Part IV

Discussion and summary
General discussion and future perspectives
In this thesis, we focused on the evaluation and improvement of burn wound healing and scar formation using dermal substitution. In the first chapters, the molecular, cellular and clinical aspects of wound healing and scar formation were reviewed. Furthermore, we investigated 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, clinical trials were performed using a dermal substitute to improve burn scar outcome.

**Part I Cellular and clinical aspects of scar formation**

To improve the quality of wound healing and minimize scarring, knowledge of the subsequent processes of wound healing and scar formation is required ([Chapter 2](#)). In this thesis, we focused on the development of hypertrophic scars in particular. Hypertrophic scars are characterized by an excessive scar proliferation within the edges of the initial wound and can be responsible for serious functional and cosmetic problems. We believe that especially in the wounds leading to these scars much can be achieved in final scar outcome (and therefore eventually patient’s quality of life), using dermal substitution. In the formation of hypertrophic scars, multiple processes in wound healing play a role. In literature, much research in this field has been reported, underlining the importance of understanding these basic processes in order to prevent or treat hypertrophic scarring.

We reviewed the different processes in wound healing and scar formation and it seems that in all the various phases of wound healing and scar formation, the normal processes can derail and lead to scar hypertrophy. Results of different in vitro, ex vivo and animal models, in combination with the observations in patients help to more closely indicate which molecular and cellular elements contribute to hypertrophic scarring. Nevertheless, it remains unclear if the described changes are a cause or a consequence of hypertrophic scar formation. Until now, it is not clarified at which moment of wound healing, processes derail and lead to hypertrophic scarring. Preventive therapies should intervene with wound healing, before the ‘hypertrophic trail’ is taken, in order to influence the subsequent processes that lead to a hypertrophic scar. Further research on the mechanisms of wound healing and scar formation is important to control the process of hypertrophic scarring.

Following the investigation of the cellular and molecular processes of wound healing and scar formation, we focused on the incidence of hypertrophic scarring and reviewed the current preventive and curative management ([Chapter 3](#)). The importance of research on hypertrophic scarring is illustrated by literature reports showing that these scars occur in 40% to 94% following burns. The reported
differences between incidences may be explained by factors such as population (i.e. race, age), wound factors (i.e. depth, grafted or not, time of healing) and the period of follow-up. However, we believe differences in the definition of the hypertrophic scar and the scar evaluation are the most important causes of the diverging incidences.

Each clinician or researcher in this field frequently faces the difficult question ‘is this a normal scar or a hypertrophic scar’? Only few authors reporting on the incidence of hypertrophic scars used validated criteria or a classification to define these scars. Although the majority of the reports refers to Peacock’s definition¹ (a scar that raises above the skin level, though stays within the confines of the original lesion), this may not always be easy to establish and therefore insufficient. In addition, scar evaluation may be the cause of the wide spread reported incidences. Subjective scar assessment scales have been used, however studies reporting on the measurement of hypertrophic scars with objective evaluation tools are scarce. We recommend to assess scars with a validated scar assessment scale and to use a certain cut-off point within the scale to define the hypertrophic scar. Only a more specific definition which is generally accepted and easily applicable, and a reliable and valid scar evaluation can provide the incidence rates of hypertrophic scars more precisely. Moreover, we feel it is of significance which percentage of the originally treated wound area became hypertrophic and to which degree of hypertrophy, especially in clinical trials. Further discussion on the importance of wound and scar evaluation will be considered later in this chapter.

In Chapter 3, we mainly focused on the current state-of-the-art preventive and curative management of scarring. To reduce the incidence of hypertrophic scarring, wounds should receive an optimal treatment, i.e. fast wound closure, grafting with small mesh expansions, and the use of dermal substitutes. In the beginning of scar formation, treatments such as silicone, pressure therapy and corticosteroids are generally used. Silicone, which can be applied to the scar in different modalities, was reported to be effective in the prevention of hypertrophic scarring²-⁴, although the contrary has also been described⁵ and O’Brien et al. recently stated that the published studies are of poor quality and highly susceptible to bias⁶. In the review described in Chapter 3, we stated that pressure garments have become a standard therapy in several burn centers, despite the fact that the effectiveness of this therapy in the prevention of hypertrophic scars had not been demonstrated in clinical trials. However, more recently, Engrav et al. reported on the beneficial effects of pressure garments investigated objectively in a clinical trial⁷.

