Perspectives on burn scar evaluation and artificial skin
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Summary

Conventional split skin graft treatment for full thickness wounds, such as burns and reconstructive wounds lead to functional, cosmetic and psychological disorders due to scar formation. Although the severity of consequences like contraction and hypertrophy is well recognized, much of the underlying mechanisms remain elusive. A paucity of suitable scar evaluation tools counteracts adequate evaluation of normal wound healing and experimental treatments.

This dissertation concerns the evaluation of different techniques for their suitability in burn scar assessment (chapter 1-3), which is followed by an objective evaluation of different aspects of scar formation after current split skin graft treatment (chapter 4-6) and after an experimental treatment involving a dermal substitute (chapter 7-9). Chapter 10 considers the suitability of different sources of autologous fibroblasts for tissue engineered skin substitution.

Perspectives on scar evaluation
chapter 1-3

Although many devices and reliable assessment tools are available for scar analysis, there is no general agreement as to the most appropriate tools for scar evaluation, because few methods have been studied sufficiently with respect to their suitability. Chapter 1 reviews the aspects of scar formation that are essential for scar evaluation and critically discusses
currently available measurement tools as well as potential devices that may be available in the future that make up the scar assessment.

Chapter 2 employs a suitability study on two practical techniques for planimetry by means photography or by tracings. Planimetry means surface area measurement, and is useful for the recording and documentation of different types of pathologic conditions of the skin, like the quantification of scar contraction over time. Although some studies already have evaluated the inter-observer reliability, the accuracy of planimetric methods for clinical application has been studied poorly. Our study shows that the inter-observer reliability of planimetry by photography is equal or superior to planimetry by tracing and has a better accuracy than tracings except for extremely curved body parts such as the forearm, which is probably due to distortion.

The analysis of collagen structure is a crucial histologic parameter of scar evaluation, because collagen is the most prevalent protein of the dermis. It provides a structural template for strength and pliability of the skin. Nevertheless, still no uniform evaluation method is available for measurement of collagen orientation. In Chapter 3, we compare conventional polarised light microscopic ratings by observers with an image analysis based technique, Fourier analysis, for their suitability. Therefore the reproducibility (reliability) and the accuracy (validity) was evaluated by both methods with respect to collagen orientation assessment.

The Fourier analysis was performed on images of scar tissue and normal skin acquired by confocal laser-scanning microscopy. Four trained observers rated both the polarised light images of sections stained with hematoxylin-eosin and the confocal microscopy images. The inter-observer reliability of the conventional light microscopy was acceptable when at least 3 observers rated pola-
rised light images whereas already two observers were sufficient for rating confocal microscopy images. The reproducibility of the Fourier analysis is perfect when the same image is used because it employs a mathematical calculation of exactly the same data (image). The accuracy of both methods was established by means rating computer-generated images with a fixed orientation. Fourier analysis was more accurate than 4 observers for the evaluation of the ‘true’ collagen orientation for different types of computer generated images. We advocate the use of Fourier analysis for collagen orientation evaluation, as it is convenient, objective and more accurate than observer ratings. However, if observers perform an evaluation by conventional light microscopy, at least 3 observers are required to attain an acceptable inter-observer reliability.

**Perspectives on scar formation**

**chapter 4-6**

It is generally assumed that burn wounds should be grafted with a split skin graft within one to two weeks post-burn to obtain the best functional result in the long-term. In chapter 4, the long-term functional result of 143 burned hands are discussed together with factors that were considered prognostic variables for the functional outcome, such as timing of surgery. We concentrated on the hand because the burned hand has a crucial effect on the performance of patients during daily activities. Twenty percent of the hands did not regain a normal function for daily activities in the long term when tested with the objective test criteria following Jebsen. Impaired function was related to advanced age, the necessity for amputations, poor graft take, and the severity of the third degree hand burn as well as the percentage for third degree burned total body surface area. The time to skin transplantation did not influ-
ence the functional outcome of hand burns, therefore we advocate the following approach to hand burns: if a full thickness burn of the hand exist, a primary excision and transplantation is advocated. Rehabilitation is commenced as early as possible and the length of hospital stay is reduced resulting in improvements in cost-effectiveness for those patients with a small TBSA. If the assessment of the burn depth is difficult to ascertain, the operation can be postponed to prevent excision of vital tissue, allowing those areas with partial thickness burns to heal spontaneously within a time limit of 2 to 3 weeks.

In the next chapter, chapter 5, a closer look is taken at the phenomenon of scar contraction after split skin grafting. Contraction of the wounded area is a beneficial process which allows fast wound closure. Unfortunately, the contraction process continues beyond the point that complete epithelialization is reached, which then leads to the development of unwanted sequels such as joint contractures and other morbidities. Although contraction of a scar is easy to perceive, it is difficult to quantify the extent of contraction in clinical situations. The study that is discussed in chapter 5 describes the course of scar contraction over time in a 'human scar model' and potential prognostic factors for the outcome of scar contraction were put into a regression model.

We established a general trend that scars contract during the first months, which is followed by expansion of the surface area as the scars mature. The range of the contraction rate was enormous for different scars. Contraction in the short term appeared to be the only predictive parameter for the long-term outcome of contraction. No other independent variables (age, location, and presence of myofibroblasts) could be correlated with long-term outcome.

