Perspectives on burn scar evaluation and artificial skin
van Zuijlen, P.P.M.

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Research on wound healing and scar formation has changed considerably during the last decades. Not only were therapeutic interventions influenced by recent developments such as tissue engineering, but also the approach to the evaluation of therapeutic and experimental interventions have become more critical. The necessity for further research is reflected by the major complications that still result from conventional treatments in functional, cosmetic and psychological areas. Nevertheless, many basic concepts of current burn and reconstructive surgery seem to rely on dogmas without substantial scientific evidence.

This dissertation attempts to identify optimal tools that are of interest for a clinical and microscopic scar evaluation and apply these tools for an accurate evaluation of the outcome of standard and experimental therapies. The first section of this dissertation starts with a review that states the relevance of different scar features for scar evaluation together with the most appropriate scar assessment tools. Some parameters, like scar contraction and collagen structure, are generally regarded as crucial parameters of scar evaluation, nevertheless, few suitability studies on scar evaluation tools are available to date. We therefore substantiated the suitability of two practical tools for the clinical evaluation of scar contraction and showed that planimetry by photography is generally more appropriate for scar contraction analysis than planimetry
by tracing. We also made considerable progress on the microscopic analysis of collagen structure by showing that observer ratings can be replaced by a mathematical computer analysis: Fourier analysis. This analysis technique runs on personal computers and allows automated analysis of a large number of images in a short time. Fourier analysis enables a standardised scar evaluation because results of different research groups can be compared objectively. Both photographic planimetry and Fourier analysis together with other appropriate tools selected from literature, such as the Cuto-meter and the 7 Objective Test Criteria by Jebsen made up our scar evaluation protocol.

The second section of this dissertation discusses objective long term evaluation of scar formation and brings prevailing dogmas concerning current burn surgery and scar formation up for discussion, making use of suitable objective criteria that are discussed in the first section of this dissertation. Interestingly, no scientific basis could be provided for generally accepted ideas on scar formation that are discussed in chapter 4, 5, and 6 respectively, that: a) skin transplantations on burned hands need to be performed in an early stage to achieve the best long term functional outcome; b) scar contraction is related to anatomical circumstances, patients' age and the presence of myofibroblasts; and c) tension over joints polarises collagen bundles of scar tissue in the direction of the load. For the study of contraction in chapter 5, and collagen orientation in chapter 6, clinical studies are sparse. This means that these prevailing ideas stem from clinical observations or experimental studies only.

The last section of this thesis is dedicated to perspectives of the development of artificial skin and a clinical trial on a dermal substitute. The review, at the beginning of this section, shows that only few clinical studies are based on an objective evaluation of the outcome. In our clinical study we concentrate on the dermal layer
of an artificial skin, the dermal substitute, as studies on epidermal substitutes elucidated the importance the dermis with respect to the outcome over the long term. A clinical trial was conducted to evaluate the effectiveness of the dermal substitute in burns and scar reconstructive wounds. Despite a relatively large number of paired wounds and the objective evaluation criteria, only a significant beneficial effect of the dermal substitute was shown for the short term (three months after surgery) in the reconstructive wound category with respect to skin elasticity.

Below, main conclusions of this dissertation will be elaborated, discussed and placed into future perspectives.

**Aiming at optimal scar evaluation**

There is no general agreement as to the most suitable tools for scar evaluation. By reviewing literature on scar formation we outlined clinical scar features that are appropriate for most clinical and research applications: scar colour, surface texture, surface area (contraction), scar thickness, tissue organisation, functional qualities of the scar (elasticity) as well as the resulting impairment and disability caused by scar formation. Crucial microscopic parameters of scar formation include the collagen structure and the evaluation of different cell types such as myofibroblasts. Most features may be evaluated both by subjective and objective assessment tools.

