Introduction and outline of thesis
The skin plays a key role in protecting the body against pathogens, fluid loss, and mechanical and physical impact. Therefore, damage of this organ may have severe consequences. Burn injury of the skin leads to loss of its protective function. The last decades, mortality rate after burns has decreased due to improved medical health care, such as enhanced control of infection and shock treatment. Patients with severe burns survive, but as a consequence face the morbidity of scarring. For this reason, there is an increasing emphasis on the result of wound healing: the functional and cosmetic scar outcome.

Both deep dermal and full-thickness burn wounds will develop into a scar. Scarred skin is usually distinguished from normal skin by an aberrant colour, less pliability, an increased thickness, and an irregular surface area. Additionally, symptoms of pruritus and pain are common complaints of the patient. All these scar aspects can play an important role in patient’s quality of life. It has been reported that up to 90% of the burn patients develop hypertrophic scars\(^1\). These scars are defined as scars that raise above the skin level, though stay within the confines of the original lesion\(^2\). Hypertrophic scars result from excessive collagen deposition and decreased collagen breakdown, however, the exact mechanism of development remains unclear.

It is necessary to understand the process of wound healing and scar formation, to improve the outcome of burns. In this thesis, we will review wound healing and scar formation in detail and apply this knowledge in clinical practice for improvement of burn scar outcome. In Chapter 2, we will make an effort to clarify and reorganize the complex molecular and cellular mechanisms that may be responsible for a hypertrophic scar, following the chronology of normal wound healing. In the formation of hypertrophic scarring, several processes seem to be derailed, such as the haemostasis, inflammation, reepithelialization, extracellular matrix production and remodelling, neovascularization, and apoptosis. A thorough understanding of the pathophysiology of the scar may help choosing the most appropriate treatment strategy. Previously, numerous studies have been published on the prevention and treatment of hypertrophic scars, however the effectiveness of therapies is not always investigated sufficiently. In Chapter 3, we will focus on the current therapies and prevention of hypertrophic scarring.

Prevention of (excessive) scar formation starts with an optimal treatment of the wound. Until now, the autologous split-thickness skin graft is the mainstay in burn wound surgery. Although this is an adequate procedure to come to wound closure, it has a limited availability in severely burned patients. In addition, scar quality of the acceptor site is often poor. The need for further improvement of methods for skin resurfacing has led to the development of skin substitutes, which could decrease the need of large donor sites and help in resurfacing the wound to achieve rapid wound
In 1975, the first epidermal substitute was developed consisting of cultured keratinocytes as an intact sheet. Although successful application of these sheets in burns was reported, the substitute showed a variable graft take and on the long term, skin fragility and blistering were frequently observed problems. In addition, the long cell culturing time and the high costs limited its clinical use.

In full-thickness wounds, the application of an epidermal substitute alone is now known to be insufficient, because a dermal component is missing. The lack of dermis may cause severe contraction, hypertrophic scarring, and instability of the skin. It was hypothesized that dermal substitutes contribute in the formation of new dermis, which would improve scar quality. Yannas and Burke were the first to report on the development of equivalents of the dermis. They stated that a dermal substitute would ideally improve wound closure and promote development of autologous dermal tissue. This would prevent infection and fluid loss and improve scar quality, respectively. Figure 1 shows a schematic image of a burn wound treated with a split-skin graft (SSG) alone, i.e. the standard treatment and Figure 2 presents a burn wound treated with a SSG combined with a dermal substitute.

**Figure 1:** Schematic image of a full-thickness burn wound treated with a split-skin graft

**Figure 2:** Schematic image of a full-thickness burn wound treated with a dermal substitute and a split-skin graft

SSG, split-skin graft
In 1981, a dermal substitute (Integra, Lifesciences Corporation, Plainsboro, New Jersey, USA) was described, which consists of a collagen-based dermal layer and a disposable epidermal silicone layer. This artificial skin is applied in a two-step grafting procedure. First, the substitute is placed on the prepared wound bed. After vascularization of the dermal layer, usually in two to three weeks, the top layer is removed and a thin SSG can be applied. Nowadays, Integra is frequently used for acute and reconstructive wounds. Good results have been reported on its clinical use, such as improved scar appearance and reduced hypertrophic scarring. However, some disadvantages are associated with the use of Integra. Due to the slow vascular ingrowth into the dermal layer, a larger risk for wound infection is present which may result in loss of the graft. Furthermore, the high costs, the specific requirements for the operation technique, the two-step procedure, and the risk of failure are reasons that Integra is not routinely used in every burn clinic today.

