet and the shaving of small pieces of the epidermis. The pieces were then applied in the first experiments on a granulation wound on the patient’s thumb. The detached epidermis stayed vital on the

¹ Skin graft means that healthy skin is harvested and transplanted to the wound to cover it.

thumb and was capable of adhering and proliferating, to create a new epithelium[13]. A couple of years later, Reverdin admitted that the graft had also contained the papillary layer of the dermis and so was not truly an “epidermis graft”[14]. Although Reverdin’s pinch grafting was a significant step forward in skin grafting, it had several limitations. The grafts caused contractures that were no better than those formed around non-grafted wounds, which limited their usefulness, particularly around joints. The grafts also healed slowly, the new scar tissue was not resistant to physical stress, and the results were cosmetically unsatisfying [15].

In 1872, the French surgeon Louis Léopald Ollier introduced the next step in skin grafting by harvesting larger grafts that included both epidermis and part of the dermis. These grafts, called split-grafts or mid-thickness grafts had several benefits when compared with Reverdin’s “pinch grafts” as they resulted in faster healing, less formation of scars, and less contraction [14, 15]. Today split thickness skin grafting is one of the most commonly-used techniques to cover wounds [15].

So, the question that arises is, are we trapped in the past of wound care? The answer is no. As we improve our knowledge, and know more about the roles of different cells and factors that inhibit and promote the wound healing process, new treatments have been developed and are constantly emerging. Negative pressure wound therapy (NPWT), protease-inhibiting dressings, tissue-engineered products created through the culture of cells from humans and animals, are just a few of the more recent developments [5].

Wound types

A wound, as we described it initially in this chapter, is an injury to the skin, but depending on the underlying cause, we usually divide wounds into two different types; ulcers and vulnus. Ulcers are “hard to heal” wounds that develop because of underlying illnesses or when healing is delayed as the result of an underlying illness. Leg and decubitus ulcers (bed sores) are typical examples of “hard to heal wounds”. Vulnus or “acute” wounds occur suddenly, spontaneously, or as a result of injury or operation. Examples of acute wounds are bites, bullet or stab wounds, burns, and wounds created during operation [16].

The focus of this thesis is on two kinds of acute wounds (**split thickness skin graft (STSG) donor sites** and **partial thickness burns**), how they usually heal, and how this process can be improved.

Anatomy of the human skin

The skin is a complex organ that covers the entire surface of our bodies. It accounts for about 15 % of the total body weight of an adult. It consists of three separate layers (Fig.1), the epidermis (upper layer), the dermis, and the hypodermis, which is also referred to as the subcutaneous fat or subcutis [17].

Figure 1. Anatomy of the human skin. Contributed by Wikimedia Commons, USGOV (Public Domain). StatPearls Publishing LLC; 2019 Jan. Copyright © 2019

The epidermis is the stratified epithelium that covers the outmost part of our bodies and it generally has a thickness of around 0.1 mm, but it varies considerably between different locations on the body. The thinnest epidermis can be found on the eyelids and the thickest in the palms and soles. About 90 % of all cells in the epidermis are keratinocytes, and the rest are melanocytes, Langerhans cells, and Merkel cells. The epidermis can be divided into four to five layers, depending on where it is sited on the body [17].

The layer closest to the dermis is called the *Stratum Basale* and it contains mainly keratinocytes, melanocytes, and stem cells. There are also Merkel cells, which are thought to work as mechanoreceptors [18]. The keratinocytes in this layer are classed as “young” because they have recently differentiated from stem cells, and

they develop and mature as they migrate towards the surface of the skin. The melanocytes produce melanin, which is responsible for pigmentation. The next layer is called *Stratum Spinosum*, comprising most of the epidermis and contains several layers of cells that are connected by desmosomes, which allow cells to remain tightly bound to each other. In this layer, Langerhans cells can be found, which are immunologically active cells. The third layer is called *Stratum Granulosum* (not shown in Fig.1) and it contains several layers of cells that contain lipid-rich granules. In this layer, cells begin to “die” as they move away from the nutrients that are located in the deeper parts of the skin. The fourth layer is called *Stratum Lucidum* and can only be found in the thick skin of the soles and palms. The outmost part, the *Stratum Corneum* contains corneocytes, which are keratinocytes at their last stage, terminally differentiated. The corneocytes and lipid-rich matrix that surround these cells make this layer a protection against the external environment and regulate the permeability of the skin.

Epidermis renews itself within a month and heals, if damaged, from epidermal appendages such as hair follicles, sweat and sebaceous glands in the dermis, and from cut edges of skin, without scarring. The epidermis does not contain any blood vessels [17-19].

The second skin layer, the dermis, is located between the epidermis and the subcutaneous fat, and primarily consists of collagen (mainly type I and III) and fibres made of elastin that create a dense connective tissue that “protects” the body from strain. The collagen in the dermis is produced by fibroblasts, the most common cell type in connective tissue. The deeper-lying fibroblasts are responsible for maintaining the extra cellular matrix (ECM) in the dermis by producing not only collagen (type I, III, and IV) but also proteoglycans, fibronectin, laminins, glycosaminoglycans, metalloproteinases, and prostaglandins. The more superficially-located fibroblasts are responsible for re-epithelialisation during wound healing. This means that the fibroblasts are critical in the reconstruction and regeneration of skin tissue and re-epithelialisation after injuries [20].

The dermis is divided into two layers, the superficial thinner layer of which is called the papillary dermis, and the deeper thicker layer of which is called the reticular dermis. The papillary dermis is tightly connected to the epidermis through a basement membrane. Hemidesmosomes, a specialised form of protein, creates a strong junction between the two layers [21]. The dermis contains adipocytes, macrophages, touch receptors, and thermoreceptors that are responsible for heat sensation as well. In addition, hair follicles, sweat glands, sebaceous glands, apocrine glands, lymphatic vessels, nerves, and blood vessels are also present. Sweat and sebaceous glands, and hair follicles are actually epidermal appendages

that extend down to the dermis and hypodermis (the subcutaneous fat) and contain epidermal skin cells. Sweat glands contribute to thermoregulation, and sebaceous glands to the lubrication of the epidermis. Blood vessels provide nourishment and waste removal for both dermal and epidermal cells in the dermis [22].

The hypodermis is the third and deepest layer of the skin and refers to the fatty tissue below the dermis. This tissue insulates the body from cold temperatures and provides shock absorption. Fat cells in the hypodermis also store nutrients and energy. The hypodermis is the thickest in the buttocks, palms of the hands, and soles of the feet. The hypodermis also contains some skin appendages like the hair follicles, sensory neurons, and blood vessels [23].

Children are thought to have a thinner epidermis than adults, fewer lipids, less melanin, and a flatter junction between the dermis and epidermis. They are also thought to have a higher water content and pH in the skin [24].

The wound healing process

The macroscopic process of wound healing has been “described” for more than 100 years and since then, there has been a constant development of new knowledge. Although considerable knowledge has evolved in this field during the last 20 years there are still large areas to be explored.

The normal process of wound healing may be described as four highly integrated and overlapping phases: the **haemostasis** and **inflammation** followed by **proliferation**, and then lastly the **maturation** phase [25, 26].

The **haemostasis phase** begins with the disruption of blood vessels, which exposes collagen in the dermis to platelets in the blood. Activated platelets stimulate the release of numerous growth factors, inflammatory markers, and cytokines, which end up in activated coagulation pathways and in the formation of the fibrin clot. The clot re-establishes haemostasis, provides a provisional extra ECM for cell migration, and releases several cytokines that are important for wound healing such as platelet-derived growth factor (PDGF), transforming growth factors (TGF), fibroblast growth factors (FGF), and epidermal growth factors (EGF). All these mediators, in their turn, attract and activate leukocytes (neutrophils), macrophages, and fibroblasts, which all together move the wound healing process to the next phase [25, 26]

The ***inflammatory phase*** includes an important increase in vascular permeability that allows leukocytes and primarily neutrophils to migrate and phagocytose “any” bacteria. Enzymes are released that digest necrotic tissue. The neutrophils are thought to be recruited by Damage-associated molecular patterns (DAMPs), hydrogen peroxide (H_2O_2), lipid mediators, and chemokines that have been released by injured cells. The rapid production of H_2O_2 in the wound is thought to minimise infections, activate keratinocyte regeneration, recruit neutrophils, and promote the formation of new vessels. Neutrophils, which are not normally found in uninjured skin, release toxic, antimicrobial granules that contain mainly proteases. Proteases are important enzymes that have antimicrobial activity, and are able to break down the basement membrane and the ECM, allowing further neutrophils to migrate to the injured area. One of the often-discussed proteases is matrix metalloproteinase (MMP). Some types of MMP are more present in the fluid of acute wounds and are therefore more likely to cause a perfect balance in collagen up and down regulation, while other MMPs are present in chronic, or slow healing wounds, and are likely to cause down regulation of collagen, which will result in a suboptimal environment for wound healing [25-27].

As mentioned earlier, a new family of wound dressings, protease-inhibiting dressings has been developed to overcome the issue of the “breaking down by MMPs”. Specific tissue inhibitors of metalloproteinases (TIMPs) work as “controllers” of MMP in the wound healing process.

The early activity of neutrophils is critical to inhibit bacterial colonisation and wound infection. Further cleansing of the tissues is undertaken by monocytes when they infiltrate the area and become macrophages. Typically, the neutrophil population is replaced within a few days by macrophages, which facilitate normal wound healing, regulate fibroblast activity, and enable the deposition and remodeling of ECM and granulation tissue. This activity involves the secretion of numerous cytokines and growth factors, including PDGF, TGF- β , tumour-necrosis factor alfa (TNF- α), and interleukins. Macrophages further stimulate inflammatory cytokines such as PDGF, TGF (alfa and beta), FGF, EGF, interleukin -1 (IL-1) and insulin-like growth factor 1 (IGF-1), which are all thought to be vitally important for the regeneration of injured tissue. Mast cells release vasoactive cytokines such as prostaglandins and histamine, which increase capillary permeability and promote local dilation to aid the migratory process. Through these mechanisms, the inflammatory phase creates a clean wound bed [25, 26].

The ***proliferative phase*** includes angiogenesis, the production of granulation tissue, the deposition of collagen, and re-epithelialisation of the wound. The crea-

tion of new blood vessels (angiogenesis) is thought to start because of the hypoxic release of growth factors such as vascular endothelial growth factor (VEGF) and PDGF, both of which stimulate the endothelial cells located in the walls of blood vessels. Stimulated endothelial cells break down the ECM in the granulation tissue then proliferate and migrate out into the tissue, creating new capillaries. The release of FGF also triggers angiogenesis, which supplies the new wound with oxygen, glucose, and other factors necessary for proper healing. As blood flow returns to the area, oxygen saturation normalises and VEGF decreases to slow the process of angiogenesis. This autoregulatory mechanism plays a role in preventing excess production of collagen and reduces the risk of abnormal scar formation.

The formation of granulation tissue starts with fibroblasts migrating into the wound and producing additional ECM. Collagen levels rise steadily for several weeks before slowing down. The tensile strength of the wound correlates with the amount of collagen deposition during this period.

As long as the epidermal appendages are intact, re-epithelialisation of the wound can occur both from the wound edges and from the stimulated epidermal appendages that contain epidermal cells and stem cells. How the body stimulates epidermal cells to migrate and proliferate in the wound area is not fully understood, but one theory is that the absence of neighbor cells (the “free edge effect”) may signal to the epidermal cells to migrate. Other factors that may be important, are the local release of growth factors and increased expression of growth-factor receptors. Partial thickness wounds (that stretch below the epidermal skin appendages into the reticular dermis, or when such appendages have been damaged) heal more slowly and the area of the wound needs to be replaced by granulation tissue [25, 26].

The maturation, or scarring, phase is the final stage of wound healing, which includes contraction, collagen cross-linking and remodeling. Contraction occurs in open wounds to reduce the surface area with the help of myofibroblasts, transformed fibroblasts, and their synthesis of alpha-smooth muscle actin. Myofibroblasts are thought to attach to collagen and pull the fibres to the edges of the wound. When the tissues are sufficiently restored, the myofibroblasts undergo apoptosis and the fibroblasts, macrophages and endothelial cells involved in healing now leave the tissues. Collagen is deposited and recreated during the scarring phase, most likely controlled by MMPs, which are secreted by macrophages, epidermal cells, endothelial cells, and fibroblasts.

As the wound is closed, a down regulation of capillaries takes place, resulting in a pale scar. After the scar matures, fibroblasts decrease in number. Epidermal appendages, such as hair follicles and sebaceous glands, and the stem cells normally found in these appendages, are often absent in the scar. This results in a dermal layer that contains few cells. The scar also contains less elastin than normal skin, which contributes to the lack of elasticity seen in scar tissue. A mature cutaneous scar consists of a large amount of collagen, 80–90% of which is type I collagen and the rest type III. The basement membrane of the epidermis that develops over scar tissue is flatter than normal because it does not contain the rete pegs that normally penetrate the dermis to keep the layers “connected”. The maximum strength of the new tissue is about 70%–80% of undamaged tissue [28]. Regarding the “re-pigmentation” of the wound, there are two potential sources from which melanocytes can be recruited to repopulate scars. Melanocytes from the surrounding unwounded skin can migrate into the wound at the edges, and epidermal elements such as hair follicles and sebaceous glands in the wound bed can also provide a source of melanocytes [25, 26, 28].

Description of the wound models of this thesis

As described earlier in the Introduction section, the focus of this thesis is on two kinds of partial thickness acute wounds; donor sites after the harvest of split thickness skin grafts and partial thickness skin burns (sometimes referred to as *second degree burns*).

Donor sites are wounds that are created under sterile conditions during operation, when healthy skin is harvested to cover wounds that will not heal spontaneously in a reasonable time (within 14 to 21 days). Skin grafts can be harvested in different thicknesses (Fig. 2) [15]. In this thesis, we focus on donor sites created when the surgeon harvests split thickness skin grafts. In Study I, skin grafts are harvested from the thigh with the dermatome (skin harvesting “machine”) set to 0.012 inches, which produces medium/intermediate thick grafts, and creates a wound approximately 0.2 - 0.3 mm deep. This should leave the epidermal appendages (glands and follicles) of the dermis almost intact [29, 30]. The most commonly-used donor site is the thigh [30].

Figure 2. Illustration of skin layers and skin graft depths²

The depth of a burn is commonly classified as superficial, partial thickness, or full thickness. Superficial burns only affect the epidermis while partial thickness burns, affects different depths (layers) of the dermis. Full thickness burns affect all layers of skin (Fig. 3) [31].

Partial thickness burns are burns that extend through the epidermis and down into the dermis. One common cause of partial thickness burns is hot liquid. Partial thickness burns are often divided into two groups; superficial and deep partial thickness burn (sometimes a third group is mentioned, mid-dermal burns).

Superficial partial-thickness burns extend into the papillary, or superficial, layer of the dermis. Blisters, both intact and ruptured, are common and the wound is wet and pink/red. When pressure is applied to the reddened area, the area will blanch and rapid capillary refill is noted upon release of the pressure. These burns are painful because the nerve ends are exposed to air (Fig. 4).