The choice for the most reliable and accurate method seems logical, but is that method always the most practical? All subjective observer evaluations and measurements performed by objective devices have their disadvantages. Studies have shown that subjective scales were reliable only after evaluation with more than three raters. Such scales can not be of use for clinical application. Also, some 'scales' appear to be no scale but a list of ordinal variables. Then, adding the outcome of separate parameters will give a mis-
leading ‘overall score’. In such cases, the data will be misinterpreted. Now that an increasing number of objective scar assessment tools have become available, scar evaluation should become more reliable and more accurate. Nevertheless subjective scar assessment tools are of paramount importance as they describe the impression of experts. Subjective scar assessment tool will become even more valuable if the opinion of the patient is evaluated. It is our opinion that objective measuring tools have improved the quality of scar evaluation. Nevertheless, conclusions based on these measurements always have to be placed in the context of the final judgment of experts and patients. We therefore disagree with the content of most scar evaluation scales when the patient’s judgment is omitted. At present, we are conducting a study to develop a reliable and accurate scar scale that includes both expert and patient judgements.

Based on literature and studies presented in this thesis we would make the following suggestions with respect to scar evaluation by means of currently available tools. Scar contraction is adequately evaluated through planimetry by photography. In extremely curved body parts, planimetry by tracing should be considered. Scar elasticity parameters are well examined by the Cutometer, nevertheless, the application of this technique is based on evaluations of sclerodermal lesions. Although sclerodermal skin shows many characteristics of scar tissue we recommend that a suitability study be conducted for the application of the Cutometer with respect to scar measurements. Scar thickness may be evaluated by ultrasound, which is the most practical and validated technique for this purpose. In this thesis it has been shown that Fourier analysis may be considered the standard for collagen orientation assessment at present instead of the microscopic evaluation by observers. For scar colour, surface texture and other parameters, no tools have been studied sufficiently to date to designate a ‘gold standard’. The
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Vancouver Scar Scale is considered the standard for subjective scar evaluation, however further adaptations are warranted to improve its suitability for scar evaluation in a research and clinical setting. Some methods for scar assessment like profilometry and collagen structure analysis by shear wave propagation, are promising as a supplement for a standard scar evaluation protocol but their suitability needs to be investigated to date.

**Scar formation: current and future perspectives**

Many chapters of this dissertation discussed different aspects of scar formation, such as scar quality, cosmetic outcome, functional disturbances and explanatory as well as prognostic factors. The use of validated measuring tools has improved the quality of analysis and therefore the accuracy of the conclusions. Nevertheless, most of the complex sequences of events that finally produce a scar need to be elucidated. Findings of this thesis are contemplated below with respect to prevailing knowledge on scar formation.

**Long term functional outcome of split skin grafting in relation to timing of surgery**

It is generally assumed that transplantation in an early stage of wound healing should serve optimal functional result in the long term. The relationship between timing of surgery and functional outcome is addressed in chapter 4 where the long term functional results of hand burns are discussed. We concentrated our study on hands as this body part is frequently involved in burn injuries; moreover, the functional outcome of hand burns has considerable influence on the performance of patients during daily activities. As we focussed on the performance of patients during activities of daily living we subjected all operated burned hands to the seven objective test criteria of Jebsen. These tests concern simulations such as writing, eating and picking up objects. Jebsen originally scored
hand function by measuring the time that patients required performing each test. In our study, the performance was evaluated by means of a 4-step scale ranging from 'normal' to 'totally disabled'. This design allowed the application of logistic regression so that factors were addressed that had prognostic value for hands to regain normal function (for all 7 tests) or disablement for one or more tests. Impaired function was related to advanced age, the necessity for amputations, poor graft take, and the surface area of the third degree hand burn as well as the third degree burned total body surface area. In contrast to the prevailing idea, we could not establish an effect of time between the burn injury and the placement of the split thickness skin graft on the functional outcome of hand burns. We therefore advocate the following approach to hand burns which might be applicable for burn wounds in general: if the diagnosis is a full thickness burn of the hand, a primary excision and transplantation is advocated. Rehabilitation is then 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 total burned body surface area. If 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. We consider that timing of surgery is not related to functional outcome in general, however, this hypothesis needs to be confirmed by randomised controlled trials. We feel, however, that the feasibility of such studies will be poor, as it will take many years, especially in case of one-centre studies, to attain a large sample size.