In the meantime, other dermal matrices were investigated. The majority of the matrices were based on collagen, which is the most prevalent protein of the human dermis. Other extracellular components, such as elastin and hyaluronic acid, have been added to improve stability of the substitute and its effect on wound healing. Elastin is a dermal element, which provides both strength and elasticity to the extracellular matrix. It was demonstrated that coating the collagen fibers of dermal substitutes with an elastin hydrolysate could stabilize the matrices in a porcine wound model. The substitute remained longer in situ compared to matrices without the elastin hydrolysate. Additionally, an increased vascularization was demonstrated one week post-wounding in wounds treated with a collagen-elastin substitute and SSG, compared to wounds treated with a SSG alone, applied in a porcine wound model. Due to the increased vascularization, a one-step procedure for application of the collagen-elastin substitute and autograft became achievable. Also, the smaller pore size of this substitute (compared to Integra) may contribute to an improved diffusion of nutrients and cell migration into the substitute. The difference in pore size between the collagen-elastin substitute and Integra is illustrated in Figure 3a and b.

In 1996, our research group (Red Cross Hospital, Beverwijk, The Netherlands) set up a controlled trial to investigate this collagen-elastin substitute in acute and reconstructive burns. In an intra-individual comparison, treatment of the wound with the dermal substitute and a SSG was compared with the application of a conventional SSG alone. First, the successful application of the substitute in a one-stage procedure was demonstrated, although take rate of the skin graft was slightly reduced and delayed in the acute burn group. This reduction was assumed to be due to the increased distance for nutrients and oxygen through the substitute to the autograft.
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In the meantime, Jescke et al. published a study on dermal substitution in combination with topical negative pressure (TNP)\(^20\). In this study, the combined application of Integra, fibrin glue and TNP therapy in reconstructive wounds could significantly reduce the time necessary for vascular ingrowth into the Integra. Additionally, the Integra + TNP group showed a significantly improved Integra take rate compared to the Integra group alone\(^20\). TNP was originally developed by Morykwas and Argenta in the early 1990s as a treatment for chronic wounds\(^21\), \(^22\). The technique involves placing foam on the wound, sealing the site with an adhesive drape, and applying subatmospheric pressure. Since several years, TNP therapy has become a generally accepted treatment, predominantly for chronic wounds, but also for acute, contaminated, and other complex wounds\(^23\)-\(^28\). Although the exact working mechanism and the effect on wound healing is not fully understood, several effects have been suggested, such as improved angiogenesis, reduction in bacterial count, and migration of endothelial cells\(^22\), \(^26\), \(^29\)-\(^33\). Furthermore, some studies showed an improved graft take after treatment with TNP therapy compared with standard dressings in skin grafted wounds\(^23\), \(^24\).

As TNP therapy was shown to improve vascularization of a dermal substitute in reconstructive wounds and improve take rate of an autograft, we hypothesized that this therapy could counteract the above mentioned disadvantage of a lower graft take in acute burns treated with a dermal substitute. In other words, we presumed TNP could increase take rate of the autograft on top of a dermal substitute and consequently improve scar outcome (illustrated in Figure 4). In acute burn wounds, the combination of these two therapies might be needed to achieve the beneficial effect of dermal substitution. Therefore, a randomized controlled trial (RCT) was initiated in the three Dutch Burn Centers (Chapter 8). In a four-armed study, the effect of the dermal substitute Matriderm in combination with TNP therapy on burn scar quality was investigated. Since the start of the initial clinical trial on dermal substitution in 1996, we have gained more knowledge on performing clinical research and evidence-based medicine. Therefore, we could improve the quality of the present trial by employing additional instruments to quantify wound and scar outcome and by performing a multicenter RCT. In this study, which will further be described as the TOPSKIN study (TOPical negative pressure and SKIN substitute), patients with acute burns were randomized to either 1. treatment with a dermal substitute, a SSG and TNP therapy; 2. treatment with a dermal substitute and SSG; 3. treatment with a SSG and TNP therapy; 4. treatment with a SSG alone.