Deep partial-thickness burns extend downward into the reticular layer of the dermis and are either mixed red or pale. Areas of redness will continue to blanch when pressure is applied, but capillary refill may be delayed when pressure is released. Blisters can be seen and the exposed surface of the wound is wet or moist, similar to superficial partial-thickness burns (Fig. 5). Oedema is marked,

²Reprinted from The Journal of Foot and Ankle Surgery, volume 49, issue 4, Christopher Bibbo. VERSAJET-Hydrosurgery Technique for the Preparation of Full Thickness Skin Grafts and the Creation of Retrograde Split Thickness Skin Grafts, issue 4 July-August 2010 pages 404-407, Copyright 2010, with permission from Elsevier.

and sensation is altered in areas of a deep partial-thickness burn [31-33]. In this thesis the term deep dermal burns (DDB) will be used for burns of this depth.

Burns are not only assessed with regard to their depth, but also by their extent, to ensure appropriate, acute burn care. The extent of the burn is described by giving the percentage of burn covering the total body surface area (TBSA). A burn covering 1 % of the TBSA is called a 1 % TBSA burn. When calculating the percentage of burn, the observer most often use either the rule of nines [34] the rule of palm [35] or the Lund and Browder Chart [36]. The rule of nines estimation of body surface area burned is based on assigning percentages to different areas of the body. Areas are “divided into nines”, where the entire head is estimated as 9%, the entire trunk as 36% (and can be further divided into 18% for anterior trunk and 18% for the back). The rule of palm estimation is used by using the patient’s palm including fingers, which is said to represent 1 % of their body. Recent publications have shown that this might lead to an overestimation of the burned area, but it is still used clinically, particularly for minor burns. The gold standard for burns that we use in our burn centre is the Lund and Browder chart - developed in 1944 - where unlike the rule of nines, the age of the patient is taken into consideration (the percentage of burned surface area for the head decreases and that for the legs increases as the child ages [37].

Figure 3. Anatomy and histology of the skin, showing the depths of burns³

³ Reprinted 2019 from Shupp, Jeffrey W.; Nasabzadeh, Teresa J. A Review of the Local Pathophysiologic Bases of Burn Wound Progression. *The Journal of Burn Care & Research*. 2010, Vol. 31, number 6, page 849-873, by permission of Oxford University Press

Figure 4. Superficial dermal burn located on the trunk of one of the children participating in Study IV (images used after written consent from guardians)

Figure 5. Areas of deep partial thickness wounds in a child participating in Study IV (images used after written consent from guardians)

Differences in healing between donor sites and partial thickness burns

Donor sites and partial thickness burns, although affecting the same skin layers, are believed to heal differently, depending on the nature of the wound [38].

As the donor site is created under clean, controlled conditions and contains very little damaged skin, the remaining tissue is vital and can therefore supply nutrients to incoming fibroblasts and epithelial cells. This results in a healing process that starts within seconds after injury with the haemostasis phase, and aims at stopping the blood flow from the healthy capillaries being “cut off”[38]. The healing process is somewhat different for burns.

In 1953, Douglas Jackson published the article “The diagnosis of the depth of burning” in the British Journal of Surgery [33]. In this well-cited article, he describes the burn wound in terms of three different zones: coagulation, stasis, and hyperaemia (Fig. 6). These zones are three dimensional, and loss of tissue in the zone of stasis will lead to the wound deepening as well as widening. Jackson described these three zones as a result of examining biopsies that were harvested from 20 healthy volunteers with experimentally-controlled burns. Even though questions have been raised regarding how the zone of stasis was depicted from such biopsies, this model is still a prevalent basis of burn wound pathology.

Figure 6. **Jackson's burn wound model.** The different zones of damage from a partial-thickness burn are shown in profile through the layers of the skin⁴

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The high heat accumulated in the centre of the tissue after a deep burn is thought to create the zone of coagulation. This zone is pale, painless, and capillaries have coagulated. This area lacks sufficient oxygen supply and so the tissue dies. For the wound to heal, the necrotic tissue needs to be removed and, depending on wound size, be replaced by a viable, autologous skin graft. Surrounding the zone of coagulation is the zone of stasis, an area where perfusion is decreased but tissue is still being perfused, which leads to fluid leakage from vessels and concomitant vascular damage. The zone of hyperaemia is the red zone, which surrounds the zone of stasis where the epidermis is partly or totally lost but the dermis remains. The vasculature is dilated and intact and the skin blanches at pressure [38-40].

Whether the healing process can continue depends on whether the capillaries in the zone of stasis can provide the burned areas with nutrition and phagocytes. It is suspected that if the cells do not get access to the wounded areas, the zone of coagulation expands, and the depth of the burn increases. Studies have shown that in deep partial thickness burns apoptotic dermal cells are found in a much higher percentage than in superficial or full thickness burns, and this difference remains for weeks. This means that dermal cells continue to die in a “programmed manner” long after the injury and this is probably one of the reasons for the progression of a burn. The lack of blood flow inhibits neutrophil access, delaying the inflammatory phase, but studies have also shown that the survival of neutrophils is prolonged in burns patients, which also prolongs the inflammatory phase [31, 39]. The exact mechanism underlying the progression of burns is not fully understood.

Factors that delay wound healing and influence scar outcome

There are several factors that influence the healing process. Some of the most commonly-mentioned systemic factors that are thought to have an impact on acute wound healing are older age [41, 42], diabetes [43], diseases that influence the circulation of the blood or mobilisation, drugs that inhibit haemostatic or immune response, or both [44], lifestyle-related factors such as smoking [45], alcohol misuse, obesity (and malnutrition, similarly) [46]. Some studies have shown that sex [41] and the levels of sex hormones have an impact on wound-healing potential. Oestrogen is thought to stimulate healing, and testosterone to induce it [47].

Local factors such as infection [42] and high levels of certain MMPs might also affect healing [27], highlighting the need for proper treatment. What “delayed wound healing” actually is can be discussed, but in acute wounds a healing time

that extends beyond 4-6 weeks is referred to as delayed healing [1]. An acute wound that shows delayed healing can become a “chronic wound” depending on the supposed reason for the delay. Many clinicians report older patients with a “chronic wound” to the lower leg after an initial “traumatic skin injury”. In these cases, perhaps an undiagnosed “vascular insufficiency” may have caused the conversion from an acute to a chronic wound.

A factor that has an impact on the wound healing process is, of course, the depth of the wound. A deeper wound requires more time for the ECM to build up, and in burns, the depth of the injury is one of the few validated factors to influence healing time [48]. The extent of the wound is another factor thought to influence healing time [42, 49].

Less is known about the factors that influence the outcome of scars. As pathological scars are common within burns care, most of our knowledge comes from this field. Pathological scarring refers to hypertrophic scars (HTS) or keloids. By definition, hypertrophic scars are confined within the margins of the original wound bed, whereas keloids extend beyond these borders [28]. Another, less-commonly discussed pathological scar is the dyspigmented scar, which can be either a hypo-pigmented or hyper-pigmented. Not only is it of cosmetic importance to the patient, it also poses a problem as the pigment protects the skin from harmful UV radiation [50].

Factors thought to cause severe or pathological scarring include: healing time extending beyond 14 [51] or 21 days [52], deeper wounds, larger extent of the burn [53], wound infection [54], female sex, young age [55], dark skin type (non-white) [56], skin grafting [55], burn on trunk or upper limb [57] and high BMI (for which there is weak evidence) [58].

The choice of dressing treatment might have a direct impact on both healing times and scarring outcome as they can affect the local environment of the wound.

Acute wound treatment

Cleaning and debridement

The cleaning and debridement of necrotic tissues are the corner stones of modern wound treatment as they reduce the bacterial burden of the wound and stimulate

healing [59, 60]. The role of antiseptics in the cleansing of acute wounds is questionable and there is not enough evidence of it being effective [11, 61].

Donor sites do not normally require debridement as damaged or necrotic tissue is absent. For the partial thickness burn, the burned tissue is necrotic and surrounded by damaged tissue, all of which require accurate debridement, which includes cleansing of the wound and the removal of any necrotic tissue to stimulate healing. Deep dermal/partial thickness and full thickness wounds (involving the hypodermis and sometimes even the muscles and bones) usually need surgical or enzymatic debridement to get rid of the necrotic tissue [59, 61].

Dressing treatments

The choice of dressing depends mainly on the characteristics of the wound in focus.

What we consider to be an ideal dressing today is a dressing that creates a moist and clean, balanced environment for the wound. One of the first reports on the positive role of a moist healing environment in wounds was published by Hinman and Maibach in *Nature* in 1963 and since then, these concepts have become standard [62].

Moist wound healing is thought to; prevent pain by protecting free nerve endings, prevent the formation of dry scabs which may contain or promote the survival of micro-organisms, and also to prevent the loss of epidermal cells. This is particularly evident when dressings are changed. Closed moist dressings are further claimed to lower the risk of contamination from the surroundings[63]. Five systematic reviews on donor site dressings were found and in four of them the authors conclude that a moist wound environment is associated with better outcomes, as in faster re-epithelialisation, less pain, and fewer infections [30, 64-67].

Seven systematic reviews for partial thickness burn wound dressings were found [68-74], showing strongly contradictory results, in which no conclusions regarding moist treatments can be drawn. Moist wound treatments have been reported to provide short healing times [70] but also to increase infection rates in burns in a recent clinical trial [75].

Examples of (claimed) moist dressings in clinical use include alginates, films, foams, hydrogels and hydrocolloid gel producing hydrofibre (cellulose)[65].

Moist wound dressings that have been evaluated for donor sites and burns in this thesis will be described in the Method section and discussed further in this thesis.

The properties of an ideal dressing for the two partial thickness wounds in focus in this thesis has been investigated in two worldwide surveys from 2012 [76] and 2013 [77] and the conclusions were that most clinicians thought that an ideal dressing does not yet exist.

The most desirable characteristics of an “ideal” donor site dressing that clinicians look for is a “one piece” dressing (composite) that is absorbent, pain-free, non-adhesive, easy to remove, and requires a minimum of changes, (no changes at all until complete healing is preferred) [77].

For burns, the desired characteristics in the ideal dressing are similar to those of donor site dressings with a few exceptions. Except for the above-mentioned characteristics, burn clinicians also ask for antimicrobial activity, and that the dressings should be available in a range of sizes. The dressing should be changed twice weekly, preferably [76]. These requests are most likely to be related to the suppression of immunity in patients with burns that leads to a high microbial burden [38] (described earlier in the section, *Differences in healing between donor sites and burns*).

Dressings with supposed antimicrobial activities have been investigated in our studies and will be presented and discussed later on in this thesis.

How to evaluate dressing treatments?

The most commonly-reported variable in wound research is the time it takes for the wound to heal, followed by variables such as infection, pain, costs, the use of resources, adverse events, mortality, and quality of life [78]. With partial thickness burns, two additional outcomes are regularly reported; Duration (or Length) of hospital stay (often referred to as LOS) and the need for operation [70].

All kinds of research designs can be seen in the evaluation of wound dressings, but to follow evidence-based medical research methods, only one design can be considered to be the proper one, the randomised controlled trial (RCT) [79].

RCTs are universally acknowledged to be the study design of choice to compare the effects of treatment, as they reduce sources of bias, such as selection bias and confounding bias. This is the reason why Cochrane reports only include RCTs [79].

AIMS

The following **aims** were addressed in this thesis:

To investigate if the choice of dressing treatment had an impact on:

The healing process of the split thickness skin graft donor site (Study I); scar outcome in the split thickness skin graft donor site (Study II, III); the healing of partial thickness burns (Study IV); scar outcome in partial thickness burns (Study V).

Our final aim, which evolved during the research project, was to investigate whether we could confirm the findings in other studies regarding factors that have affected healing time and scarring, by using the data collected in Studies I-V.

Hypotheses

In Study I we hypothesised that there was a superior type of dressing than our standard of care dressings at that time.

In Study IV we hypothesised that there was a superior type of dressing than our standard of care at that time.

METHODS

Study population

All patients included in these studies were admitted to the Department of Hand Surgery, Plastic Surgery and Burns, at the Linköping University Hospital, Linköping, Sweden and all participated after given written informed consent.

The inclusion criteria in Studies I and IV and demographic data collected were selected with the aim of homogenising the study groups as much as possible.

Study II, III and V are follow-up studies that comprised patients originally recruited in the RCTs (Study I and IV), who participated again after providing informed consent.

Inclusion criteria for the study participants in Study I:

- In-patients planned for split thickness skin grafting from the thigh
- A planned donor site ranging from 30 cm² to 400 cm²
- Graft to be harvested with dermatome at a standard depth
- 18 years or older
- Swedish speaking
- Written informed consent given

Inclusion criteria for study participants in Study IV:

- Admission within 72 hours after burn injury
- Children aged 6 months to 6 years
- Partial-thickness scalds, suitable for dressing within standard of care (SOC); a porcine xenograft, as judged by the burn surgeon on duty
- Written informed consent from guardians given

In Study I, the following data were collected; age, sex, weight, and height (BMI calculated), treatment with cortisone or anticoagulants, autoimmune diseases (systemic lupus erythematosus, Crohn's disease, ulcerative colitis, rheumatoid arthritis, and diabetes mellitus) and smoking habits.

In Study IV the following data were collected; age, sex, weight, previous illnesses, medications, allergies, depth of burn, and TBSA %.

Design

Study I. *Dressing the split-thickness skin graft donor site: a randomised clinical trial*, comprised 67 patients aged 18 years or older who had been admitted for elective surgery, in which split thickness skin grafts were harvested from the thigh. After giving written informed consent the patients were randomly assigned to be given one of the three different dressings: polyurethane foam (Allevyn, Smith & Nephew, St Petersburg, Florida), hydrofibre (Aquacel, ConvaTec, Skillman, New Jersey) or porcine xenograft (Mediskin, Mölnlycke, Health Care AB, Gothenburg, Sweden). Patients were recruited between October 2008 and December 2009.

Study II. *Scarring at donor sites after split-thickness skin graft: a prospective, longitudinal, randomised trial* was a follow-up study investigating the outcome of scars in the patients originally recruited to Study I. All 34 patients who were still alive and registered in Sweden from the original RCT were contacted by letter and asked to participate. Twenty-seven patients completed the follow-up study. Study participants gave their subjective opinion on the outcome of donor site scars using a scar scale named the Patient and Observer Scar scale (POSAS), which will be explained later in this section. Patients were recruited in 2017, approximately eight years after participating in the original Study I.

Study III. *Scarring at donor sites after split-thickness skin graft:*

A prospective, longitudinal, randomised trial; the observer view consisted of 17 patients from Studies I and II. All living patients who had participated in Study II were contacted by letter and asked to participate in this last part of the donor site project. Donor site scar outcome was then evaluated using the scar scale POSAS and an instrument for measuring the firmness and elasticity of the skin (Cutometer® MPA 580), described in Paper III. Seventeen chose to return to our Department in 2018 for this last follow up.

Study IV. *Superiority of silver-foam over porcine xenograft dressings for treatment of scalds in children: A prospective randomised controlled trial* comprised 58 children between the ages of 6 months and 6 years, who were admitted with partial thickness scalds. After guardians gave written informed consent, the patients were randomly assigned one of two study dressings; porcine xenograft (EZderm, Mölnlycke Health Care AB, Gothenburg, Sweden) or silver-foam dressing (Mepilex Ag, Mölnlycke Health Care AB, Gothenburg, Sweden). Patients were recruited between May 2015 and 2018.