**Scar contraction and relaxation**

Contraction is a continuing process that starts within days after wounding and proceeds often for months after wound closure. The beneficial effect of this process, accelerated wound closure
and reducing the size of an unsightly scar can also be detrimental for skin and joint function, depending on anatomical circumstances. It would be helpful for operative procedures, like scar releases, if the rate of contraction after split skin grafting could be predicted. The size of the release wound could then be adjusted. The only prognostic factor that we identified for the extent of contraction after one year was the contraction after three months. This finding is of no use at the time of surgery, but it may help clinical decision making whether to operate when a joint becomes dysfunctional a few months after grafting.

No consensus exists on the mechanisms that cause contraction in the burn scar. Over the past several decades, multiple theories have been proposed to explain which cell type or structure is responsible for contraction and its location in wounds. Two theories survived to date: the 'picture frame theory' by Grillo and Watts and the 'pull theory' by Abercrombie and co-workers. The 'picture frame theory' hypothesises that cells located at the wound margin grow into the wound and generate forces that cause contraction whereas the 'pull theory' hypothesises that cells throughout the granulation tissue generate the forces to pull the wound margins together. The clinical experience that contraction continues after wound closure, as discussed in chapter 5, is inconsistent with the 'picture frame theory' and supports the 'pull theory' because the granulation tissue, the new dermal tissue, still may contract underneath a complete epithelium.

There is no consensus on the type of cell that is responsible for contraction. Generally two cell types are considered candidates: fibroblasts or myofibroblasts. The concept of the myofibroblast as contracting element has been proposed by Gabbiani and Majno, who found the presence of fibroblasts-like cells with morphologic attributes of smooth muscle cells. Although it seems natural that such cells generate forces that cause contraction, Ehrlich and co-
workers reported studies that suggest that fibroblasts are capable of generating enough forces to obtain wound closure and that myofibroblasts are not required for contraction. Our study on scar contraction could not support the key role of myofibroblasts with respect to contraction, as we showed no relationship between the presence of myofibroblasts at the end of the proliferation phase and the extent of wound contraction. At this time, approximately three months after surgery, contraction is believed to come to an end. Future studies however are warranted that investigate the concentration of myofibroblasts in relation to contraction at an earlier stage of scar formation.

In vitro models posed other predominantly single local factors to cause contraction, such as transforming growth factor β1, interferon α-2b, platelet derived growth factor, tensional forces, free fatty acids, serum factors, mast cells and photolysis. However, their value for the clinical situation is unclear.

More recently, mathematical computer models have been challenged on contraction mechanisms. Data of in vitro studies were applied to the mathematical model and so many models could be analysed. We feel that such mathematical approaches have their place under certain circumstances, but oversimplification of the in vivo situation may lead to inaccurate conclusions. Once the model is validated, infinite simulations can be undertaken to investigate all sort of effects that might be of interest. Then computer-models will be cost saving, fast and might even replace in vitro and animal studies.

In chapter 5, we addressed the phenomenon 'scar relaxation', meaning that the surface area of the contracted scar finally relaxes, which may have a beneficial effect on the functional outcome especially at joints. Clinical experience shows that scar relaxation commences a few months after wounding and parallels the tissue-remodelling phase of wound healing. In our study it was shown that the contracted state of scars (63 percent of the original wound area on average) increased significantly to 75 percent of the original surface area after one year. Literature regarding scar relaxation
exists, however, these publications sparsely mention this topic12,13. We fail to comprehend the paucity of studies on this clinically relevant issue. Also we noticed that studies generally have a follow-up of a few months. Most studies therefore missed the relaxation phase.