A quantitative analysis on collagen structure was performed by means of Fourier analysis, to gain a better understanding of the 3-dimensional organisation of scar and normal dermis. It is generally hypothesised that collagen bundles become aligned
in the direction of mechanical tension during scar formation. These conclusions were based on simple observations instead of experimental or clinical studies. Our data, which are discussed in chapter 6, do not support the dogma that mechanical tension over joints polarises collagen bundle orientation in the direction of stress. Novel findings of this quantitative analysis include that there was more random collagen organisation in deeper layers compared to superficial layers and that there was more random organisation in the plane parallel to the epidermis when compared to the perpendicular plane.

**Perspectives on the development and application of artificial skin**

**chapter 7-10**

Skin substitutes might be the solution for complicated wound healing problems in order to improve the outcome of current traditional treatments and to substitute the patient’s own skin when donor sites are lacking. However, the status of skin substitutes remains unclear. As reviewed in chapter 7 still no general agreement exist on the effectiveness of full thickness skin replacements, epidermal substitutes or dermal substitutes. We believe that there are mainly two reasons for the lack of clarity. Firstly, studies so far seldomly employed adequate evaluation tools, and secondly, there is paucity of large clinical trials that might provide the evidence for clinical effectiveness.

We initiated a clinical trial to evaluate an acellular collagen/elastin dermal substitute for burn wounds and scar reconstructions by means of objective and subjective clinical and microscopic parameters, as discussed in chapter 8 and 9. This substitute was selected after extensive animal studies and a clinical study on uncomplicated, small acute wounds at the Department of Dermatology.
of the Academic Medical Centre, Amsterdam, the Netherlands. The dermal substitute was combined with a split skin graft and compared to the conventional split skin autograft. We showed that the dermal substitute could be applied in a one step procedure, as it did not impair survival of the autograft on top of the substitute for reconstructive wounds and only gave a small reduction of the take in burn wounds. In the short term (i.e. three months after surgery) a statistically significant increase of skin elasticity parameters was found in the reconstructive wounds category. The clinical effectiveness of the collagen/elastin dermal substitute in terms of improving skin elasticity is diminished in time as both substituted and control areas become suppler and approached elastic qualities of normal skin over the long term. We could not establish statistical evidence for long-term clinical effectiveness in neither acute burns nor scar reconstructions with respect to elasticity measurements, scar contraction, the Vancouver Scar Scale, and for the opinion of the patient. Several findings of this clinical trial merit highlighting for the discussion on dermal substitution and future wound healing research. Firstly, it has been shown that the substitute has the qualities to interfere with tissue repair as statistically significant differences were found in the short term for reconstructive wounds in favor of the substituted area. Further adaptations might increase its effectiveness in the long-term. The second detail worth mentioning is the clinical impression that the dermal substitute was more effective in combination with a larger expansion of the overlying graft compared to a graft with a small expansion. The need for dermal substitution increases in case of extensive wounds, when large expansion ratios are used. The dermal substitute 'replaces' the dermis of the graft, which is otherwise lacking in these gaps. Further studies are required to focus on the application of the dermal substitute together with widely expanded autografts.
Chapter 9 concerns the microscopic evaluation of the study on dermal substitution that is described in chapter 8. Biopsies were taken of the control and substituted site approximately one year after surgery and were analysed by conventional light microscopy and confocal laser-scanning microscopy in combination with Fourier analysis. Parameters evaluated by conventional light microscopy (thickness of epidermis, rete ridge formation, maturation of basement membrane, (myo-)fibroblasts, and maturation of the extracellular matrix) did not show a statistically significant beneficial effect of the acellular dermal substitute for acute burn wounds and burn scar reconstructions. Fourier analysis was employed for structural analysis of collagen. The dermal substitute seemed to be beneficial with respect to collagen structure of the deep dermis, however, despite the fact that our study is one of the largest on this topic (44 pairs of reconstructive wounds and 42 pairs of burn wounds) we did not reach statistical significance in these areas. Extensive evaluation on the effectiveness of the collagen/elastin dermal substitute in the animal model indicated a beneficial effect of adding cultured fibroblasts to the substitute. Preferably the fibroblasts have to be of autologous origin for the preparation of a ‘living dermal substitute’ as allogenous fibroblasts might evoke immunogenic rejection. Chapter 10 discusses different sources for autologous fibroblasts with respect to isolation, growth, and contraction characteristics. Obviously, dermal tissue is the logical choice as source for fibroblast harvesting, but then a donor site is needed which may leave a scar. Extensively burned patients who require large donor sites to prepare a living dermal substitute paradoxically have the fewest sites available. Fat tissue, which is easy accessible, and burn eschar, which is normally discarded after burn surgery, have shown to be optional sources for culturing autologous fibroblasts. The best source however remains dermal tissue as it
results in the highest yield per gram tissue. This type of tissue contained the lowest number of myofibroblasts and gave the lowest contraction of the collagen/elastin dermal substitute in an in vitro model. At present, a clinical trial is being conducted to test the fibroblast seeded dermal substitute for acute burns and reconstructive surgery.

In the general discussion, conclusions of this thesis are reviewed and integrated into future perspectives. The importance of both objective scar evaluation tools and subjective scores by experts and patients is stressed. Generally accepted ideas on scar formation are discussed with respect to the conclusions of this thesis such as functional outcome and timing of surgery, scar contraction and relaxation, and the relationship between collagen orientation and mechanical forces. Mathematical computer models are discussed which allow the study of contraction and tissue organisation. Lastly, our clinical experience with the collagen/elastin dermal substitute is reviewed and recommendations for future research are made.