Study V. *Burn scar outcome at 6 and 12 months after injury in children with partial thickness scalds: did the dressing treatment matter?* was a follow up study investigating the outcome of burn scars in the children originally recruited to Study IV. Guardians gave informed consent to this follow-up study during the Study IV procedure and the patients were at the completion of this study scheduled for 6 and 12 months' scar evaluations (Study V). Thirty nine of the 58 children participated in the follow up at 6 months and 34 at 12 months. Scars were evaluated using the scar scales described later in this section.

Variables and clinical assessments

Variables chosen and investigated in this thesis are the ones commonly presented in systematic reviews and Cochrane reports for wound treatments [70, 78]. The following variables were investigated in both Studies I and IV: healing time, infection, pain and change of dressing times (part of the variable: *ease of use* in Study I) and dressing frequency (part of the variable: *cost* in Study I). In Study I, impact on everyday life and costs were also evaluated. In Study IV, the need for operation and LOS was also examined. These variables were added as they are considered to depict the ability of the dressing to reduce the need for both operation in partial thickness burns (not relevant in Study I), and in-patient hospital care.

Healing time

In Studies I and IV *healing time* was assessed by a plastic surgeon; complete wound closure also had to be confirmed by a nurse attending the change of dressings. In Study I healing was assessed at post-operative day 14 and 21. As the donor site wound was standardised and localised on a flat surface, wound tracing was used to calculate percentage of re-epithelialisation. Donor sites were evaluated as healed if re-epithelialised to 98 % or more. As the calculation was done retrospectively patients not completely healed at day 14 also attended the day 21 visit. Healing times were corrected for the 98 % limit and presented in Paper I. As the wounds were irregular and located at convex and concave sites in Study IV, wound tracing was abandoned for technical reasons and healed areas were calculated using the rule of the palm and then later a calculation of percentage of re-epithelialisation was done. Burns were considered healed if re-epithelialised to 97 % or more. Children were followed until complete (100 %) wound closure.

Infection

In Studies I and IV *infection* was diagnosed based on clinical signs such as: redness, heat, swelling, pain, odour, fever, and a positive wound swab. In Study IV, we also assessed levels of C-Reactive Protein (CRP) with the help of a validated and calibrated instrument (Quick read go, Orion Diagnostica Oy, Espoo, Finland).

Pain

In Study I *pain* was assessed at rest, during activity, and during dressing changes using a Numeric Rating Scale (NRS; 0-10). This scale has been validated and recommended as the “best practise” pain assessment instrument for dressing changes [80, 81].

In Study IV, conducted in small children, we used the Face, Legs, Activity, Cry, and Consolability (FLACC) scale (Table 1) [82] . The FLACC was used to assess pain before, during, and after dressing changes together with a Parents’ Postoperative Pain Measure (PPPM) diary [83] in which guardians registered signs of their children’s pain during the day. The FLACC scale contains five categories, each of which, is scored from 0 to 2, to provide a total score ranging from 0 to 10. It is clinically used and validated in a variety of settings such as pain after operation and injury in children up to the age of 7 (Study IV). However, it has not been validated for procedural pain in those under the age of five [82, 84].

In Study IV, also the need for analgesia was recorded. This, however, was not considered relevant for Study I, because it was thought to be problematic to differentiate between analgesia taken for pain at the donor site compared with analgesia taken for pain at the transplanted wound area.

Table 1. THE FLACC SCALE

Category	Scoring		
	0	1	2
Face	No particular expression or smile	Occasional grimace or frown, withdrawn, disinterested	Frequent to constant quivering chin, clenched jaw
Legs	Normal position or relaxed	Uneasy, restless, tense	Kicking or leg drawn up
Activity	Lying quietly, normal position, moves easily	Squirming, shifting back and forth, tense	Arched, rigid or jerking
Cry	No cry (awake or asleep)	Moans or whimpers; occasional complaint	Crying steadily, screams or sobs, frequent complains
Consolability	Content, relaxed	Reassured by occasional touching, hugging or being talked to, distractible	Difficult to console or comfort

Dressing time

In Study I and IV, the time taken to change dressings was measured using intervals of 10 minutes. This was noted in the Case Report form (CRF), by the nurse who was doing it. Frequency of dressing changes was recorded as a part of the cost variable in Study I, and separately in Study IV. We were able to do a cost calculation in Study I, as the donors sites were of similar sizes (the same size of dressing was required for all patients), on the same location in the body and applied in a similar procedure (dressings used after day 21 were not included in study). In Study IV, children had burns on different parts of the body and were followed up until complete wound closure. This resulted in differently-sized dressings and different timings for each change, so no cost calculations were done. Data on the number of dressing changes in Study IV, was extracted from the local Burn Unit Database (BUD) [85] and also confirmed in the the unit's medical records.

LOS and operations

In Study IV, data for the LOS and need for operation was extracted from BUD and the patients' medical records. The design of the Study I made it inappropriate to examine the need for operation (as this was an inclusion criteria) and LOS was also affected by the healing status of the transplanted area.

Scar variables

The variable investigated in Study II was the patient's subjective opinion of the donor site scar outcome. For this purpose, the patients used the Patient scale of the POSAS (Fig.7a). In Studies III and V, the Observer scale of POSAS was used to evaluate the scar outcomes (Fig.7b). We used a validated Swedish version of POSAS distributed by the Uppsala Burn Centre, Sweden in 2016.

Each scale contains six items that are scored numerically and when added, constitutes "Total Score" of the Patient and Observer Scale. Each of the six items on both scales has a 10-point range (1 - 10), with 10 indicating the worst imaginable scar or sensation. The lowest score, "1" corresponds to the normal skin. The Patient Scale contains six questions that address the items: pain, itching, colour, stiffness, thickness and relief. The Observer rate addresses: vascularity, pigmentation, pliability, thickness, relief, and surface area. Besides the 10-step scale, category boxes are available to score nominal parameters (such as the type of pigmentation) for the observer. The patient and observer can also score their "Overall Opinion" on the scar compared to normal skin with a 10-step scale, with 10 indicating a scar very different from normal skin. POSAS is one of the most commonly-used scales for evaluating scars [86].

POSAS Observer scale

The Patient and Observer Scar Assessment Scale v2.0 / EN

Date of examination:

Observer:

Location:

Research / study:

Name of patient:

Date of birth:

Identification number:

	1 = normal skin worst scar imaginable = 10										
PARAMETER	1	2	3	4	5	6	7	8	9	10	CATEGORY
VASCULARITY	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	PALE PINK RED PURPLE MIX
PIGMENTATION	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	HYPO HYPER MIX
THICKNESS	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	THICKER THINNER
RELIEF	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	MORE LESS MIX
PLIABILITY	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	SUPPLE STIFF MIX
SURFACE AREA	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	EXPANSION CONTRACTION MIX
OVERALL OPINION	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	

Explanation

The observer scale of the POSAS consists of six items (vascularity, pigmentation, thickness, relief, pliability and surface area). All items are scored on a scale ranging from 1 ("like normal skin") to 10 ("worst scar imaginable"). The sum of the six items results in a total score of the POSAS observer scale. Categories boxes are added for each item. Furthermore, an overall opinion is scored on a scale ranging from 1 to 10. All parameters should preferably be compared to normal skin on a comparable anatomic location.

Explanatory notes on the items:

- **VASCULARITY** Presence of vessels in scar tissue assessed by the amount of redness, tested by the amount of blood return after blanching with a piece of Plexiglas
- **PIGMENTATION** Brownish coloration of the scar by pigment (melanin); apply Plexiglas to the skin with moderate pressure to eliminate the effect of vascularity
- **THICKNESS** Average distance between the subcuticular-dermal border and the epidermal surface of the scar
- **RELIEF** The extent to which surface irregularities are present (preferably compared with adjacent normal skin)
- **PLIABILITY** Suppleness of the scar tested by wrinkling the scar between the thumb and index finger
- **SURFACE AREA** Surface area of the scar in relation to the original wound area

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Figure 7b. The POSAS Observer scale used in Paper III and V. Permitted for reuse 23 of august 2019. Copyright holders P.P.M.van Zuijlen, Beverwijk-NL

In Study V, the scars were also examined with the other commonly-used scar scale, the Vancouver scar scale (VSS)[86]. This scale was developed in the 1990's and is based on four items: pigmentation, vascularity, pliability, and height of scar, and each is assessed independently, with an increasing score assigned to the more pathological condition. Normal skin has a score of 0. The highest possible VSS total score is 13 (see Table 2)[87].

Table 2. The Vancouver scar scale

1	Vascularity	Normal	0
		Pink	1
		Red	2
		Purple	3
2.	Pigmentation	Normal	0
		Hypopigmentation	1
		Hyperpigmentation	2
3.	Pliability	Normal	0
		Supple	1
		Yielding	2
		Firm	3
		Ropes	4
		Contracture	5
4.	Height	Flat	0
		< 2mm	1
		2-5 mm	2
		> 5 mm	3

In Study II, III and V, the scars were also evaluated for hypertrophic scarring (HTS). In Studies II and III, this was done with the help of two techniques: either a total score on POSAS above the median for the group and/or an individual score higher than 1 on the item thickness (POSAS). For Study V, we also included the combined opinion of two specialists, who had been blinded to the details of the dressings, where both had to agree whether or not the scarring was hypertrophic.

In Study III, the viscoelasticity of the donor site was also investigated using a Cutometer MPA580 (Courage + Khazaka electronic GmbH, Köln, Germany). This instrument is described in detail in Paper III.

Predictive factors for wound healing and scarring

As described in the *Aims* section, we also decided to explore the predictive power of the variables we have examined, and their relation to the healing and scar development variables registered in the studies.

The following factors/variables were studied:

For ***healing time***: Sex, age, infection, presence of autoimmune disease (systemic lupus erythematosus, Crohn's disease, ulcerative colitis, rheumatoid arthritis, and diabetes mellitus), use of systemic cortisone or anticoagulants, smoking habits, BMI (reflecting both the nutritional status and obesity), burn wound depth and extent (size) of donor site or burn.

For ***scarring*** (as diagnosed, or registered by POSAS and/or VSS scores): Sex, age, infection, healing times, the depth and extent of the burn, burns on trunk or upper limb, the skin type according to the Fitzpatrick skin type system (see Table 3) [88] and previous grafting. When examining predictive factors for scar outcome in the children (Study V) we used the highest total scores reported with POSAS and VSS. When a child had more than one scar, we chose the one with the highest score (worse scarring), a strategy which has been used in a similar study [51].

Table 3. Fitzpatrick Skin Type Classification Scale Categories

Skin type	Skin colour	Characteristics
1	White; very fair; red or blonde hair; blue eyes;	Always burns, never tans
2	White; fair; red or blonde hair; blue, hazel or green eyes;	Usually burns, tans with difficulty
3	Cream white; fair with any eye or hair colour	Sometimes mild burn, gradually tans
4	Brown; typical Mediterranean Caucasian skin	Rarely burns, tans with ease
5	Dark brown; Middle Eastern skin types	Very rarely burns, tans very easily
6	Black	Never burns, tans very easily

Variable description

In the ensuing Tables 4 and 5, the dependent and independent/predictive variables used are described. We analysed data for adults (Study I) and children (Study IV) separately, with regards to predictive power, for healing and scarring (donor sites or burns). We also decided to investigate any factor proposed to have an effect on healing and/or scarring, regardless of the type of dermal wound investigated. For this purpose, the healing variable in Study IV, originally a continuous variable was converted to a categorical variable (Table 4).

Predictive variables collected for both adults and children were used, such as: healing times, sex, and infection. As no child presented with: autoimmune disease, use of systemic cortisone, or anticoagulants, or had smoked, these variables were left out. All children were of normal weight for their age (no obesity recorded) and because data were missing for their height in most, the estimate of BMI was not calculated. No data on Fitzpatrick skin type were collected for the adults (Studies I-III). The burn-specific predictive factors: TBSA %, DDB, and skin grafting were not used for the donor site group for obvious reasons.

We examined age as continuous and also divided age groups into categories (in speared analyses). As there were few patients in each “age-group”, the purpose of using “age categories” was to increase group sizes and improve power.

We examined donor site size (factor not used for burn group) as continuous and also divided size groups into categories (in speared analyses) for the same reasons as for the factor age.

In the section Statistical methods we describe the methods used to analyse relations between factors, criteria for inclusion in the models, and the significance levels chosen.

Table 4. Variables used in the multivariate regression analysis for Healing time

Study	Variable name	Type of variable	Scale level	Regression analysis used	Result displayed with
I	Healing 98 %	Dependent	Categorical (0=14 days or less, 1=15-21 days, 2= more than 21 days)	Ordinal regression	Odds ratio (OR) and 95 % confi- dence interval.
I	Healing 100 %	Dependent	Categorical (0=14 days or less, 1=15-21 days, 2= more than 21 days)	Ordinal regression	OR and 95 % con- fidence interval.
IV	Healing 97 %	Dependent	Continuous	Linear regression	Regression coeffi- cient with 95 % confidence inter- vals
IV	Healing 100 %	Dependent	Continuous	Linear regression	Regression coeffi- cient with 95 % confidence inter- vals
Regr*	Healing 100 %	Dependent	Binary Healed at day 14 (Yes= 1, No= 0) Healed at day 21 (Yes= 1, No= 0)	Logistic regression	OR with 95 % confidence inter- vals
I	Age**	Independent	Categorical (adults) Age cat1= 20-43 Age cat 2=44-67 Age cat3=68-90 Categorical (children) Age cat1= 6-25 Age cat 2=26-45 Age cat3= 46-66 Also analyzed as continuous		
I,IV	Sex	Independent	Binary (female=0, male=1)		
I, IV	Infection	Independent	Binary (No=0, Yes=1)		
I	Smoking	Independent	Binary (No=0, Yes=1)		
I	Systemic cortisone	Independent	Binary (No=0, Yes=1)		
I	Anti- coagulants	Independent	Binary (No=0, Yes=1)		
I	BMI	Independent	Continuous		
I	Autoimmune disease	Independent	Binary (No=0, Yes=1)		
I	Donor site size in cm ^{2**}	Independent	Categorical Size cat1= 29-138 Size cat 2=139-247 Size cat3=248-357 Also analyzed as continuous		
IV	TBSA %	Independent	Continuous		
IV	Presence of DDB	Independent	Binary (No=0, Yes=1)		
IV	DDB %	Independent	Continuous		
IV	Skin grafting	Independent	Binary (No=0, Yes=1)		

* This variable was used for a model where we looked at all patients from Studies I and IV

**Variable changed to categorical for analysis purpose

Table 5. Variables used in the multivariate regression analysis for Scarring

Study	Variable name	Type of variable	Scale level	Multivariate analysis used	Result displayed with
II	POSAS Patient part Total score	Dependent	Ordinal	Linear regression	Regression coefficient with 95 % confidence intervals
III, V	POSAS Observer part Total score	Dependent	Ordinal	Linear regression	Regression coefficient with 95 % confidence intervals
V	VSS Total score	Dependent	Ordinal	Linear regression	Regression coefficient with 95 % confidence intervals
V	Presence of hypertrophic scarring (HTS)	Dependent	Binary (No=0, Yes=1)	Logistic regression	OR and 95 % confidence interval.
II,III, V	Age*	Independent	Categorical (adults) Age cat1= 20-43 Age cat 2=44-67 Age cat3= 68-90 Categorical (children) Age cat1= 6-26 Age cat 2=27-47 Age cat3= 48-66 Also analyzed as continuous		
II , III, V	Sex	Independent	Binary (female=0,male=1)		
I,IV	Infection	Independent	Binary (No=0, Yes=1)		
I	Healing time	Independent	Categorical (0=14 days or less, 1=15-21 days, 2= more than 21 days)		
IV	Healing time	Independent	Continuous		
I	BMI	Independent	Continuous		
IV	Skin grafting	Independent	Binary (No=0, Yes=1)		
IV	TBSA %	Independent	Continuous		
IV	Presence of DDB	Independent	Binary (No=0, Yes=1)		
IV	DDB %	Independent	Continuous		
V	Fitzpatrick* skin type	Independent	Categorical/binary Skin cat 1= skin type 1-3 Skin cat 2= skin type 4-6 Also analyzed as continuous		
V	Burn on trunk or upper limb	Independent	Binary (No=0, Yes=1)		

*Variable changed to categorical for analysis purpose

Dressing materials evaluated in this thesis

In this thesis we compared our current SOC dressings with other dressings of potential interest. When we started this project, we hypothesised that we did not always use the most optimal dressings for donor sites, and that there might be room for improvement. This was based on the observation that numerous patients returning to our outpatient clinic did so with unhealed donor sites long after 21 days postoperatively. Examination of our medical records from 2006 (data not published) showed that 41 out of the 184 patients who had had elective skin grafts returned after 21 days with an unhealed donor site.