The potential value of computer models for future studies on wound healing and scar formation becomes evident when we consider the study by Olsen et al.14 who developed a mathematical model to study contraction. In this manuscript they stated: ‘The model predicts that the contracted state is a transient (albeit long) phenomenon, in contrast to real wound healing, in which a permanently contracted state is normally observed’. Their numerical simulations confirmed that the repaired tissue finally relaxes. The authors were puzzled about the outcome of this model. Apparently, they were unacquainted with scar relaxation. Although the validity of such computer models remains to be established, this model was able to predict scar relaxation that was not accounted for. Moreover, scar relaxation has not been described properly in vitro and in vivo studies to date. These findings proclaim innovative future developments of computer models. Together with inevitable experimental and clinical studies, computer models may increase the knowledge and understanding of scar contraction and relaxation, thus influencing clinical decision making significantly.

Collagen structure

Many authors have hypothesised that collagen bundles become aligned in the direction of mechanical tension during scar formation14,15. Tensional load seems to be of significance at joints were contractures and ‘ropes’ develop. Ropes have an orientation in the direction of tension at the macroscopic level (see Figure 1b, chapter 1). The evaluation of the relationship between tensional load and collagen orientation is complicated by the lack of suitable tools in clinical studies to record the total amount of load on an anatomical
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location over time. Furthermore, data from animal models are not applicable to humans as most animals have a well-developed layer of cutaneous muscle which is loosely connected to underlying tissues\textsuperscript{1}, which is known as the panniculus carnosus. This layer permits contraction without interfering with mechanical function of the skin\textsuperscript{2}. It is therefore not ideal for one to use an animal model to investigate the influence of mechanical tension on collagen structure. We restricted our study to comparing collagen orientation at joints and compared these findings to the collagen structure of adjacent areas where tensional load is considered to be lower; moreover, ripples and contracture generally do appear less frequently in the area in-between joints. Our findings do not support the hypothesis that mechanical tension polarises collagen bundle orientation in the direction of stress. For the first time, an objective validated method was employed (Fourier analysis) to study the collagen structure of human scar tissue and also of normal skin, which was analysed as control. Novel findings of this quantitative analysis mainly concerned normal skin: the organisation in deeper layers of normal skin showed a more random collagen organisation compared to superficial layers and collagen appeared to have a more random organisation in the plane parallel to the epidermis compared to the perpendicular plane.

Scar tissue apparently lacks the characteristics of normal skin at an overall microscopic level (parallel versus random orientation) and at a more refined level, because normal skin shows considerable variation within its dermal architecture. We propose that scar tissue collagen may be composed of ‘collagen planes’ instead of single fibres. This ‘collagen plane theory’ may account for the aspect of images taken from different angles, which shows that ‘fibres’ always run parallel to the epidermis. If the fibres had a predominant direction, we would have found transected fibres that appear as small dots.
A computer model was developed by Dallon to study collagen alignment that showed that cell flux, cell density and initial matrix orientation can have a significant impact on the overall alignment of the collagen. They concluded that cell flux is the most significant alignment mechanism. Unfortunately tractional forces were not considered in this model. Additional experimental and clinical studies are necessary to gain a full understanding of the processes that determine collagen alignment. Biopsies obtained by clinical studies might be evaluated for parameters other than collagen structure such as the presence of fibroblasts, myofibroblasts, inflammatory cells, growth factors and many other components that may play a role in scar formation. In addition, these clinical data may be utilised to make up more appropriate computer models.

**Skin substitutes: current status and future research**

Much research has been conducted on different types of skin substitutes, frequently with promising results. Statistical evidence for clinical effectiveness of skin substitutes has, however, not been established through clinical trials due to insufficient number of patients. We criticise the approach of most reported studies that have been to show clinical effectiveness of skin substitutes as small study sizes are used and frequently a control treatment group is absent. It is therefore much too early to come to definitive conclusions on the clinical effectiveness of dermal substitution. Moreover, clinical trials are mandatory that utilise both objective and subjective scar assessment tools. Initially, most studies, which employed skin substitutes, focused on epidermal replacement. These studies indicate that cultured keratinocytes may be applied in burn patients who have limited donor-sites available for split thickness skin grafts due to the large
extent of their burned surface area. Cultured keratinocytes, survive poorly during the grafting procedure and are susceptible to contamination by micro-organisms. The long term functional and cosmetic outcome also remained poor, which is related to the absence of a dermal substitute.