The preventive therapies, silicone, pressure therapy and corticosteroids can also be used for the curative management of hypertrophic scars. Although the effectiveness
of silicone has been demonstrated in several (randomized) controlled trials\(^8\),\(^9\), the level of evidence for the effectiveness of pressure therapy in the treatment of existing hypertrophic scars is low. The treatment of hypertrophic scarring with corticosteroids is a possible approach, with high reported response rates\(^10\),\(^11\), however this treatment is not suitable for extensive burn scars. Finally, promising results have been described of several new developments in the prevention and treatment of hypertrophic scars, such as interferon\(^12\), 5-fluorouracil\(^13\), bleomycin\(^14\), enalapril\(^15\), Transforming Growth Factor (TGF)-β blockade\(^16\), FGF-2 solution\(^17\), and TGF-β3 injection\(^18\). Concluding, the optimal treatment for the prevention and management of hypertrophic scarring has not been developed. This may be caused by the lack of understanding the complete pathogenesis of wound healing and scar formation. Although multiple therapies have been described, no treatment can repair the (hypertrophic) scarred skin completely. To prevent hypertrophic scars, it seems important to intervene at the start of wound healing. In this thesis, we focused on the clinical application of dermal substitution in acute burns to improve wound healing and reduce scar formation, which will be discussed at the end of this chapter.

**Part II Clinimetry: wound and scar evaluation**

The second part of this dissertation concentrates on the clinimetric aspects of wound and scar evaluation. Both subjective and objective measurements of wound and scar features are indispensable, not only to record the individual progress of healing or scar formation, also to compare the effect of applied treatments in a research setting. Subjective assessment methods are usually more convenient to use, are often freely accessible, and for these and other reasons more frequently used. An advantage of subjective assessment tools is that the opinion of the observer and the patient can be included. On the other hand, objective tools are generally preferred over subjective scales, especially in a research setting. Objective tools are considered to be more reliable and less prone to errors, compared to subjective tools. However, we feel the measurements of both the subjective and objective methods can be influenced by the experience or the coping skills of the observer. For example, the amount of pressure applied by the observer with the DermaSpectrometer (Cortex Technology, Hadsund, Denmark) can cause differences in the objective measurements of scar color. Therefore, in our opinion, a combination of subjective and objective tools should be used in clinical trials. More importantly, the primary outcome parameter of a study should indicate the most relevant measurement tool for that study.

In the past decade, more research was focused on the clinimetric properties of measurement tools. In evidence-based medicine, instruments should be reliable, valid, and feasible. Using reliable and valid measurement tools, the probability will
be increased that the effect of a therapy is measured correctly. Consequently, to perform a randomized controlled trial (RCT) on dermal substitution, we required reliable and valid, subjective and objective measurement tools. The assessment of wound parameters *graft take and rate of epithelialization* are important to discriminate between the different therapies. In general, these parameters are evaluated subjectively, however, this evaluation has not been tested on its clinimetric properties. Despite this, in clinical practice and in research setting, this assessment is still most frequently used. For this reason, we investigated the reliability and validity of this wound evaluation. In clinimetric research, reliability of measurement methods is investigated first, after which validity is tested. If a method is not reliable, there is no point in testing its validity, as unreliable measurement tools should not be employed. Accordingly, we first investigated the intra- and interobserver reliability of the assessment of graft take and rate of epithelialization (Chapter 4). Intraobserver (test-retest) reliability refers to whether the same result is obtained when an observer uses the instrument for the second time on the same subject. Interobserver reliability illustrates the degree to which multiple observers obtain the same result with the instrument when measuring the same subject at the same time. In this study, we additionally investigated the influence of clinical experience of the observer on the reliability of the assessment.