Dressing selection in Study I

For the donor sites investigated in Study I, the current SOC was polyurethane foam (Allevyn®), a non-adhesive, foam, absorbent dressing, that can absorb up to ten times its own weight (10 gr of fluid/gram of dressing) [89]. It is claimed to preserve moisture as an occlusive dressing, and thus promotes moist wound healing. Allevyn® is multi-layered foam, comprising three layers, a polyurethane contact layer, a middle layer of absorbents, and an outer polyurethane film, the latter of which is claimed to be waterproof. It was used as a single dressing under fixation. The use of Allevyn® at donor sites has been previously reported [90-92].

To critically examine our SOC we introduced hydrofibre (Aquacel®) dressing covered with a semipermeable film (Tegaderm, 3M Health Care, St Paul, Minnesota), which is also claimed to create a moist wound healing environment. The advantages of a moist wound healing environment have been briefly described in the *Introduction* section of this thesis and will be further addressed in the *Discussion* section. Aquacel® is a primary non-adhesive wound dressing made from sodium carboxymethylcellulose and is produced as a textile fibre and presented in the form of a fleece held together by a needle bonding process. The dressing has a large fluid-absorption capacity, twice as high as Allevyn®, (20 gr fluid/gram of dressing) [89] and interacts with exudates from the wound to form a soft, hydrophilic, gas-permeable gel that provides a micro-environment claimed to facilitate healing. The vertical absorption properties help to maintain the moist area over the wound and reduce the risk of maceration. The use of Aquacel® at donor sites has been reported previously [93-95].

The third dressing we decided to use in these investigations was a biological dressing, a porcine xenograft (Mediskin®) that was already in use for donor sites at our burn centre, with good clinical results. Mediskin® consists of meshed frozen epidermis and dermis from pigs. It primarily attaches to the wound bed, but

is later rejected as re-epithelialisation occurs underneath. The use of porcine xenograft in donor site treatment was first reported in the 1970's [96-98].

Dressing selection Study IV

The SOC at our department for children with partial thickness burns for at least the last 20 years has been porcine xenografts, as described above. The use of porcine skin in burns is better documented than its use in donor sites [99, 100]. After 2013, the department stopped using Mediskin®, and began to use a similar product, EZderm®, that is produced by the same company. The EZderm® consists of aldehyde cross-linked dermis from pigs [98] and has the advantage compared to Mediskin®, that it can be stored in room temperature.

In 2008-2009 a new synthetic silver-containing dressing was introduced in Sweden; the Mepilex®Ag. It is a silicone foam containing water soluble silver sulphate as a main layer, and a hydrophobic silicone membrane with micropores as a contact layer; the outer layer consists of a waterproof film such as that of the Allevyn® dressing. Mepilex® Ag can absorb about 10 times its own weight [89], as is seen also with Allevyn®. The silver component is active for up to seven days, enabling its use without the need for daily dressing changes [101]. With the aim of lowering the bacterial burden in burn wounds and thereby stimulate healing this antimicrobial dressing was selected in Study IV.

In summary, we have investigated four dressings in this thesis, the hydrofibre with film, polyurethane foam with and without silver, and a biological dressing in the form of a porcine xenograft.

Statistical methods

The choice of research design of the included studies was clinical RCTs, which is the recommended way to evaluate the efficacy of treatment. Studies were analysed on an Intention-to-treat (ITT) basis, with the exception of patients who did not receive the allocated dressing at the initial visit. These patients were excluded from the study and no data was collected.

As the data in this thesis are mainly of a qualitative nature, non-parametric tests have been used primarily. Demographic data about participants are mainly described using median and percentiles or quartiles (where appropriate). When data are displayed with means ranges or standard deviations are used. Probabilities of 0.05 or below were accepted as significant.

To detect any significant differences between the three study groups in Studies I-III, we used the Kruskal-Wallis one-way analysis of variance (ANOVA) for qualitative data and one-way ANOVA for those judged to be quantitative and normally distributed. If a significant difference was detected, we made a post hoc analysis with Tamhane's T2 test in Study I and for Study II, we used the Benjamini-Hochberg test. To detect differences between the two study groups in Studies IV and V, we used the Mann Whitney U test for independent samples. In Study V, we examined repeated measurements on the same group (pairwise comparison) and therefore used the Wilcoxon signed rank test. For all studies, we used the Chi squared tests (Fisher's exact test if expected numbers were below five) for nominal data (frequencies) When data contained ties (the same value given many times), the Chi squared test was used as a complement.

To analyse factors that affected scar outcomes in Study II, we used a multivariable regression model. Independent variables (predictors) selected were: age, sex, healing time, type of dressing, and the items from the POSAS evaluation. The cut-off point for inclusion of an item in the model was set at a $p < 0.05$, and the variables were added in a forward stepwise manner, except for: sex, age, and healing time, which were all kept in the model as its baseline.

For the purpose of investigating the relation between healing and scarring outcomes with potential predictive factors, we used multivariable regression models. Linear regression was used when examining continuous dependent variables. Prior to the analyses normal distribution of residuals, extreme outliers and linear correlation (with scatter plot) were checked. Ordered logistic regression was applied when dependent variables were categorical/ordinal and when three categories were analysed. Ordered logistic analysis was only carried out for variables if they were significant ($p \leq 0.05$) in the presiding Chi squared test. Dependent variables of an ordinal type, such as a POSAS or VSS total scores, with more than three categories, were examined with linear regression to enable us to better interpret the results. Logistic regression was used if the dependent variables were binary.

For linear regression models, coefficients with 95 % confidence intervals are presented. Only models with a p-value ≤ 0.05 are displayed. Odds ratios (OR) with 95 % confidence intervals are used for logistic regressions. In our prediction models, we decided to include only variables that independently had a significant relation with the dependent variables, such as healing times or scarring. With the aim of having a maximum number of variables, not exceeding 10 % of the number of observations in the model, a manual backward elimination of predic-

tive/independent variables in the models was conducted, excluding the non-significant variables first, and then if needed, the remaining variables that were the least significant. Linear regression models were checked for multicollinearity using Variance inflation factor (VIF). A VIF higher than 5 indicates multicollinearity but as the aim of the regression models are “prediction”, models are displayed regardless of multicollinearity or variables being non-significant in the model.

For the logistic regression models the Hosmer-Lemeshow test, a goodness of fit test, was used. Small p-values means that the model is a poor fit and only models with a p-value > 0.05 are displayed. To estimate the coefficient of determination in these models the Nagelkerke's R^2 was used.

Statistical software

Study I: Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS Inc, Chicago, IL, USA).

Study II: STATA version 12.0 (Stata Corp, College Station, Texas, USA)

Study III: STATA version 14.0 (Stata Corp, College Station, Texas, USA)

Study IV: STATA version 15.0 (Stata Corp, College Station, Texas, USA)

Study V and regression analyses: SPSS version 25.0 (IBM Corporation, Armonk, NY, USA)

Ethical considerations

All studies (I-V) were approved by the Regional Ethics Review Board and conducted in compliance with the Guidelines of International Conference on Harmonisation of Good Clinical Practise, ICH-GCP. Studies III and IV, was registered in the public register: clinicaltrials.gov. Regarding the choice of dressings, they were all CE-marked⁵ in Europe, and used according to their indicated purpose. There were ethical considerations regarding the use of the porcine xenografts that are described in Study IV. Regarding the choice of keeping our SOC for donor sites in the RCT, even though the examination of medical records showed poor healing results, the RCT was done to find evidence for the association between poor' healing outcomes and the dressing.

⁵ CE (Conformité Européenne) marking is a certification mark that indicates conformity with health, safety, and environmental protection standards for products sold with the European Economic Area (EEA)

RESULTS

Study I

Dressing the split-thickness skin graft donor site: a randomised clinical trial

A total of 73 donor sites were included in this study; 24 were randomly assigned to Aquacel® (hydrofibre), 25 to Allevyn® (polyurethane foam) and 24 to Mediskin® (porcine xenograft). The three groups compared were similar in distribution of sex, age, BMI, main diagnosis, medication, smoking habits, and size of the donor sites.

Regarding the primary outcome variable: **Healing time**, the Aquacel® and the Mediskin® showed significantly shorter healing times than the Allevyn® ($p=0.01$).

At two weeks (14 days), 15 donor sites were healed in the Aquacel® group, 12 in the Mediskin® group and 6 in the Allevyn® group. At the next, and last visit (day 21) two additional donor sites were healed in the Aquacel® group (a total of 17 donor sites healed), 11 in the Mediskin® group (a total of 23 donor sites healed) and 8 in the Allevyn® group (a total of 14 donor sites healed). This means that in the Aquacel® group four donor sites were not healed at 21 days, one in the Mediskin® group, and nine in the Allevyn® group.

For the secondary outcome: **pain, infection, impact on everyday life, ease of use, and costs** some additional differences were found. At day 14, pain was significantly higher at dressing changes for the Allevyn® group than for the Aquacel® group ($p=0.002$). Aquacel® was reported as significantly easier to apply and remove than the other two dressings on this occasion (Aquacel® -Allevyn® $p=0.001$ and Aquacel® -Mediskin® $p=0.002$). Aquacel® also took less time to apply and remove than the other two dressings (Aquacel® -Allevyn® $p=0.03$ and Aquacel® -Mediskin® $p=0.007$). Results for secondary outcomes (published and unpublished) are presented in Table 6.

Table 6. Secondary outcomes in Study I

	Aquacel®	Allevyn®	Mediskin®	p values
Infection	4	3	0	0.236
Pain at day 3				
Rest	0 (0-1)	0 (0-2.5)	0 (0-1)	0.954
Activity	0 (0-2.8)	1 (0-3.5)	1 (0-3.8)	0.161
Pain at day 14				
Rest	0 (0-0)	0 (0-2)	0 (0-1)	0.264
Activity	1 (0-2)	1 (0-5)	1 (0-3.8)	0.258
Dressing change	1 (0-2.5)**	4 (1.5-5)**	1.5 (0-4)	0.009 *
Pain at day 21				
Rest	0 (0-3)	0 (0-0.5)	0 (0-0)	0.755
Activity	0 (0-4.5)	0 (0-2.5)	0 (0-0)	0.245
Dressing change	0.5 (0-1.3)	0 (0-4)	0 (0-0)	0.185
Impact on everyday life				
Day 3	0 (0-0)	0 (0-1)	0 (0-1)	0.880
Day 14	0 (0-0)	1 (0-4)	0 (0-2.25)	0.051
Day 21	0 (0-1.5)	0 (0-0.5)	0 (0-0)	0.265
Ease of use (0-5)				
Day 3	1 (0-1)	Not changed	1 (0-2.25)	0.202
Day 14	0 (0-0)***	1 (1-3)***	1 (0-2)***	<0.001*
Day 21	1 (0-1)	0 (0-2)	0 (0-0)	0.077
Total cost in US dollars	114	57	261	0.001*

Data are presented as median and IQR 25-75. * Significant values. ** Pairwise comparison shows p=0.002. *** Aquacel®-Allevyn® p= 0.001 and Aquacel® -Mediskin® p = 0.002.

Study II

Scarring At Donor Sites After Split-Thickness Skin Graft: A Prospective, Longitudinal, Randomised Trial

The study participants (consisting of patients who were originally recruited in Study I) were evaluated with regards to their scar outcome. The three dressing groups (a total of 29 donor sites), were similar in: sex distribution and age, but, as in the original RCT group, the healing times were significantly longer for the Allevyn® group ($p = 0.046$), than the Aquacel® group ($p = 0.018$), but no other differences were found with regard to healing time.

As for the donor site **scar outcome**, the Aquacel® group was significantly more satisfied with their scars ($p = 0.03$). Correction with Benjamin-Hochberg showed that the Aquacel® group scored better on the POSAS than the Allevyn® ($p = 0.03$) and the Mediskin® ($p = 0.02$) groups. There were no significant differences between the latter two ($p = 0.47$).

The multivariable analysis showed that treatment with Mediskin® was associated with a higher overall opinion (less like normal skin) on the POSAS score than the other two dressings. The “pigmentation” item was the only item that affected the overall POSAS scores.

Study III

Scarring At Donor Sites After Split-Thickness Skin Graft: The Observer view

In this study, participants from study II were evaluated regarding the **scar outcome** from the observer's perspective and compared with the patient's own experience. The group consisted of 17 donor sites, five from the Aquacel® group, six from the Allevyn® and Mediskin® groups. They were similar in age and sex distribution and also, contrary to the two previous studies, in healing time of the donor site. A figure showing the eligibility of patients assessed for the long-term follow ups studies (Study II and III) is shown in Paper III.

Results showed no differences between the groups with regard to any of the items used in POSAS, or firmness and elasticity (measured with the Cutometer® MPA 580). When regrouping participants (regardless of previous dressing treatment) according to their healing time (categorised as follows: 0 = 14 days or shorter, 1 = 15 to 21 days, and 2 = longer than 21 days) there were no differences for any of the POSAS items or Cutometer® values.

Study IV

Superiority of silver-foam over porcine xenograft dressings for treatment of scalds in children: A prospective randomised controlled trial.

A total of 58 children were included in this study, 30 randomly assigned to porcine xenograft (EZderm®) and 28 to silver-foam (Mepilex® Ag). The two treatment groups were similar in sex distribution, age, weight, previous illnesses, TBSA% burned, and depth of burn.

Regarding the primary outcome **Healing time**, the median (10th-90th percentiles) time to 97% healing for the silver-foam group was 9 (7-23) days compared to 15 (9-29) days in the porcine xenograft group ($p = 0.004$). The median time to complete healing for the silver-foam group was 15 (9-29) days and for the porcine xenograft group 20.5 (11-42) days ($p = 0.010$), this significance remained when we excluded children with TBSA % more than 10 %, and 20 %, respectively ($p=0.045$ and $p=0.034$, respectively).