To determine the effectiveness of these therapies, wound and scar measurement tools are required to quantify wound healing and scar quality. Ideally, evaluation of the outcome parameters consists of both subjective and objective measurements,
especially in clinical trials\textsuperscript{34}. Reliable and validated evaluation instruments are needed to increase the level of evidence of clinical trials. Therefore, a part of this thesis focuses on examining several wound and scar evaluation methods on their clinimetric properties, i.e. the reliability, validity, and feasibility (Chapter 4-6).

**Figure 4:** Schematic image of a full-thickness burn wound treated with a dermal substitute, a split-skin graft and topical negative pressure therapy

![Schematic image of a full-thickness burn wound treated with a dermal substitute, a split-skin graft and topical negative pressure therapy](image)

SSG, split-skin graft

In burn wounds, important wound parameters are take rate of the graft and epithelialization. These wound parameters are mainly assessed by the clinician (i.e. subjectively). The subjective assessment is often criticized, as the evaluation of the clinician can be influenced by for example (in)experience or coping skills\textsuperscript{34}. However, we feel that the use of objective tools needs experience and coping skills as well and therefore both subjective and objective evaluation methods should be considered when performing a clinical trial. So far, subjective wound assessment in burns had not been tested clinimetrically. For this reason, two clinimetric studies were set up. First, reliability of the subjective evaluation of graft take and epithelialization was investigated. Reliability refers to the repeatability of the assessment or measurement, the degree in which the instrument measures the same way, each time it is used under the same condition. Following, the validity of the assessment of wound parameter epithelialization was examined. Validity refers to the best available approximation to the true parameter. In other words, do we measure what we intend to measure? Both studies are described in Chapter 4 and 5, respectively.

Besides wound assessment, emphasis of our RCT was put on scar outcome. In our first clinical trial on dermal substitution in 1996, scar aspects were evaluated subjectively by means of the Vancouver Scar Scale (VSS). Although, the Vancouver Scar Scale (VSS) is still the most widely used and evaluated burn scar assessment tool, this
scale does not meet the criteria of a suitable assessment scale i.e. reliable, feasible, consistent, and valid. In 2004, a reliable and valid scar assessment scale (the Patient and Observer Scar Assessment Scale) was developed by our group, with superior clinimetric properties compared to the VSS. Besides a subjective assessment, scar aspects should ideally be measured objectively as well. Objective evaluation of scar outcome was only performed in a few trials on dermal substitution. In our previous clinical trial, scar elasticity was already measured objectively using the Cutometer Skin Elasticity Meter (Courage and Khazaka GmbH, Cologne, Germany). However, in recent years, several additional objective tools have been developed and tested. For instance, the DermaSpectrometer (Cortex Technology, Hadsund, Denmark) was found reliable for the quantification of scar pigmentation and vascularization and was subsequently used in the TOPSKIN study. So far, several objective measurement tools for skin surface roughness had been described, however none of these tools complied with all clinimetric characteristics. For this reason, scar roughness is usually evaluated subjectively by means of a scar assessment scale. We considered that the quantification of this scar aspect in particular, is very important. First of all, it was demonstrated that scar roughness had a significant effect on the patient’s and observer’s opinion of the scar, which indicates that this aspect is an important parameter in scar appearance. Second, it was noted previously that scars treated with a dermal substitute appeared to have a smoother surface, however at that time no objective tool was available to objectify this finding. Since several years, the Phaseshift Rapid In Vivo Measurement of the Skin (PRIMOS) (GFMesstechnik GmbH, Teltow, Germany) has become available. The PRIMOS is a measuring device that in vivo produces a three-dimensional image of the microtopography of the skin. Previously, this device was used in the cosmetic industry to measure volume of wrinkles and in the dermatologic field to measure depth of acne scars. However, its use in healthy skin and burn scars was not investigated and clinimetric properties had not been described. We performed a clinimetric study to investigate the reliability, validity and feasibility of the PRIMOS for surface roughness in healthy and scarred skin. This study is presented in Chapter 6.

Summarizing, the focus of this thesis is on the evaluation and improvement of burn wound healing and scar formation using dermal substitution. First, we reviewed the molecular, cellular and clinical aspects of wound healing and scar formation. Second, we tested the clinimetric performance of several wound and scar evaluation tools which are needed to determine the effectiveness of therapies. With the acquired knowledge on scar formation and evaluation methods, we performed clinical trials investigating the effects of a dermal substitute on burn scar outcome.
References

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