Experienced observers obtained intra- and interobserver intraclass correlation coefficient (ICC) scores that were excellent and higher than the scores of the medium- and inexperienced observers. According to our expectations, scores of experienced observers were higher than inexperienced observers, as experience is thought to give a more consistent assessment. The intraobserver ICC data indicate that if an experienced or medium experienced observer evaluates graft take or rate of epithelialization, only one (photographic) assessment is needed. The interobserver scores showed that only one experienced observer was needed for fair to good reliability for the evaluation of both wound parameters. Based on the results of our study, we can recommend performing the evaluation of graft take and rate of epithelialization by a single experienced observer. However, in clinical trials, the evaluation by two experienced observers is recommended for a higher reliability.

As reliability was good, we accordingly investigated the validity of the assessment of rate of epithelialization (Chapter 5). We choose to investigate the validity of the parameter epithelialization only, as this seemed a more general wound healing parameter compared to the parameter graft take, which is used merely in transplanted wounds. The validity of a parameter refers to whether the assessment measures what it is intended to measure. In contrary with testing the reliability, research on validity is more difficult to perform. Ideally, the validity of a measurement technique
is analyzed by correlating its measurements to the “true value” (of that wound or scar aspect). However, the true value is frequently not known. For the validity of a subjective assessment, this assessment is usually correlated to an objective measurement. On the other hand, the validity of an objective tool is generally analyzed by correlating it with a subjective assessment. It seems that validity only approximates the true value.

As there is no standard tool to assess the rate of epithelialization, we considered the assessment of epithelialization with digital image analysis to be the most valid tool. Therefore, to test the validity of the subjective assessment of epithelialization, it was compared to epithelialization measured with digital image analysis. Preceding the investigation of the validity of the subjective assessment, the interobserver reliability of digital image analysis was tested, as this was not performed previously for this parameter. Interobserver scores of parameter epithelialization measured by digital image analysis were good. Clinical epithelialization scores correlated highly with data of the digital image analysis, indicating an excellent validity. Measurement with digital image analysis is relatively time-consuming, and for this reason may prove less feasible in a clinical setting. Therefore, we conclude that subjective clinical assessment remains the most feasible method for these parameters in burn wounds. The findings described in Chapter 4 and 5 are important for future clinical research. The use of the subjective assessment in clinical trials, performed by the clinician or researcher, is often criticized as it would not be reliable or valid. With the outcomes of these studies, we demonstrated that this subjective assessment is reliable and valid.

Besides research on the clinimetric properties of wound assessment, the clinimetric performance of scar evaluation was also investigated in this thesis. The evaluation of scar outcome is essential to determine the effectiveness of wound and scar therapies, such as dermal substitution. All scar aspects are clinically relevant and relate to the quality of a scar. In general, subjective scar assessment scales contain the majority of the scar features, such as color, pliability, thickness, relief, surface area, and the symptoms pain and itching. Preferably, also objective measurement tools are available for all these scar aspects. The use of objective devices for scar color, pliability, thickness, and surface area has been described. In this thesis, we focused on scar parameter surface roughness as it was demonstrated to be an important parameter in scar evaluation for both the clinician and the patient. Objective measurement tools for skin surface roughness have been described previously. However, the described techniques are not easily applied in a clinical setting and have not been clinimetrically evaluated. Several years ago, the Phaseshift Rapid In vivo Measurement Of the Skin (PRIMOS) (GFMesstechnik GmbH,
Teltow, Germany) became available for the measurement of skin surface roughness. In **Chapter 6**, we investigated the reliability and validity of this objective tool for surface roughness. The PRIMOS was found to have an excellent intra- and interobserver reliability on healthy and scarred skin. Concerning the validity, the PRIMOS showed a high correlation with the relief score of the Patient and Observer Scar Assessment Scale (POSAS) on scars. Furthermore, the PRIMOS is accurate, noninvasive, easy and fast, compared to indirect profilometry methods. Despite the high price, the PRIMOS seems to be a good choice for measuring scar surface roughness objectively.

In addition to reliability and validity, measurement tools should be tested on responsiveness. This means an instrument should be able to identify any degree of change of the wound or scar in time, i.e. detect a clinically minimal important change. Frequently, these aspects are considered as part of validity and not as separate attributes. In the above described clinimetric studies, responsiveness of the evaluation tools was not examined, as wounds and scars were only assessed at one time point. Future studies on the responsiveness of tools will improve the measurement of wounds and scars further.