The silver-foam dressing group also showed better outcomes in **time for application and removal** and **number of dressing changes required** ($p = 0.03$ and $p = 0.03$, respectively). For the secondary outcome variables: **Infection, Pain, Duration of hospital stay** and **need for operations**, the results were similar between groups. See Table 7 below.

Table 7. Secondary outcomes in Study IV

	Porcine xenograft	Silver-foam dressing	p values
Operations	4	2	0.671
Infection	9	10	0.643
Antibiotic use	11	12	0.630
Duration of hospital stay (days)	2 (0-13)	3 (0-8)	0.944
Number of dressing changes	5 (3 – 9)	4 (3 - 8)	0.031*
Dressing time (minutes)	20 (10-50)	20 (10-30)	0.030*

Data are number, or median (10-90 percentiles). * Significant values

Study V

Burn scar outcome at six and 12 months after injury in children with partial thickness scalds: did the dressing treatment matter?

A total of 39 children from Study IV returned for scar follow up at six months and 34 for the 12 months' follow up.

As in the original RCT group (Study IV), the two treatment groups in this follow up study of scars were similar in sex distribution, age, weight, previous illnesses, TBSA% burned, and depth of burn. Unlike the original RCT study (Study IV) they were also similar in healing times.

The results for the evaluation of burn **scar outcomes**, at six and 12 months' follow up, did not show any differences between dressing groups regarding POSAS or VSS total scores, or the incidence of hypertrophic scarring or dyspigmentation.

For the 27 children returning for both scar follow ups, there was a significant reduction ($p < 0.001$) in total scores for both POSAS and VSS between six and 12 months, indicating that scars got better with time according to the Observer.

There was a significant correlation between days required to complete healing and final scar outcome, in which longer healing times gave higher scar scores and increased the risk of **HTS**.

Results for regression analyses looking at predictive factors for healing and scarring outcomes

Predictive factors for *healing times*, *burn scar scores* and *hypertrophic scarring* were analysed using regression analysis.

All variables and p values including coefficients or OR and 95% confidence intervals are displayed in result tables. Only models showing significance ($p < 0.05$) are shown.

Healing time outcome models

Study I (n = 73)

When using the Chi squared test for all potential predictive variables; age categories, sex, infection, smoking, the use of cortisone or anticoagulants, BMI, autoimmune disease and donor site size, none of the variables were found to be affecting the time to healing of the donor sites (time categorised: 0 = 14 days or shorter, 1 = 15 to 21 days, and 2 = longer than 21 days). We therefore decided to create two new binary variables, healed at day 14, and 21, and we looked for associations between the dependent variable (healing at day 14 or 21) and the predictive variables listed above.

Healed at day 14

When analysing potential predictors for donor sites that had healed at day 14, the logistic regression showed that men had a 3.6 times higher chance (OR 3.6 95 % CI 1.14 to 11.34, p value= 0.029) of having their donor site healed at day 14 than women. No other variable was independently related to complete healing of the donor site at day 14.

Healed at day 21

When analysing potential predictors for donor site healed at day 21, none of the variables turned out to be significant.

Study IV (n=58)

When analysing potential predictors for longer healing times for the 58 children with scalds in Study IV, there were three variables that were independently associated with healing time (97 % healed); larger TBSA %, presence of DDB and skin grafting, all prolonged healing. When adding the significant variables into the model, variable DDB and skin grafting remained significant (Table 8).

When looking at complete healing (100 % healed) of the burn, the same three variables remained significant and the variable, infection, also had a significantly negative impact on healing, but as with the former model, only the presence of DDB and skin grafting remained significant when adding the variables to the model.

When we excluded children who had had skin grafts (n = 6) or outliers (two with healing times beyond 42 days), TBSA % and DDB remained as independently related variables, but only the presence of DDB remained significant when the variables were added together (Appendices 1 and 2).

As the presence of DDB had an impact on healing time in all groups and we had data to show the percentages of DDB in all children from the Lund and Browder charts, an examination of this variable was done independently to see how much each per cent of DDB affected the number of days to complete healing.

When looking at all children (58), each per cent of DDB increased the number of days to 97 % healing with 2.4 days (95% CI 0.77 to 3.92 $p = 0.004$) and to complete healing with 4.9 days (95 CI % 3.4 to 6.6 $p < 0.001$), respectively. For the group that did not have skin grafts the results were similar: for 97 % healing each per cent added 2.5 days (95 % CI 1.0 to 4.0 $p = 0.001$) and for complete healing 4.6 days (95 % CI 2.8 to 6.5 $p < 0.001$), respectively.

Sex and age were not found to be significant in all models, regardless if age was used as a categorical or a continuous variable.

Calculation of VIF showed no signs of multicollinearity in the healing models seen in Table 8 (VIFs below 1.44).

Table 8. Predictors for longer healing times in Study IV for all children

		Coefficient	95 % CI	p value
Independently related				
(Days to 97 % healing)	TBSA %	0.62	0.08 to 1.17	0.026
	DDB	8.99	3.69 to 14.28	0.001
	Skin grafting	18.03		<0.001
In a model				
(Days to 97 % healing)	TBSA %	-0.08	-0.64 to 0.48	0.769
	DDB	6.718	1.35 to 12.82	0.015*
	Skin grafting	15.66	7.01 to 24.31	0.001*
Model				<0.001
Adjusted R square				0.314
Independently related				
(Days to complete healing)	TBSA %	1.37	0.78 to 1.96	<0.001
	DDB	14.30	8.39 to 20.22	<0.001
	Skin grafting	21.67	11.73 to 31.61	<0.001
	Infection	8.27	1.14 to 15.41	0.024
In a model				
(Days to complete healing)	TBSA %	0.592	-0.02 to 1.21	0.059
	DDB	9.556	3.76 to 15.35	0.002*
	Skin grafting	11.649	1.69 to 21.61	0.023*
	Infection	2.880	-3.08 to 8.84	0.337
Model				<0.001
Adjusted R square				0.445

* Significant in the model

Healing times Study I and IV.

As part of the analysis we chose to examine all participants from study I and IV with regards to potential predictors of dermal wound healing (both donor sites and partial thickness burns). For this purpose, healing times for burns in study IV were reorganised to: 0 = 14 days or shorter, 1 = 15 to 21 days, and 2 = longer than 21 days. Unpublished data on time to complete (100 %) healing of donor sites was used for this purpose.

A total number of 127 study participants had data on complete (100 %) healing.

Neither sex, nor infection were independently or together significantly related to time to complete healing.

Scar outcome models

Study II

POSAS Total score – Patient's opinion (n=29)

When analysing potential factors for the patient's opinion on donor site scarring, we found that only sex was independently associated with subjective opinion. Female patients provided higher POSAS values, meaning that they were less satisfied with the outcome (Table 9). The remaining variables analysed were; age, healing time, infection (at donor site) and BMI, and none was significant.

Study III

POSAS Observer - Study III

When analysing potential factors for the observer's opinion on scarring at donor sites, we had a similar finding; only sex was independently associated. Female patients had, according to the observer, more severe scarring (higher scores). Sex explained about 25% of the variation in total scores (Table 9). There was a tendency ($p = 0.053$) for older patients to rate higher than those of a younger age (coefficient 2.10, 95% CI -0.03 to 4.23).

The remaining variables analysed; age, healing time, infection (at donor site) and BMI were not significant.

Table 9. Predictor models of scarring in Study II, III

	Coefficient	95 % CI	p value
Study II (n=29)			
POSAS Patient total score			
Sex (1=male)	-7.36	-12.62 to -2.12	0.008
<i>Model, R Square</i>			0.235
Study III (n= 17)			
POSAS Observer total score			
Sex (1=male)	-3.129	-6.15 to -0.11	0.043
<i>Model, R Square</i>			0.246

Study V

For all scar analyses of children in Study V, we used the measure days to complete (100 %) healing of the burn (healing time in days).

POSAS scores at six months

POSAS Observer total scores at six months in Study V

There were several factors independently related to higher scar scores at the six months' follow up; longer healing times, previous infection, larger TBSA%, DDB, and skin grafting (Table 10). Sex, age, burns on trunk or upper limb and Fitzpatrick skin type (regardless of being used as categorical or continuous variables) were not found to be related to POSAS scar scores.

Prediction model for six months' scar scores

Healing time, DDB and skin grafting remained significant predictors when added into the model. The variables together explained 55% of the variation in the Observer's scores on the POSAS. As the variables TBSA % and infection were the least significant, they were excluded from the model (maximum of three variables) (Table 10). Exclusion of the outliers (two children with healing times that exceeded 42 days) did not alter the findings.

Exclusion of the six children with skin grafts showed that healing time, infection, and DDB were significantly related to higher scar scores. When the variables were added into the model, only infection remained significant. The variables together explained 44 % of the variation in POSAS Observer's scores for children who had healed spontaneously (Appendix 3).

POSAS scores at 12 months

POSAS Observer total scores at 12 months in Study V

Having longer healing times, larger TBSA%, DDB, and skin grafting were independently related to higher scar scores at the 12 months' follow up (infection was not to be significant) (Table 10). Sex, age, burns on trunk or upper limb and Fitzpatrick skin type (whether being used as a categorical or a continuous variables) were not found to be related to POSAS scar scores.

Prediction model for 12 months' scar scores

When the significant variables were added into the model, none remained significantly related to the scar score; even though the model itself was significant ($p=0.001$). As DDB was the least significant of the four significant variables, it was excluded from the model (maximum of three variables). There was a tendency ($p = 0.051$) in the model for longer healing time to result in higher scar scores (Table 10). Excluding the outliers showed similar results as for the entire group, except for healing time, which remained significant ($p=0.047$). Excluding children who had had skin grafts, the only variable significantly related to scar scores was healing time. This variable alone explained 27 % of the variation in the POSAS Observer's scores for children who had healed spontaneously (Appendix 4).

Calculation of VIF showed no signs of multicollinearity in the POSAS models seen in Table 10 (VIFs below 1.68)

Table 10. Predictors for scarring at 6 and 12 months for all children using POSAS Observer scores

	6 months (n=39)			12 months (n=34)		
	Coefficient	95 % CI	p value	Coefficient	95 % CI	p value
Independently related						
Healing time (days)	0.38	0.19 to 0.57	<0.001	0.25	0.12 to 0.38	<0.001
Infection	8.41	3.46 to 13.36	0.001	Non-significant		
TBSA %	0.68	0.26 to 1.09	0.002	0.62	0.23 to 1.02	0.003
DDB	9.10	4.55 to 13.66	<0.001	6.04	1.89 to 10.19	0.006
Skin grafting	13.38	7.27 to 19.49	<0.001	8.29	2.96 to 13.61	0.003
In a model						
Healing time (days)	0.18	0.02 to 0.34	0.030*	0.16	-0.00 to 0.32	0.051
Infection	Excluded in this model			Non-significant		
TBSA %	Excluded in this model			0.29	-0.16 to 0.73	0.196
DDB	5.08	0.89 to 9.26	0.019*	Excluded in this model		
Skin grafting	7.91	2.06 to 13.76	0.009*	2.76	-3.54 to 9.05	0.379
<i>Model</i>						
<i>Adjusted R Square</i>						

*Significant in the model

VSS scores at six months*VSS total scores at six months in Study V*

The same five predictors as for the POSAS scores remained independently related to the total VSS scores at six months; longer healing times, previous infection, larger TBSA%, DDB, and skin grafting (Table 11). Sex, age, burns on trunk or

upper limb and Fitzpatrick skin type (regardless of being used as categorical or continuous variables) were not found to be related to VSS scar scores.

Prediction model for six months' scar scores

When adding the significant variables into the model, only DDB remained significantly related to the VSS scar score. The three variables together explained 44% of the variation in the Observers' scores (Table 11). Because infection and TBSA % were the least significant of the five variables, they were excluded from the model (maximum of three variables). The exclusion of outliers showed similar results as those for the entire group of 39 children, but no variable turned out to be significant when added into the model.

When repeating the analysis for children who had not required skin grafts ($n = 33$) the variables of healing time and DDB were independently related. Adding them into the model showed that only healing time remained significantly related to the scar scores for children who had healed spontaneously (Appendix 5).

VSS scores at 12 months

VSS total scores at 12 months in Study V

Longer healing times, larger TBSA%, DDB, and skin grafting remained independently related to the total VSS scores at 12 months' follow up. Sex turned out to be significantly related to scar scores; in which boys had scored higher than girls (Table 11). Age, burns on trunk or upper limb and Fitzpatrick skin type (regardless of being used as categorical or continuous variables) remained non-significant.

Prediction model for 12 months' scar scores

When adding variables into the model, only healing time remained a significant predictor. The variables together explained 39% of the variation in VSS scores (Table 11). As TBSA % and sex were the least significant of the five variables, they were excluded from the model (maximum of three variables).

Excluding children who had had skin grafts, the factors of longer healing times and male sex were independently related to higher scar scores at the 12 months' follow up; only healing time remained significant when added into the model (Appendix 5).

Calculation of VIF showed no signs of multicollinearity in the VSS models seen in Table 11 (VIFs below 1.71)

Table 11. Predictors for scarring at 6 and 12 months for all children in Study V using VSS scores

	6 months (n=39)			12 months (n=34)		
	Coefficient	95 % CI	p value	Coefficient	95 % CI	p value
Independently related						
Healing time (days)	0.13	0.08 to 0.19	<0.001	0.12	0.07 to 0.18	<0.001
Infection	2.44	0.34 to 4.55	0.024	Non-significant		
TBSA %	0.26	0.10 to 0.43	0.003	0.21	0.02 to 0.39	0.030
DDB	3.65	1.85 to 5.45	<0.001	2.70	0.86 to 4.55	0.005
Skin grafting	4.24	1.60 to 6.90	0.003	3.23	0.77 to 5.68	0.012
Sex (male=1)	Non-significant			2.00	0.02 to 3.98	0.047
In a model						
Healing time (days)	0.07	-0.00 to 0.14	0.053	0.09	0.02 to 0.16	0.013*
Infection	Excluded in this model			Non-significant		
TBSA %	Excluded in this model			Excluded in this model		
DDB	2.27	0.43 to 4.12	0.017*	1.32	-0.48 to 3.12	0.145
Skin grafting	2.00	-0.58 to 4.58	0.124	0.81	-1.71 to 3.33	0.517
Sex (male=1)	Non-significant			Excluded in this model		
Model,						
Adjusted R Square						

*Significant in the model

Hypertrophic scarring in Study V

Children who had healed within 14 days showed no hypertrophic scarring. Five of the six children who had had skin grafts showed hypertrophic scarring at the grafted areas. The 15 children that showed HTS at the six months' follow up had persistent HTS at the 12 months' follow up, which was in contrast to the findings of Study V for POSAS and VSS scores that were significantly lower at 12 months than at six months. All children who had hypertrophic scars had been prescribed pressure garments within the first two months after injury. None of the children with Fitzpatrick skin type 4-6 showed HTS (there were few observations, however).

The predictive factors for scarring, as registered with POSAS and VSS, also remained independently significant for hypertrophic scarring; healing time, DDB, TBSA %, infection, and skin grafting (Table 12). Sex, age, burns on trunk or upper limb and Fitzpatrick skin type (regardless of being used as categorical or continuous variables) were not found to be related to HTS.