We studied a dermal substitute for the application in a one-stage grafting model in acute burn wounds and in reconstructive surgery. This dermal substitute was selected after it had been subjected to a number of experimental studies and in punch biopsy wounds in humans.

For the short-term clinical follow-up, a promising and highly significant increase of skin elasticity was measured in the category of reconstructive wounds for dermally substituted wounds compared to control wounds. We provided evidence that the dermal substitute has features which improve the quality of tissue repair. However, in its current composition, it is not considered to have clinical effectiveness over the long term, as no statistical significance could be established for both the burn wound and reconstructive category with respect to skin elasticity. The same conclusion was drawn from the array of other clinical and microscopic parameters as no statistically significant differences were found.

The circumstances in which the dermal substitution is applied are of eminent importance. Firstly, the dermal substitute gives better result in the reconstructive category compared to acute burn wounds. We considered that bacterial contamination and potentially toxic products, present in acute burn wounds, would accelerate the degradation of the dermal substitute. Nevertheless we were not able to demonstrate differences between contaminated wounds and wounds that were clean at surgery (unpublished data). The collagen/elastin dermal substitute seemed to be effective in a
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subcategory of burn wounds when it was applied in combination with a large expansion of the overlying graft. This finding bears clinical relevance as the need for dermal substitution increases in case of extensive wounds, when large expansion rates of the autograft are used. From this perspective it would be interesting to evaluate the dermal substitute in combination with large graft expansions for reconstructive wounds.

The findings of our clinical trial bring new, more specific premises that may lead to a dermal substitute with long term clinical effectiveness. Studies in animal models indicate that significant improvement in outcome is obtained when autologous cultured fibroblasts are added to the dermal substitute\textsuperscript{21,22}. The beneficial effect of these cells is explained by their production of wound repair-stimulating factors that promote proliferation and migration of resident cells\textsuperscript{23}. We are therefore conducting a clinical trial following the same protocol as described in Chapter 8, making use of the collagen/elastin dermal substitute seeded with autologous fibroblasts. We concentrate on cells of autologous origin, as allogeneic cells seemed capable of arousing unwanted immune responses\textsuperscript{24}. We showed that different cell sources might be considered for the isolation and cultivation of fibroblasts: fat tissue, burn eschar and dermal tissue. Fat tissue, which is easy accessible, and burn eschar, which is normally discarded after burn surgery, have shown in our study to be optional sources for culturing autologous fibroblasts. The best source however, remains dermal tissue as it results in the highest yield per gram of tissue.

Allogeneic keratinocytes should not be dismissed completely. They may serve as a temporary cover, thus overcoming a serious disadvantage in the application of autologous cells: the time delay required for culturing a sufficient number of cells. Moreover, allo-
generic keratinocytes provide a good environment for the growth of autologous keratinocytes.\footnote{25} Based on our current knowledge we propose the development of an ‘optimal’ skin substitute for the extensively burned patient that allows fast wound covering together with improved long term functional and cosmetic outcome. At the time of surgery, an ‘off-the-shelf’ skin substitute is applied on the wound, consisting of allogeneic keratinocytes together with a dermal substitute. During the same operation, a skin biopsy, or split thickness skin graft, is harvested for isolation and cultivation of autologous keratinocytes that can be applied to the wound after one or two weeks. Essential information remains elusive to date and needs to be elucidated in order to establish a definitive skin replacement. We have to integrate our present knowledge of wound healing, scar formation, computer models, skin substitutes, and tissue engineering to improve the overall outcome from the patient’s perspective.

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