Despite the use of measurement methods with the required clinimetric properties, variability may still remain in wound and scar measurements. As mentioned above, an observer bias can cause an error in the measurements in both subjective and objective measurement tools. In addition, the results may be influenced by the location of the scar that has been assessed or measured. Is this the most representative part, the worst part or the center point of the wound or scar? It is important to clarify and document this measuring location accurately. This is essential in clinical trials in which the effectiveness of therapies is compared, but also in individual patients that are measured at different time points: subsequent measurements need to be measured on the same location. Especially in clinical trials, measurements should be performed according to a protocol (e.g. each measurement is performed in the scar center) in order to prevent a bias. We believe that objective measurement tools are more sensitive to location changes than subjective assessment tools, because their measuring probes often measure smaller parts of the scar compared to the overview of the observer applying the subjective assessment on a scar.

**Part III Clinical application of dermal substitution**

The third part of this thesis concentrates on the clinical application of dermal substitution to improve outcome of burns (**Chapter 7 and 8**). It is generally accepted nowadays that dermal substitution can provide a beneficial effect on burn scar outcome, especially in the prevention of hypertrophic scarring. In recent years,
several clinical studies have been performed on dermal substitution in reconstructive and acute wounds. In general, good results were demonstrated, such as an improved scar appearance and scar elasticity. However, the majority of these studies involved only small patient numbers and was not carried out in a randomized controlled design. In addition, usually study outcome was evaluated in a subjective manner only and long-term data on scar quality are lacking.

Since many years, our research group has investigated dermal substitution in several formats. Animal experiments showed the improved vascularization in the collagen-elastin substitute, enabling a single-stage grafting procedure. In the clinical trials that followed, this single-stage procedure was successfully used in acute and reconstructive wounds. In the clinical trial performed in 1996 to 1998, acute and reconstructive burn wounds treated with a dermal substitute and a split-skin graft (SSG) were compared to wounds treated with a SSG alone. Take rate of the autograft was significantly lower in acute burn wounds treated with the substitute compared to the wounds treated with a SSG alone, although the difference was very small\(^41\). In reconstructive wounds, no difference in graft take was seen between substituted and non-substituted wounds. By increasing the distance between wound bed and the graft, the substitute may hamper vascular ingrowth and diffusion of nutrients, which could endanger the survival of the overlying graft. Burn wounds are characterized by unfavorable wound conditions compared to reconstructive wounds. For this reason, the autograft in acute burn wounds may require more time to achieve complete revascularization.

We hypothesized that the delayed and reduced take rate of the autograft in acute burns treated with a dermal substitute, could be improved by means of topical negative pressure (TNP) therapy, as it was previously reported that this therapy contributed to an improved take rate of skin grafts\(^42, 43\) and of a dermal substitute\(^44\). In the TOPSKIN (TOPical negative pressure and SKIN substitute) study described in Chapter 8, graft take and epithelialization showed no statistically significant differences between the four treatment groups (patient example in Figure 1a-g). These findings are essential for future implementation of Matriderm in the treatment of acute burn wounds. The possibility to apply this substitute in a single-stage procedure is an important advantage towards some other substitutes, as a two-step procedure brings additional costs, risk of infection and potential loss of the autograft. In other reports on acute burns, Matriderm (with 1mm thickness) was applied in a single-stage procedure as well, without the use of TNP pressure. The take rate of the autograft on top of the substitute was described to be between 83\% and 97\%\(^45, 46\). Goutos et al. reported a mean graft take of 94\% in a patient series including reconstructive scars and acute burn wounds treated with Matriderm and TNP therapy\(^47\).
Figure 1: Example of a patient randomized for treatment with dermal substitute and topical negative pressure

Fifty-nine year-old woman with flame burn on the hand. A, At admittance, day of burn; B, post burn day 14, before excision; C, After wound bed preparation and haemostasis; D, E, F, Application of the dermal substitute, the split-skin graft and the topical negative pressure; G, wound assessment 5 days postoperatively.
In the TOPSKIN trial, it was shown that wounds treated with the substitute, a SSG, and topical negative pressure (TNP) therapy had a higher take rate of the autograft compared to wounds treated with the substitute alone. Previously, several reports demonstrated that TNP is a good method for securing the skin graft and improving the graft take. In our clinical trial, we could not demonstrate this improvement with significant differences. On the other hand, graft take was high in all groups, which made it difficult to improve this even further. Comparable with our results, the literature reports a graft take of more than 90% with the use of TNP therapy. The improved take rate of the autograft or substitute is thought to be due to an improved vascularization and increased diffusion of nutrients caused by negative pressure. This will be discussed later in this chapter.