Prediction models for six months' HTS

The model could perfectly predict non-HTS using the variable of skin grafting (all observed non-HTS also predicted by the model). Converting the healing time variable to “not healed by day 14” showed that the model could perfectly predict HTS also (all observed HTS were also predicted by the model). For this reason, these variables could not be analysed and were excluded from the models even though they were perfect predictors.

As TBSA % was the least significant (maximum of three variables) it had to be excluded. Adding significant variables into the model showed that only infection remained a significant predictor. Having had a previous infection increased the risk of HTS at the six months' follow up by more than nine times. Excluding outliers showed similar results as those of the entire group of 39 children.

For the children who did not have grafts, healing time and infection remained significantly related to HTS, when added into the model only healing time remained a significant predictor. For each day required to heal, the risk of HTS increased by 12 % (Appendix 6).

Prediction models for 12 months' HTS

At the 12 months' follow up, only healing time and DDB remained significant. Converting healing time to “not healed by day 14” showed that having a healing time that exceeded 14 days increased the risk of HTS by more than 15 times (Table 12). Skin grafting remained a perfect predictor (all observed non-HTS had been predicted in the model). Adding the variables of healing time and DDB into the model showed a significant Hosmer and Lemeshow goodness of fit test ($p=0.007$). Therefore, we used the converted healing time variable of “not healed by day 14” instead in the model, and the converted variable was the only significant predictor. Not being healed by day 14 increased the risk of HTS by more than 10 times (Table 12). Excluding outliers showed similar results as those of the entire group of 34 children.

For the children who did not have grafts, the only significant predictor of HTS was longer healing time (Appendix 6).

Table 12. Predictors for HTS at 6 and 12 months for all children

	<u>6 months (n=39)</u>			<u>12 months (n=34)</u>		
	OR	95 % CI	p value	OR	95 % CI	p value
Independently related						
Healing time (days)	1.13	1.04 to 1.23	0.006	1.18	1.04 to 1.32	0.008
Not healed by day 14	Perfect prediction of HTS (not possible to analyse)			15.56	1.69 to 143.17	0.015
Infection	10.00	2.19 to 45.64	0.003	Non-significant		
TBSA %	1.24	1.01 to 1.54	0.045	Non-significant		
DDB	6.66	1.47 to 30.21	0.014	6.86	1.43 to 33.01	0.016
Skin grafting	Perfect prediction of non HTS (not possible to analyse)					
In a model						
Healing time (days)	1.10	0.99 to 1.23	0.070	10.46	1.05 to 103.82	0.045**
Infection	9.24	1.40 to 60.93	0.021*	Non-significant		
TBSA %	Excluded in this model			Non-significant		
DDB	3.56	0.50 to 25.60	0.207	4.28	0.77 to 23.85	0.097
<i>Model.</i>	<0.001			0.002		
<i>Model prediction %</i>	79.5			73.5		
<i>Nagelkerke R Square</i>	0.557			0.400		
<i>Hosmer-Lemenshow goodness of fit</i>	0.104			0.609		

* Significant in the model ** Converted variable "Not healed by day 14".

Deep dermal burns

A diagnosis of DDB was a significant predictor of POSAS and VSS scores at both six and 12 months' follow up, and for HTS. As data on percentage body surface area of DDB were available for all children, separate analyses were made looking at the relation between DDB % and scar outcomes. Each percentage body surface area of DDB had a significant impact on all scar score outcomes. Each percentage body surface area of DDB increased the risk of HTS by four times (Table 13).

Table 13. Percentage of DDB as a predictor of scar outcomes

	<u>6 months (n=39)</u>			<u>12 months (n=34)</u>		
	Coefficient	95 % CI	p value	Coefficient	95 % CI	p value
POSAS						
DDB %	2.39	1.25 to 3.54	<0.001	1.84	0.77 to 2.90	0.001
VSS						
DDB %	0.97	0.52 to 1.42	<0.001	0.84	0.37 to 1.31	0.001
	OR	95 % CI	p value	OR	95 % CI	p value
HTS						
DDB %	4.18	1.71 to 10.21	0.002	4.00	1.55 to 10.32	0.004

DISCUSSION

In this thesis, I have studied factors that affect wound healing, and those that could lead to long-term scarring. The two wound models chosen were split thickness skin graft donor sites and partial thickness scalds in children. Different types of dressings were studied to investigate their effect on healing and scarring, using randomised controlled clinical trials with follow-up that ranged between one and eight years.

I. *Wound healing – donor sites*

Study I (Hydrofibre/Aquacel®)

In Study I (on split thickness donor sites) the hydrofibre (Aquacel®) covered with film and porcine xenograft (Mediskin®) provided shorter healing times than our standard of care dressing, which was, at that time, polyurethane foam (Allevyn®). As a result of this, the ease of application, and lower cost, we implemented the hydrofibre (Aquacel®) into our clinical practice as the new standard of care.

To the best of our knowledge, there are four RCTs examining the Aquacel® dressing as a donor site dressing and they were done by Barnea et al. in 2004 [93], Dornseifer et al. in 2011 [94], Brölmann et al. in 2013 [95] and Brenner et al. in 2015 [92]. See Table 14 where the data from our Study I is also presented.

In these studies, the depths of harvested grafts and the locations of donor sites were similar, and also comparable to those of Study I. However, donor sites differed considerably in average size (from 37.5 cm² to 233 cm²), as did the healing criteria used, which made a more detailed comparison difficult.

Healing time

In the study by Barnea et al. [93] healing was evaluated at days three, seven, 10, and 14. The donor site treated with Aquacel® healed up to seven days earlier than its comparator, paraffin gauze, and was found to be superior overall. The mean percentage of re-epithelialisation at day 15 was about 96 % at donor sites treated with Aquacel® compared with 83 % at day 14 in in our Study I. Dornseifer et al. [94] evaluated Aquacel® at day 10 in which 54.5 % of the donor site halves that had been treated with Aquacel® were healed, similarly to the to 71 % that we found at day 14 in our Study I. The comparator, polyurethane film, was considered to be superior, however, as 86.4 % healing was seen at day 10. In the

study by Brölmann et al. [95] the Aquacel® group had a median time to complete healing of 22 days, similar to the 21 days seen in our Study I. They concluded that of the six comparators, hydrocolloids were superior because they had the shortest healing time of all, with a median of 16 days. Brenner et al. [92], on the other hand, showed a short mean healing time of only 8 days for the Aquacel® group. The short healing time was assumed to be related to the fact that the study participants were young and healthy children. Brenner et al. concluded that Aquacel® and Kaltostat® (with 0.5 days shorter healing time than Aquacel®) were suitable for dressings at donor sites, but Kaltostat® was preferred, as it is less costly.

Infection rates

The infection rates reported at donor sites treated with Aquacel® in these four studies were all lower than those we saw in Study I (17 %). Barnea et al. reported no infections compared with Brenner et al. (5 %), Dornseifer et al. (6 %) and Brölmann et al. (15 %), respectively, the latter two did not state the criteria for the assessment of infection or the incidence of microbial swabbing [92-95].

Pain scores

In our study (Study I) NRS 0-10 was used, whereas Barnea et al. and Brölmann et al. used a Visual Analogue Scales (VAS) [93, 95]. Dornseifer et al. used a Likert scale from 0 - 5 [94] and Brenner et al. FLACC and the Wong Baker faces [92]. All but the study by Barnea et al. showed similar results to those found in our Study I; consistently low pain scores for the area treated with Aquacel® (median scores $\leq$ of 1). Barnea et al. reported VAS values of up to 2.5 and although higher, they are still in the low range [93].

Dressing application technique

It is noteworthy that in none of the above described RCTs was the Aquacel® applied under a semi-occlusive secondary dressing, such as the Tegaderm® used in our Study I, but a dry gauze was used instead. The Tegaderm® applied on top of the Aquacel® helped to keep the wound moist by blocking 98 % of the evaporation [102]. In 2010, Blome-Eberwein et al. [103] compared Aquacel® Ag covered with film (referred to as “gelled”) with Aquacel® Ag covered with gauze (adherent) (Table 14). As the Aquacel® Ag (including silver) was used, the results may have been affected by the exposure of the wound to the silver. Interestingly, though, the design and results for the “gelled” variant were similar to those seen in our Study I, and, the “gelled” version resulted in significantly more healed donor sites at day 14 than the dry version. This supports our use of a semi-occlusive technique.

Table 14 : RCTs examining Aquacel® treatment for donor site

First author	Published	Design	Comparator(s)	Participants(n) Age (range)	Mean donor site size(range) Harvest depths Most common location	Healing criteria	Median healing time and/or percentage of donor sites healed	Authors Conclusion
Barnea	2004	intra- individual	Paraffin gauze	N=23 51(19-86) years	> 160 cm ² 0.3 mm Thigh	No residual exudate and no pain when exposed to air	Aquacel® 7-10 days* Mean re-epithelialisation day 15 96 % Paraffin gauze 10-14 days Mean re-epithelialisation day 15 88%	Aquacel® superior
Blome- Eberwein ***	2010	inter- individual	Aquacel®Ag covered with gauze	N=70 42(5-82) years	308 cm ² 0.1-0.3 mm Thigh	Re-epithelialised ≥90%	Aquacel ® Ag with film 88 % healed at day 14 Aquacel® with gauze 67 % healed at day 14	Equally good
Brenner	2015	inter- individual	Kaltostat® Allevyn ®	N=56 4 (1-16) years	63.5 ^{##} (8-600) cm ² 0.2mm	No dressing re- quired	Aquacel ®8 days Kaltostat® 7.5 days Allevyn ® 9.5 days	Kaltostat® superior
Bröhlmann	2013	inter- individual	Alginate Film Gauze Silicone Hydrocolloids	N=289 60-62 years	37.5- 50** (10-800) cm ² 0.3 mm Thigh	Full re- epithelialisation without remaining scab according to the patient.	Aquacel ® 22 days (IQR 15-27) Alginate 22 days (IQR 19-29) Film 23 days (IQR 14-36) Gauze 22 days (IQR 18-33) Silicone 26 days (IQR 18-33) Hydrocolloids 16 days (IQR 12-21)	Hydrocol- loids superior
Dornseifer	2011	intra- individual	Polyurethane film	N= 50 63 (18-95) years	233 (90-732) cm ² 0.2-0.3 mm Thigh	No residual exudate and no pain when exposed to air or antiseptic solution	Aquacel ® 54.5 % healed at day 10 Polyurethane film 86.4 % healed at day 10	Polyurethane film superior
Karlsson Study I	2014	inter- individual	Allevyn ® Mediskin®	N=67 62 (20-90)	112 (30-356) cm ² 0.2-0.3 mm Thigh	Re-epithelialised ≥98% only a protective dressing required	Aquacel ® 71 % healed at day 14, 81 % healed at day 21 Mean re-epithelialisation day 14 83 % Mean re-epithelialisation day 21 77 % Allevyn ® 27 % healed at day 14 59 % healed at day 21 Mean re-epithelialisation day 14 81 % Mean re-epithelialisation day 21 92 % Mediskin ® 50 % healed at day 14 96 % healed at day 21 Mean re-epithelialisation day 14 90 % Mean re-epithelialisation day 21 99 %	Aquacel® superior

* Mean values. ** Medians *** Aquacel Ag

Study I (Porcine xenografts)

The use of porcine xenograft (PX) provided the most amount of healed donor sites at 21 days. As the PX used in the study was meshed and therefore allowed fluid to come out of the wound, we think that it did not completely fulfil the criteria of a moist-wound dressing [104]. As we were unable to find any RCTs using porcine xenografts for the dressing of donor sites, the studies reported below may be considered to be of lower quality.

We found five studies that examined the use of PX dressings at donor sites:

Salisbury et al. [96] and Chang et al. in 1973 [105], Breach et al. in 1979 [97] and Vanstraelen [98] and Gao et al. in 1992 [106]. Only Vanstraelen reported the type of the PX used (EZderm®) [98]. In Study I Mediskin® was used. See Table 15 where the data from our Study I is also presented.

Healing time

Healing criteria were described only by Gao et al., as spontaneous detachment of the PX dressing and complete re-epithelialisation [106]. Comparisons of healing are therefore difficult. Complete healing in all donor sites were reported at day 10 by Breach et al. [97], whereas Chang et al. reported 5-14 days for the PX, which is 3-7 days shorter than the comparator Xeroform® [105]. Vanstraelen described a mean healing time of 11.3 days for PX, three days longer than that seen when using the comparator Kaltostat® [98]. Gao et al. presented a mean time of 10.3 days, which was four days shorter than the comparator petroleum jelly [106]. The reported healing times in these studies were all shorter than those seen in Study I, in which 50 % were reported as healed at day 14.

Infection & Pain scores

As in our Study I, low infection rates was reported by Salisbury et al. [96], Breach et al. [97] and Gao et al. [106]. Vanstraelen reported a 20 % infection rate at day 7 in donor sites treated with PX [98]. The criteria for the assessment of infection are not stated in any of these studies. The incidence of microbial swabbing is reported by Salisbury et al. and Vanstraelen. Pain in areas treated with PX was reported in two studies as “not less than with other dressings” [96] and “eliminated” [105]. Consistently low pain scores were reported in our Study I for the PX group (median VAS ≤ 1.5).

The reason for not recommending PX, apart from longer healing times, was related to biopsy findings of incorporation of the PX or a marked inflammatory reaction in the area in which it was used, or both [96-98].

Table 15 : Studies examining porcine xenograft treatment for donor site

First author	Published	Design	Comparator(s)	Participants (n) Age (range)	Mean donor site Harvest depths Most common location	Healing criteria	Healing times	Authors' Conclusion
Salisbury	1973	intra-individual	Fine mesh gauze Air exposure (no dressing)	N= 17 Age not stated	Size not stated 0.25-0.30 mm Thigh	Not stated, biopsies to state epithelialization (well-formed epithelium)	11 healed within 7-14 days, six showed abnormal healing at PX area with incorporation of PX.	PX not recommended
Chang	1973	intra-individual	Xeroform®	N= 52 (donor sites) Age not stated	Size not stated 0.35 mm Thigh	Not stated	PX: Separation within day 5-14 Xeroform® 3-7 days longer	PX superior
Breach	1979	intra-individual	Bovine dermis	N= 20 Age not stated	Size not stated Depth not stated Thigh	Not stated, tough adherent dressing indicating not healed.	Complete healing in 10 days (range 8-10 days) for both dressing	Discontinuation of both dressings
Vanstraelen	1992	intra-individual	Kaltostat®	N= 20 Age not stated	Size not stated 0.15-0.25 mm Thigh	Re-epithelialized	Kaltostat® mean healing time of 8.1 (±1.1) days, 50 % healed at day 7. PX mean healing time of 11.3 (±1.6) days, 0 % healed at day 7.	Kaltostat® superior
Gão	1992	intra-individual	Petroleum gauze	12 24 (3-38) years	Size not stated Depth not stated Thigh	Spontaneous detachment	Petroleum gauze mean 14.5 (±1.0) days PX mean 10.3 (±0.7) days	PX may be more effective
Study I Karlssoon	2014	inter-individual	Allevyn® Mediskin®	N=67 62 (20-90)	112 cm ² 0.2-0.3 mm Thigh	Re-epithelialized ≥98% only a protective dressing required	PX (Mediskin®) 50 % healed at day 14, 96 % healed at day 21 Aquacel® 71 % healed at day 14 81 % healed at day 21 Allevyn® 27 % healed at day 14 59 % healed at day 21	Aquacel® superior (lower cost and more manageable)

Cytotoxicity of Aquacel® and Mediskin®

Regarding cytotoxicity, Aquacel® has shown a small, but significant cytotoxic effect on keratinocyte survival and proliferation in “in vitro”[107] testing. In vitro studies evaluating the cytotoxicity of Tegaderm® have concluded that the film had no impact on the survival or proliferation of human keratinocytes or fibroblasts, and could be classified as non-toxic [108, 109]. No study evaluating the cytotoxicity of Mediskin® was found.