**Figure 2:** Example of a patient randomized for treatment with dermal substitute and topical negative pressure; continued

A, B, C, D. Scar outcome at respectively 3, 5, 12 and 18 months postoperative (patient previously shown in Figure 1).

Besides the measurement of graft take and epithelialization, we investigated the effect of dermal substitution on scar outcome in the long term with several reliable and valid scar assessment tools (patient example in Figure 2). The most important finding of the clinical trials performed by our research group was demonstrated in the measurements of scar elasticity. In the clinical trial that started in 1996, an improved elasticity was found in reconstructive scars treated with the dermal substitute and a
SSG compared to scars treated with a SSG alone, 3 months postoperatively. As long-term effects of dermal substitution had previously not been reported, our research group performed a long-term follow-up of the patients treated in this clinical trial (Chapter 7). Twelve years postoperatively, we measured scar elasticity of these patients and found that the difference between substituted and non-substituted scars was still present, although not statistically significant anymore. The presence of the substitute in the early phase of wound healing seems to contribute to a higher elasticity, even after 12 years. The beneficial effect in scar elasticity had not been found in acute burns at both the short and long-term follow-up of this clinical trial. In the TOPSKIN trial however, we did find a significantly improved elasticity in scars of acute burns treated with the substitute and TNP therapy, one year postoperatively. Elasticity was not improved in scars treated with the substitute alone, which is comparable with our results found earlier. In contrast to these findings, several other studies have reported an improved scar elasticity with the use of dermal substitution only, although often not measured objectively 45, 46, 53-56.

Previously, we hypothesized that the presence of potentially toxic products from bacteria and lysosomal enzymes from the dead autologous cells in acute burns, can degrade the collagen present in the substitute 41. Therefore, dermal substitutes in burns might degrade, before they can play a role in the repair of the dermis. This process may have long-term consequences on scar outcome. These findings are of importance for future use of dermal substitutes as they suggest that wound conditions play a role in the effectiveness of dermal substitution and that TNP therapy is necessary to obtain beneficial effects of dermal substitution in acute burns. Besides the effect of dermal substitution and TNP therapy on scar elasticity, we demonstrated the correlation between the effect of dermal substitution in acute burns and the applied expansion of the autograft. In a separate analysis of the acute burn group included in the initial trial of 1996, it was found that scars treated with a dermal substitute and a largely expanded autograft (mesh 1:3 or mesh 1:4) were significantly more pliable, compared to scars treated with a largely expanded autograft alone. This improved skin elasticity was not seen in substituted scars treated with a small expanded autograft (mesh 1:1.5 or mesh 1:2). Measurements of elasticity at the long-term follow-up performed 12 years postoperatively also demonstrated this significant difference. This finding is of paramount importance and has clinical relevance as the need for dermal substitution increases when large expansion rates of the autograft are used, usually in severely burned patients. We expect that the effectiveness of dermal substitution on the quality of scars treated in the TOPSKIN trial would have increased even further, when applied in combination with larger expansion rates of the autograft.
Another effect of dermal substitution was the improvement of scar surface roughness. In the initial clinical trial (1996-1998), we noticed that scars treated with the substitute were smoother compared to scars treated with a SSG alone. At the follow-up of 12 years post operatively, we were able to measure scar surface roughness objectively and consequently, it was quantified that these scars indeed were smoother. In the TOPSKIN study, only a small part of the patients was measured with this objective tool, as this device was not available in all burn centers. Scars treated with the substitute were smoother compared to scars treated without the substitute, however, no statistical differences were found. This could be due to the small patient numbers, but also due to the fact that all wounds were treated with a small mesh expansion (1:1.5). We hypothesize that the dermal substitute creates a beneficial effect in surface roughness by bridging the interstices of the split-skin graft, which is where usually the hypertrophy of the scar develops. To prevent scar roughness, the use of a dermal substitute can be beneficial, especially in wounds that will be treated with a largely expanded mesh graft. Boyce and Warden mentioned a smoother surface roughness in a patient treated with the substitute Integra\textsuperscript{57}, however, further data on the effect of dermal substitution on scar roughness have not been described.