Study I. Predictive factors for donor site healing

As described in the introduction section, there are several factors, either known or suspected, that influence wound healing. Analyses of the variables involved in the outcome of wound healing in Study I showed that female sex was related to longer healing times at the donor sites. A clinical study from 2016 on donor site healing found similar results [41]. It may be debated if this result is related to sex hormones, because oestrogen and testosterone are both thought to affect healing. In our studies, both women and men had a high median age suggesting that the corresponding sex hormone levels (oestrogen/testosterone) were low, which may be claimed as potentially good for males (there are less unfavourable effects of testosterone as it decreases) and negative for females (because of the less positive effects of the reduction of oestrogen) [110].

I. Wound healing - partial thickness burns

Study IV (Silver foam)

In Study IV, the silver foam dressing (Mepilex®Ag) provided a shorter healing time than the standard of care dressing at that time; porcine xenograft (EZderm®). As a result of this, we implemented the silver foam dressing (Mepilex®Ag) into clinical practice as the new standard of care.

Mepilex®Ag (silver foam) was evaluated for its efficacy in the treatment of partial thickness burns in three RCTs (Table 16) published by Silverstein et al. in 2011[111], Gee Kee et al. 2015 [112] and Hundeshagen et al. 2018 [113]. In the RCTs from 2011 and 2015, Mepilex®Ag was compared with other dressings that contained silver [111, 112] and in 2018 with Suprathel® (polylactic acid membrane) [113]. Mepilex® Ag was the preferred dressing in the studies to compare silver-containing dressings [111, 112], and was evaluated to be equally as good as Suprathel® [113].

Healing time

Similar healing criteria as in Study IV were used in these RCTs. The average TBSA % was similar to that of the 5 % reported in Study IV, except in the study by Gee Kee et al, in which it was considerably lower at 0.5-1 % TBSA [112].

Silverstein et al. [111] reported a mean time to complete healing of 13 days in the Mepilex® Ag group, a similar result to that of 15 days seen in our Study IV, and 3.5 days shorter than the comparator silver sulfadiazine (SSD). Hundeshagen et al. [113] presented a median time to complete healing of 12 days for both Mepilex® Ag and Suprathel®, which was three days shorter than in our Study IV. As for the study by Gee Kee et al. [112], in which TBSA % was smaller and the healing criteria set to burns that had re-epithelialised to 95 %, median time to healing was only seven days.

Rates of infection

The reported rate of infection in Study IV was considerably higher than those described in the other RCTs. Silverstein et al. reported one case of infection in 49 patients (0.02 %) [111], compared with 10/28 (35 %) in our Study I. Gee Kee et al. reported no infections in 96 children [112], and Hundeshagen et al. presented no infections in 30 patients (adult and children) [113]. Initial swabs were taken in the study by Silverstein et al., and only 14 % contained microbial growth [111], compared with 86 % prevalence of growth in our Study IV. Silverstein et al. did not specify at which time the swab had been taken (before or after cleansing) or which cleansing agents were used. It is not stated in the protocol [114] or the final publication [112] by Gee Kee et al. how burns were cleaned or if any antibiotics were used. In this trial, children who presented with colds, flu, or viral symptoms (such as upper respiratory tract infections) were excluded, but such children were not excluded in our Study IV. It is noteworthy that in none of these studies were microbial swabs taken regularly, but only on clinical signs or suspicion of infection, and no CRP levels were presented. This could potentially have been the reason for a higher reported incidence of infections seen in our Study IV.

Pain scores

Silverstein et al. used different pain scales, none of which were the FLACC that we used in Study IV [111]. As for the study by Gee Kee et al. the FLACC scale was used, together with several other pain scales, and only differences in pain scores between the dressing groups were shown [112]. In this study in which

Mepilex®Ag was compared with Mepitel®/Acticoat® and Acticoat® alone; the use of Mepilex®Ag and Mepitel® reduced pain significantly more than Acticoat®. This is suggested by the authors to be a result of the Safetac® technology used in the dressings. In the study by Hundeshagen et al. the children rated their pain using the face-scale Wong-Baker Faces. Pain was initially rated lower in the Suprathel® group (first five days) [113]. Similarly to our Study IV, the authors noted a steady decline in pain in both groups over time.

LOS, costs and dressing time

In the study by Silverstein et al, the median LOS for the Mepilex®Ag group was three (range 1-30) days [111], which was similar to our Study IV, which had a median LOS of 2.5 (10-90 percentiles 0-8) days. Treatment with Mepilex® Ag was reported to be less expensive than its comparator in two of the RCTs, which is the same conclusion as in our Study I [111, 113]. Regarding change of dressing times, the Mepilex® AG group in the study by Gee Kee et al. required a median of five minutes, which was shorter than the 10 minutes reported in the other groups [112] and considerably shorter than the 20 minutes seen in our Study IV. The reason was most likely to be a result of the more extensive burns seen in Study IV.

Cytotoxicity of Mepilex®Ag

In “*in vitro*” studies from 2015, 2017, and 2018, it was shown that Mepilex® Ag was highly effective against some of the most common bacterial pathogens in burns, such as staphylococcus aureus and pseudomonas aeruginosa [115-117]. However, it has also shown to have a negative effect on cell survival [115, 118]. In 2017, Mepilex® Ag was compared with three other silver foams (Betafoam®, PolyMem-Ag® and Allevyn-Ag®) and the survival of mouse fibroblasts was examined after exposure to the dressing. Mepilex® Ag showed the second largest reduction of fibroblast survival, with a reduction in cell count of 75% [115]. “*In vitro*” studies, such as the ones described, must however be interpreted with caution, as they might not be the most accurate way to evaluate antimicrobial properties or cytotoxic effects “*in vivo*” on human skin.

Table 16 : RCTs examining Mepilex® Ag for partial thickness burns

First author	Published	Design	Comparator(s)	Participants(n) Age (range)	TBSA % burned	Healing criteria	Median healing time and/or percentage of burns healed	Authors' Conclusion
Silverstein	2011	inter-individual	SSD (Silvadene®)	N= 100 Mepilex® Ag 31.5 (8.4-88.1) *years SSD 36.5 (8.7-86) * years	Mepilex® Ag 4.5(range 2.5-24) * SSD 4 (range 2.5-15) *	100% healed	Mepilex® Ag 13.44 days** At day 7: 35 % healed At day 14: 76 % healed SSD 17.11 days ** At day 7: 20% healed At day 14: 56 % healed	Mepilex® Ag superior
Gee Kee	2015	inter-individual	Acticoat® Acticoat®+Mepitel®	N= 103 1 (IQR 1-4/5)*years	0.5-1 % *	≥ 95% re-epithelialised	Mepilex® Ag 7.00 (IQR 4-8) days Acticoat® 9.50 (IQR 7-14) days Acticoat®+Mepitel® 10 (IQR 8-13) days	Mepilex® Ag superior
Hundesdøgen	2018	inter-individual	Suprathel®	N= 62 Mepilex® Ag 20±20 years** Suprathel® 24±23 years**	Mepilex® Ag 5.9 ±5.8** Suprathel 5.5 ±4.6**	Completely re-epithelialised no further dressing	12 days both groups	Both dressings feasible and efficacious
Study IV Karlsson	2019	inter-individual	EZderm®	N=58 Mepilex® Ag 17 (6-52) months EZderm® 21 (6-66) months	Mepilex® Ag 5 (10-90 th 2-8) EZderm® 5 (10-90 th 3-22)	97% re-epithelialised 100% re-epithelialised, with no need for further dressings other than a protective covering	Mepilex® Ag 97 % 9 (10-90 th 7-23) days 100 % 15 (10-90 th 7-30) days EZderm® 97 % 15 (10-90 th 9-29) days 100 % 20.5 (10-90 th 11-42) days	Mepilex® Ag superior

* median ** mean

Study IV. Factors affecting the healing time of partial thickness burns

When important factors for predicting the healing times of burns were sought, several potentially predictive factors were found. Children with larger TBSA burned, DDB, infected burns, and skin grafts showed longer healing times. Including them in the analysis showed that the only two variables associated with healing time in the final model, were DDB and skin grafting. Each percentage of body surface DDB increased the number of days to complete healing of the burn by up to five days. As a DDB might require operation, we also excluded the children who had skin grafts, and the association between DDB and longer healing time remained. Infection was associated with longer healing times, when examining the entire group until complete healing, (including children with skin grafts), but not otherwise.

II. Scarring outcome

Study II

In Study II, it was concluded that patients in the hydrofibre group were the most satisfied with their donor site scars, according to the subjective evaluation done with the POSAS Patient scar scale instrument.

To our knowledge, three RCTs have reported on scarring outcomes with the use of Aquacel® on donor sites. Barnea et al. evaluated scar outcome at 30, 60, 90 days, and 1 year after surgery using a modified VSS [93]. Aquacel® was found to be superior at all time points compared to paraffin gauze. Dornseifer et al. reported on a 60 day scar follow up using VSS where Aquacel® gave similar good results as polyurethane film [94]. Brölmann et al. reported on a three months' follow up where POSAS was used [95]. The Aquacel® group in that study had a median POSAS score of 10 (IQR 7-15), which was similar to that seen for alginates, film, gauze, silicone, and hydrocolloids, and similar to Study II where the median score for the Aquacel® group was 8 (7-11).

Surprisingly, in Study II, the PX group had the highest scar scores, which indicated that they were the least satisfied with their scar. Previous studies (though not RCTs), have also reported on less satisfactory scarring seen after the use of PX [97, 98].

To our knowledge, two studies (one RCT) have reported on scar outcomes with the use of Allevyn® [90, 119], none of the supporting the less satisfactory scarring seen with Allevyn® in our Study II. In both studies Allevyn® was com-

pared with Acticoat® regarding scar outcome at 1, 2 and three months after surgery using VSS. Dressings were found equally good in one [90] and Allevyn® superior at one and two months in the other study [119].

The overall opinion of the scar compared with normal skin (on a scale of 1-10 where 1 = scar like normal skin) rated by the patient in Study II (not used by the observer in Study III) was 1 in the Aquacel® group, 2 in the Allevyn® group and three in the PX group. This gave a significant difference in POSAS total score in which Aquacel® had a median score of nine, Allevyn® 15, and PX 19.

A multivariable regression analysis showed that a higher overall opinion (scar less like normal skin) was significantly associated with treatment with PX and abnormal pigmentation. Healing time, age and sex did not contribute significantly to this model, which could explain 47 % of the variance in overall opinion score.

What the actual reasons were for patients in the two latter groups recording worse overall opinions in Study II can only be guessed. The groups were small, 11 patients in the Aquacel® group and nine patients in the other two. Even though not significant in the regression model sex might have influenced the overall opinion as the percentage of women was somewhat higher in the two latter groups, 56 % (Allevyn®) and 44 % (PX) compared with 36 % in the Aquacel® group. As seen in the result section female sex was significantly related to the total scores on POSAS given by the patients.

Regarding age, also non-significant in the model, the PX group were more than 17 years younger than the other two groups in Study II, which could have affected the overall opinion scores. Older age has been shown to be associated with lower scar scores [120] (something that we found a tendency towards ($p=0.053$) in Study III.

Several donor sites in Study II were suspected to be HTS, using two earlier suggested models on what defines a hypertrophic scar (which are described in Paper II and III) but at the last evaluation, the scars were judged not to be hypertrophic. This highlights the need for detailed visual inspection of scars to diagnose HTS properly, and that measurements of height are needed for a proper diagnosis.

Study III

In Study III the donor sites that were assessed by the patient in Study II were further evaluated by a blinded observer, and with the use of a Cutometer® MPA 580. Neither the Observer POSAS, or the Cutometer could help to confirm the

previous satisfying scar results (hydrofibre), nor the unsatisfying results for PX, or polyurethane foam such as those described by the patients in Study II. No differences in any of the items could be seen between dressing groups.

As discussed in Study III, a potential reason for not being able to confirm previous results may be that the patients who returned for the last part (Study III) were not fully representative of the group examined in Study II. The ones returning in the hydrofibre (Aquacel®) group, (such as those who had higher patient-reported scores than those in the other two groups, in which the returners had lower patient-reported scores than the non-returners).

Looking closer at the scores given by the patients in Study II confirmed that consistently lower scores (median = 1) were given for all items except pigmentation, in which the median was three (on a scale of 1-10) for the Aquacel® group, and five in the other two groups. The pigmentation item (together with surface area) was the only one that was rated higher than 1 (1 = normal) by the Observer.

Hypopigmentation has been reported earlier in a donor-site study, but with a lower incidence than was noted in Study III. Melanin levels have been reported to be lower, but not significantly so in donor sites compared with uninjured skin [121].

There are several possible causes that can explain the high incidence of hypopigmentation. One could be that the surgeons harvested skin grafts deeper than was thought (removing the epidermal skin appendages from the donor site [122, 123]. An (animal) study showed that in biopsies from partial thickness wounds, melanin is found mainly at the level of the basal membrane, and not at all in epidermal layers, as is seen in healthy uninjured skin, which could potentially result in macroscopic hypopigmentation [124]. In 2001, Velangi et al [125], presented a study in which biopsies were taken from long-term hypopigmented scars in humans and they found that there were no differences in the number or activity of melanocytes in pale scars compared with those of healthy skin in the same individual. Instead, their theory was that the pale outcome could have been related to reduced vascularity, or a second possibility was that abnormal organisation of collagen within the dermis, or epidermal changes, or both, could have resulted in a greater reflection of light back towards the observer.

Factors predicting the development of scarring at donor sites

As reported in the result section, we found that female sex was the only factor that seemed to negatively influence the self-reported total POSAS scar scores. Two recent studies, from 2018 and 2019 [120, 126], supported this finding, showing that women rated their overall scar score significantly higher than men. Another study from 2014, showed that women least preferred donor sites on the thigh, compared with all other locations [127]. This might indicate that the women in this study were unsatisfied with the location of the scar, which led to a higher POSAS score. Interestingly, when analysing factors that are important for the prediction of the observer's view on the scarring at donor sites (Study III), a similar finding was made; that is to say, only sex was independently associated with a worse scar estimate. Female patients (according also to the observer), had more severe scarring (higher scores). It must, however, be stressed that scar scores were relatively low and severe scarring was hardly described at all. The main scarring issue reported was, as has been discussed, hypopigmentation.

Werdin et al presented in 2018 [128] a donor site scar follow up at three and 12 months on a population similar to that of Studies II and III. They found that there was a linear relation between healing time and scarring, a finding that could not be reproduced in this study. It could possibly depend on the smaller sample size, or the fact that only three possible healing times were evaluated in our Study I (less than 14, 14 to 21, and more than 21 days) or that scars actually got better with time. In the study by Werdin et al., no scar evaluation was made beyond 12 months.