In the TOPSKIN trial, the combined application of dermal substitution and TNP therapy seemed necessary for a beneficial effect of the substitute. Therefore, we will briefly discuss the supposed mechanism of actions of this additional technique. TNP was originally developed as a treatment for chronic wounds, however, it has become an accepted treatment for acute, contaminated, and burn wounds. In these wounds, TNP therapy has been used for several purposes, such as the formation of granulation tissue\textsuperscript{58, 59}, reduction of wound contamination\textsuperscript{60, 61}, improvement of blood flow\textsuperscript{58, 62}, prevention of burn wound progression\textsuperscript{63}, wound bed preparation and the fixation of an applied autograft\textsuperscript{42} or dermal substitute\textsuperscript{44}.

The beneficial effect of TNP therapy on the ingrowth of dermal substitutes and autografts has not been fully explained. Previously, it was reported that TNP reduces the time to vascularize the dermal substitute Integra\textsuperscript{64}. In addition, it was shown in vitro that endothelial cells show improved proliferation and migration in Integra treated with TNP therapy\textsuperscript{65}. We hypothesize that TNP therapy improves take rate of the dermal substitute by means of stimulating the diffusion of nutrients to the substitute. The increase in skin perfusion and the improved angiogenesis have been demonstrated in experimental studies\textsuperscript{58}. We assume an increase in skin perfusion leads to an increased diffusion of nutrients as a result of the applied negative pressure to the tissue. An improved angiogenesis is functional in wound healing as the overlying substitute or skin graft will receive increased nutrients. However,
in scar formation, this improved angiogenesis should not prevail, as it may lead to hypervascularization and scar hypertrophy. Also, we believe that the beneficial effect of TNP therapy on the ingrowth of a dermal substitute may partly rely on the adequate fixation of the substitute to the recipient bed during the critical period of capillary ingrowth. Shear forces between the wound bed and the overlying substitute or fluid collections resulting in separation of the two surfaces could result in disruption of the revascularization process and consequently, a reduction of the take rate\textsuperscript{66}.

Despite the great interest in wound treatment with TNP, the optimal protocol for the level of negative pressure and the mode of application has not been determined. A pressure level of 125mmHg was reported to produce the most significant increase in wound blood supply\textsuperscript{67}. However, more recently, it has been described that the same effect can be reached with lower pressures\textsuperscript{65, 68-70}. The results of the TOPSKIN study, might therefore have been obtained using lower negative pressures, however, this was not investigated. The optimal level of negative pressure remains unknown, especially with the use of dermal substitutes. We suppose that TNP therapy with lower pressures may not be sufficient when an additional layer (the dermal substitute) is applied between the wound bed and the autograft.

The mode of application of TNP therapy also remains under discussion. Although originally the PU (polyurethane) foam was applied on top of the wound, later the white polyvinyl alcohol (PVA) foam was developed. The use of PVA foam was reported to reduce the growth of granulation tissue into the foam surface and reduce patients pain scores, compared to the PU foam\textsuperscript{62}. More recently, good results with the application of a gauze-based negative pressure system were described\textsuperscript{47}. Nevertheless, it seems that further research is needed to define the optimal parameters of TNP therapy, especially in combination with dermal substitutes.

In general, the use of TNP therapy is associated with higher costs compared to the conventional dressings, due to the costs of the device and its disposable materials. A RCT on TNP therapy performed in patients with acute or chronic wounds investigated the total costs associated with this treatment, compared to conventional dressings\textsuperscript{71}. It was shown that material costs of TNP therapy were significantly higher compared to conventional dressings. However, the total costs of TNP therapy were not significantly higher, as wounds treated with this treatment showed a shorter healing time and costs of the labor time of the nursing staff were lower\textsuperscript{71}. Others have also reported on the higher material costs that were compensated for by fewer dressing changes, reduction in nursing time, faster healing rates and shorter hospital stays\textsuperscript{60, 72}. Furthermore, the use of TNP therapy by means of simple gauze-based dressings instead of the more expensive foams, has been reported to reduce the material
costs\textsuperscript{47}. In our opinion, it is important that future studies on TNP therapy, either or not in combination with dermal substitution, include aspects of cost-effectiveness. So far, studies on the cost-effectiveness of skin substitution have not been described. The use of dermal substitutes is more expensive than the standard treatment of burns (i.e. the application of an autograft alone) and this may not be affordable for all patients or healthcare providers. Nevertheless, it has to be considered that in the long term, dermal substitution may prevent expensive reconstructive surgery. Research on the epidemiology of reconstructive surgery after burns and cost-effectiveness analyses of dermal substitution should be performed to demonstrate cost-effectiveness of this therapy.

Considering the above described chapters, where do we now stand in tissue engineering of the skin? Experimental and clinical research has lead to a dermal substitute that is available on demand, can be applied successfully in several types of wounds, and improves burn scar outcome. Unfortunately, today’s skin substitutes do not repair the skin without scar formation; therefore, further development of skin tissue engineering is necessary. In severely burned patients, substitution of the dermis can improve scar quality; however, the need for an overlying autograft is still present. When donor sites are limited, the replacement of the epidermis by cultured keratinocytes may lead to wound closure. However, the absence of a dermal layer can lead to blistering, hypertrophic scarring, and contractions, which is a disadvantage of this therapy. An artificial skin, containing both dermis and epidermis, would then ideally be used. At present, we are attempting to find a therapy for severely burned patients with a limited availability of donor sites, by conducting a RCT on the effectiveness of cultured autologous keratinocytes seeded in a dermal substitute. Proliferating keratinocytes are cultured within 10 to 14 days and seeded onto Matriderm which enables easy and stable transport of the cells. This technique has already shown promising results in vitro\textsuperscript{73}. The application of cultured keratinocytes in combination with a dermal matrix may provide a future means of obtaining improved wound healing, when donor site skin is limited.

To develop the next generations of an artificial skin, we should first focus on improving the quality of the epidermal and dermal substitutes. In the future, further improvement of artificial skin may be reached by adding the lacking intradermal epithelial structures to the artificial skin. Ideally, a skin substitute not only contains an epidermis and dermis, also the sebaceous glands, sweat glands, and hair follicles may play a role in the development of the optimal skin substitute. Another challenge is the addition of endothelial cells to the artificial skin that may contribute to an improved angiogenesis of the substitute.
Summarizing, in this thesis we reviewed the different phases of wound healing and scar formation that can derail and lead to hypertrophic scarring. The optimal treatment for the prevention and management of these scars has not been developed. Nevertheless, it seems important to intervene at the start of wound healing, to prevent hypertrophic scars. To improve prevention and treatment of scars, wound and scar assessment with the required clinimetric properties are mandatory. The results of the three clinimetric studies described in this thesis are important for future clinical trials and contribute to an extensive array of subjective and objective measurement tools that are reliable and valid. Using these tools, the clinical trials described later in this thesis have brought new insights into the clinical effectiveness of artificial skin. The subjective and objective measurements indicated that the use of an artificial skin is a promising therapy in burns. First of all, due to increased control over the composition of the substitute, problems of vascularization have been overcome, which means the substitute can be applied in a single-stage procedure. Second, the short and long-term effectiveness of dermal substitution on scar elasticity was demonstrated in reconstructive burn wounds. In acute burn wounds, the positive effect of dermal substitution was shown when applied in combination with TNP therapy. In this thesis, it was emphasized that wound conditions are important for the effects of skin substitutes, which was shown in the difference between acute and reconstructive wounds and the need for additional TNP therapy. Furthermore, the long-term follow-up study showed that dermal substitution has a long-lasting effect on scar quality with improved scar parameters in both acute and reconstructive substituted wounds. The obtained results of this thesis are an important step in improving burn wound healing, reducing scar problems, and achieving a better quality of life for the patient. Additionally, these findings may lead to a better understanding of the indications of dermal substitutes and their mode of application.
Chapter 9

References