Study V

In Study V burn scar outcomes for the children participating in Study IV were evaluated. The data suggested that neither dressing had a more favourable impact on scar outcome, and this was supported by the fact that no significant differences were found in POSAS or VSS scores, or the incidence of HTS.

Only two RCT studies could be found with follow up on scar outcomes after dressing treatments for partial thickness burns [129, 130] and none of these were undertaken with follow up as long as 12 months after injury, such as we did in Study IV. In 1989 Cockington compared [130] scar outcomes for up to three months after injury in an unknown number of children treated with SSD, paraffin gauze or transparent adhesive film. It was concluded that all scars were similar, and that dyspigmentation persisted in a few scars up to three months after injury.

This is in contrast to our findings in Study V. The lower incidence of dyspigmentation might have been related to the short healing times reported in the study by Cockington, in which all children were healed at three weeks after injury.

In 2016, Gee Kee et al [129] did a follow up on 43 children who had been recruited originally in an RCT study [112] in which Mepilex® Ag was compared with Acticoat® or Mepitel® with Acticoat® on top. The dressing type was identified as having a significant effect on the POSAS scores. Other important factors were healing time, burn depth, and TBSA %. However, as multicollinearity was noted and the dressing type in itself, was finally ruled out as being unimportant for the scar outcome. This statement closed the discussion in this study and, surprisingly, it was not further commented upon or explained. In the same study, burn scar thickness was also evaluated and no relation between the type of dressing and the thickness of the scar was found.

Factors predicting scarring of partial thickness burns (Study V)

When examining factors that were important in the development of scarring, findings from similar populations were confirmed. Longer healing times were associated with higher POSAS and VSS scar scores and higher incidences of hypertrophic scarring in burns [51-53, 55, 57, 129, 131-133]. Also, the model confirmed that a larger TBSA % and deeper burns had a negative effect on scarring, as had also been reported before [51, 53, 55]. Interestingly, infection seemed to be related to both higher scar scores and incidences of HTS at six months' follow up. This could, however, not be confirmed at the 12 months' follow up,

As reported in earlier studies [55, 57, 132], skin grafting had a major impact on scar scores and the rate of hypertrophic scarring in the children in Study V. They presented with significantly worse and hypertrophic scars. Lonie et al. [57] found that skin grafting offered no protection from HTS in children who had healed after 22-30 days. It seems to suggest that if skin grafting is not done in an early stage so that the child is healed within <21 days it might be a better alternative to leave the burn to heal spontaneously, even if the healing time is significantly longer. Finlay et al. showed in 2017 [132] that surgical interventions had a significant impact on scarring, but did not impose the same influence that healing time does on scar scores. This suggested that operation should be done at an early stage to shorten the healing time[132]. Animal models (porcine) have indicated the same thing, and that early skin grafting (at day three) lowers the risk of HTS scarring compared with delayed operation >14 to 21 days)[134] .

The children in our Study IV were given split skin grafts rather late (days six, 14, 15, and 21 after injury) when looking at the suggested healing times to lower the risk of HTS.

In 2006 Cubison et al presented data on 281 children with scalds. They found that the incidence of HTS in the group who healed spontaneously within 14 days was lower than children with scalds who had been operated on with split skin grafts and healed within 14 days after injury [52]. These findings were supported by Hassan et al in 2014, who suggested that burns up to 5 % might be left to heal spontaneously beyond 21 days without increasing the risk of hypertrophic scarring [48]. It is also worth mentioning in this context that both Cubison and Hassan used a retrospective design, which might have influenced the validity of the results. In our Study V, the incidence of HTS was 38 % (15/39) which was similar to what Cubison reported [52].

Infection, in our regression analysis, was also found to be of relevance to the scar scores registered and hypertrophic scarring at the six months' follow up. The association between infection and scarring has been supported by most studies [48, 54, 135-137], but has also been questioned [131]. It has been argued that infection prolongs the inflammatory phase, thus resulting in the formation of HTS. Further, it has been claimed that infection also delays the onset and duration of epithelialisation, which is known to be associated with HTS, as has been described in earlier studies [58].

Potential ways to prevent or reduce scarring may be to harvest skin grafts from other locations, such as the back or buttocks (less visible scars). Using the scalp or harvest thinner grafts could potentially shorten healing times at the donor sites [138, 139] and save melanocytes in epidermal appendages, the latter to reduce the risk of hypopigmentation. Other surgical techniques such as dermis grafts can be advocated [140] or resurfacing the donor sites with thin grafts [141]; minced grafts [142] or by adding autologous skin cells [143].

III. *Strengths and Limitations*

Strengths

A methodological strength in this thesis is the use of the RCT design to reduce the risk of allocation bias. The patients' baseline characteristics were similar between groups at the start of the trials. To ensure equal-sized treatment groups we used blocked randomisation. This led also to favorable groups for comparisons. Applying wide inclusions criteria and thereby reducing exclusions also facilitated the clinical implementation as well as increased the generalisability of the study findings. As the wound healing and scarring prediction analyses on our scald study participants in Study IV-V confirmed previous results from studies mainly from Europe, we have reason to think that our "group" was representative of all children with burns in Western, developed countries.

Another strength is the attempt to report items according to the Consolidated Standards of Reporting Trials (CONSORT), which are claimed to improve the reporting standards of RCTs [144, 145].

As for the choice of outcome variables, we chose those most commonly used in previous publications to facilitate comparisons between studies. The number of articles on donor site scarring is, however, limited and Studies II and III can therefore contribute to the knowledge in this field, even though the number of observations were low.

The use of "more" objective markers for healing and scarring such as laser doppler flowmetry or imaging, narrow-band reflectance spectrophotometers measuring erythema and melanin, tissue biopsies or Cutometers would have been advantageous, but many of these still require a "subjective" interpretation, which could still introduce bias.

Another strength of the present studies is the relatively high return rate. Seventy-nine per cent of the surviving patients in Study I were included in Study II and of these 68 % returned for Study III. In Study V, 79 % of the children from Study IV were included, and 67 % returned. Another strength is that the Observers of scar outcome were blinded.

Limitations

The limitations of these studies are issues that are often encountered in the evaluation of wound dressings in general, such as the lack of "solid" data, making reproducibility a challenge. Researchers in this field often use different nomencla-

ture and varying evaluation methods, which makes homogenisation difficult, as has been pointed out by the Cochrane group [70, 146] and shown in the discussion section. Research into wound healing is hampered by the relative lack of consensus regarding objective endpoints at which to assess healing and scarring. In the latter case there are two generally used outcome measures, the VSS and the POSAS.

In our RCT studies we have used two different techniques for the evaluation of healing, neither of which can be claimed to be totally objective. In Study I we used wound tracing and from this could calculate the percentage of the wound that had healed, because the donor sites were standardised in a particular form (a square) and located in an accessible area. In Study IV, we tried using the same method but realised early that this could not be done, because the burns were located in both convex and concave surfaces, and so were often less accessible for to measure. Therefore, the burn surgeon instead estimated the surface area burned by the rule of the palm (1 % of the TBSA is roughly the size of the injured person's palm), and later the surface area healed using the same technique. The use of wound tracing on irregular surfaces has been reported to be associated with a significant risk of errors, which supports our choice of method [112].

Another limitation was the difficulty in blinding the observers for the evaluations of healing. The reason for this is that porcine xenografts adhere to the wound bed, and when finally rejected (with a healed wound surface underneath) a temporary mesh pattern can sometimes be seen. Hydrofibre dressings can also sometimes leave remnants, which is visible as white "threads" in the wound bed. It could be argued that the result has then become biased (assessment bias). To reduce such risks, it was decided that both surgeon and nurse had to agree on the healing status. The most accurate evaluation would, of course, be based on a strictly blinded procedure, but the nature of the dressings used made blinding difficult.

Most preferably we should have used intra-individual comparisons of dressings to reduce any patient-selection bias and effects of inter-individual variations. For Study I we concluded, as early as the planning phase, that the three different donor site dressings of choice would not be possible to apply side by side in the same donor site in a satisfactory way. As for the children in Study IV, their burns were irregular, of different depths and locations, making an intra-individual complete dressing evaluation difficult, not to say impossible.

A general limitation in studying scars and scar maturation is the lack of consensus on what constitutes normal scar healing, maturation of a scar, and clearly pathological scarring. Modified scar scales, approaching a level of properly defining a non-pathological and pathological scars are being developed [147]. The most appropriate scar studies or use of scar scales is likely to be pairwise comparisons in the same scar, investigating the effects of potential treatments.

Use of the Cutometer has only recently been introduced at the department and was therefore not used in Study V. In the future, as our experience increases, the instrument is more likely to be used further in both adults and children, although suspected to be more difficult to use in small children, who are less compliant.

A significant limitation, which is out of our control, is loss to follow up in Studies II and III. As reported in Study II almost 50 % of participants in Study I were deceased at the time of enrollment to Studies II and III. As described in Paper II this group was significantly older than the returning participants. Eight patients did not respond, or declined to participate in Study II and III for unknown reasons. A potential reason, we think, for declining to participate was the travelling distance.

CONCLUSIONS

The results of the data presented and the ensuing prediction analyses made in this thesis are as follows:

- The choice of dressing does have an impact on the healing process of the split thickness skin graft donor site as well as in partial thickness burns. As hypothesised, the hydrofibre Aquacel® dressing was superior to the standard of care dressing. Donor sites treated with the hydrofibre Aquacel® covered with the Tegaderm® - film or with the porcine xenograft Mediskin® showed significantly shorter healing times compared with the standard of care dressing, the polyurethane foam Allevyn®. Partial thickness burns treated with the silver-foam Mepilex®Ag showed shorter healing times than the standard of care dressing, the porcine xenograft EZderm®.
- The choice of dressing had an impact on the patient's opinion of the donor site scar quality, and those who had Aquacel® together with Tegaderm® were more satisfied. This finding could not be confirmed by a blinded observer or in the data gathered using the Cutometer® MPA 580.
- The choice of dressing for partial thickness burns did not have an effect on scar outcome.
- One specific and predictive factor associated with improved healing - and scar outcomes at donor sites - could be clearly identified. Females had longer healing times and also scored higher in their own scar evaluations as well as those done by the observer.
- Specific, and predictive factors associated with improved healing - and scar outcomes of partial thickness burns could also be identified. Previous known factors responsible for longer healing times in burns were: higher TBSA %, the extent and presence of DDB, skin grafting, and infection. The same factors were identified to be important for higher scar scores with the special addition of longer healing times (>14 days) that impacted negatively on the final scar outcome.

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APPENDIX

Appendix 1. Predictors for longer healing times in Study IV in Children not skin-grafted (n=40)

		Coefficient	95 % CI	p value
Independently related				
Days to 97 % healing	TBSA %	0.50	0.02 to 0.98	0.026
	DDB	5.86	2.29 to 9.43	0.002
In a model				
	TBSA %	0.23	-0,27 to 0,72	0,368
	DDB	5.11	1.16 to 9.06	0.012*
<i>Model</i>				0.005
<i>Adjusted R square</i>				0.162
Independently related				
Days to complete (100 %)healing	TBSA %	1.01	0.40 to 1.63	<0.001
	DDB	10.75	6.42 to 15.08	<0.001
In a model				
	TBSA %	0.54	-0.05 to 1.14	0.071
	DDB	9.03	4.40 to 13.66	<0.001*
<i>Model</i>				<0.001
<i>Adjusted R square</i>				0.351

* Factor significant in the model

Appendix 2. Predictors for longer healing times in Study IV without outliers

		Coefficient	95 % CI	p value
Independently related				
Days to 97 % healing	TBSA %	0.58	0.21 to 0.97	0.003
	DDB	6.34	2.90 to 9.78	0.001
In a model				
	TBSA %	0.21	-0.29 to 0.70	0.408
	DDB	2.19	0.27 to 4.10	0.026*
<i>Model</i>				0.001
<i>Adjusted R square</i>				0.202
Independently related				
Days to complete healing	TBSA %	1.01	0.40 to 1.63	<0.001
	DDB	10.75	6.42 to 15.08	<0.001
In a model				
	TBSA %	0.49	-0.11 to 1.10	0.109
	DDB	3.25	0.90 to 5.59	0.008*
<i>Model</i>				<0.001
<i>Adjusted R square</i>				0.335

* Factor significant in the model

Appendix 3. Predictor models of scarring at 6 months in Study V not skin-grafted (n=33) using POSAS Observer scores

		Coefficient	95 % CI	p value
Independently related				
	Healing time (days)	0.38	0.17 to 0.60	0.001
	Infection	6.86	1.98 to 11.74	0.007
	DDB	6.44	2.15 to 10.72	0.004
In a model				
	Healing time (days)	0.19	-0.48 to 0.44	0.112
	Infection	5.05	0.57 to 9.53	0.028*
	DDB	4.10	-0.31 to 8.50	0.067
<i>Model</i>				<0.001
<i>Adjusted R Square</i>				0.443

* Factor significant in the model

Appendix 4. Predictor models of scarring at 12 months in Study V not skin-grafted (n=28) using POSAS Observer scores

	Coefficient	95 % CI	p value
Independently related			
Healing time (days)	0.33	0.11 to 0.54	0.005
<i>Model</i>			0.005
<i>Model R square (only one variable included)</i>			0.269

* Factor significant in the model

Appendix 5. Predictors for scarring at 6 and 12 months for children not skin-grafted using VSS scores

	6 months (n=33)			12 months (n=28)		
	Coefficient	95 % CI	p value	Coefficient	95 % CI	p value
Independently related						
Healing time(days)	0.16	0.07 to 0.25	0.001	0.18	0.10 to 0.28	<0.001
DDB	2.57	0.72 to 4.43	0.008	Non-significant		
Sex (male=1)	Non-significant			1.93	0.01 to 3.90	0.049
In a model						
Healing time(days)	0.12	0.01 to 0.23	0.028*	0.17	0.07 to 0.26	0.001*
DDB	1.37	-0.67 to 3.40	0.181	Non-significant		
Sex (male=1)	Non-significant			1.29	-0.34 to 2.91	0.116
<i>Model,</i>			0.003			0.001
<i>Adjusted R Square</i>			0.281			0.400

* Factor significant in the model

Appendix 6. Predictors for HTS at 6 and 12 months for non-grafted children

	<u>6 months (n=33)</u>			<u>12 months (n=28)</u>		
	OR	95 % CI	p value	OR	95 % CI	p value
Independently associated						
Healing time(days)	1.13	1.03 to 1.25	0.013	1.17	1.04 to 1.32	0.012
Not healed at day 14	Perfect prediction of HTS (not possible to analyse)					
Infection	6.25	1.15 to 34.12	0.034	Non-significant		
In a model						
Healing time(days)	1.12	1.01 to 1.24	0.034*	1.17	1.04 to 1.32	0.012*
Infection	4.09	0.62 to 26.84	0.143	Non-significant		
Model.			0.006	0.003		
Model prediction %			75.8	75.0		
(Nagelkerke R Square)			0.381	0.372		
Hosmer-Lemenshow goodness of fit			0.819	0.210		

* Factor significant in the model

Papers

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