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

Citation for published version (APA):
van Zuijlen, P. P. M. (2002). Perspectives on burn scar evaluation and artificial skin
Scar contraction in humans: long term evaluation and prognostic factors

Paul PM van Zuijlen 1,2,3, Monique H Suijker 1, Antoine JM van Trier 4, Frits Groenevelt 3,4, Robert W Kreis 1,2,5,6, Esther Middelkoop 1,6

From the Burn Centre 1 and the Department of Surgery 2, Beverwijk, The Department of Plastic, Reconstructive and Hand Surgery 3 of the Academic Medical Centre, Amsterdam, The Department of Plastic and Reconstructive Surgery 4, Beverwijk, The Department of Surgery 5, VU University Medical Centre, Amsterdam, and the Dutch Burns Foundation 6, Beverwijk, The Netherlands.
Abstract The autologous split skin graft is still considered the mainstay for the treatment of large burn wounds and for skin defects that are created during scar reconstructions. Scar contraction occurs as part of the wound healing process after the application of a split skin graft and frequently impairs the function of the skin and underlying joints. Knowledge of the aetiology and pathophysiology of scar contraction may be of great help for the reconstruction of such deformities.

In a prospective clinical study, we established the course of contraction of split skin grafts in time. In addition, potential prognostic factors for scar contraction on the long term were studied, such as age, location of treatment, the presence of myofibroblasts and the short term contraction.

We analysed the outcome of 48 split thickness autografts applied to cover wounds that were created to release a burn scar for reconstructive procedures. The surface area was assessed by planimetry at different time points for at least one year and was expressed as percentage of the surface area at surgery. Biopsies, obtained after three months (n=30), were evaluated for the presence of myofibroblasts (anti-α-smooth-muscle-actin staining).

A statistically significant reduction of the surface area was found three months after surgery to a remaining surface area of 62.8 percent with a standard deviation (SD) of 28.2 percent (p<0.001). The contraction period was followed by a statistically significant increase of the surface area to 75.4 percent (SD:36.8, p=0.004) after one year. Four parameters that were presumed to influence contraction on the long term were fitted into a multiple linear regression model. Age of the patient, the presence of myofibroblasts, and location of the split skin graft (flexion versus non-flexion surfaces) were not found to be prognostic factors for the extent of contraction after one year. The contraction on the short term was found to be the only predictor.

In a 'human scar model', scars show a contraction phase during the first months that convert to scar relaxation. There was no explanatory relation between the extent of scar contraction and the presence of myofibroblasts, age or location.
Introduction

Wound healing and scar formation are issues that almost every surgical speciality has to deal with. Especially in extensive full-thickness wounds, it is essential to consider the consequences of scar formation that occur on the long term, as it may render a disfiguring and poor functional outcome. One of the most severe and clinically relevant consequences of scar formation is contraction of the scar. Studies on contraction are predominantly based on in vitro evaluation with short-term follow up. No studies are available of a long term clinical evaluation of this item to date. Therefore, we initiated a prospective clinical study with a follow-up of one year to evaluate the contraction process in a group of patients that have been treated with a split skin graft.

In this communication, 'scar contraction' is defined as the diminution of the originally injured surface area. We advocate the use of the term 'scar contraction' instead of 'wound contraction' as this study predominantly concentrates on contraction that occurs after the wound has been closed. Scar contraction should not be confused with 'contracture', a term that indicates the deformity that remains as end result of scar contraction.

What mechanism causes contraction? In 1956, Abercrombie was the first to propose that connective tissue cells of the repair tissue induce wound contraction. In the beginning of the nineteen-seventies, much attention was focused on granulation tissue fibroblasts, the so-called myofibroblasts, which developed features of smooth muscle cells. These cells were considered to play a crucial role in contraction. However, despite an extensive number of in vitro and animal studies, there is no definite proof provided by clinical studies. Other factors for the occurrence of contraction have been considered. Age was supposed to have a relation with contraction. Peacock highlights the relation to the anatomical locations and the influence of tension and mobility of the skin. These factors should explain contraction at joints in the direction of flexion, which finally results in the so-called 'flexion contracture'.

We have evaluated a substantial number of split skin graft treatments prospectively by means of a standardised scar evaluation.
protocol. The data enabled us to evaluate contraction in time in a ‘human scar model’, and the relation between the extent of contraction and parameters of interest.
Firstly, the surface area of scars was assessed at surgery, after three months and after one year to measure and plot the course of contraction in time. Secondly, potential prognostic parameters for the contraction rate after one year such as age, location, presence of myofibroblasts and short term contraction results were fitted into a regression model that weighted the influence of the separated parameters.

Patients and methods
Patients were eligible who required a split thickness skin autograft during a reconstruction of a burn scar between August 1996 and March 1998. The protocol was approved by the ethics committee of our hospital. All patients gave informed consent before surgery. Forty-four consecutive patients (65 reconstructions) enrolled the study protocol. After one year, seventeen scars were considered ineligible for planimetry, as the margins became difficult to delineate accurately during the follow-up. These patients (study areas) were withdrawn from the analysis. In the remaining group of 33 patients (17 male and 16 females) with 48 reconstructed scars were included. Split skin graft treatments were performed within a range of 77 days to 39 years after the burn injury (average: 5.7 years). The mean age was 34.2 years with a standard deviation (SD) of 17.7 years. The initial total body surface area (TBSA) burned was 28.3 percent (SD:17.3) of which 19.8 (SD:14.8) was a third degree burn. No statistically significant differences were found regarding these characteristics between the group of included and excluded patients. Locations for interventions were: neck (n= 17), thorax (n= 6), axilla (n= 8), arm (n= 4), elbow (n= 1), wrist (n= 3), hand (n= 4), groin (n= 1), leg (n= 1), knee (n= 2) and foot (n= 1).

Operation procedure
The contracted burn scar tissue was released by a double-Y incision resulting in a clean full-thickness wound with clear edges and a wound bed of fat and muscle tissue. The split-thickness grafts were always obtained from unburned skin of an upper leg with a Zimmer
The dermatome (Zimmer Inc., Dover, Ohio, USA). The dermatome was adjusted to obtain grafts with a thickness of the 0.12 - 0.14 inch (the thickness was indicated by the scale of the dermatome). After hemostasis was obtained, the graft was applied and fixed with staples. Forty-three grafts were meshed to a ratio of 1:1.5, and applied without spreading onto the wound bed. Five grafts were not meshed. Paraffin gauze and absorbent cotton, soaked in a solution of 30% polyethylene glycol, 20% sorbitol and 1% Povidoniodine, were applied on top of the graft. The gauze and cotton were removed after five to seven days.

**Planimetry**

The scar surface areas were measured during the operation, after three and twelve months. Besides these time points, scars were measured during every other outpatient visit. With a Polaroid Macro 5 SLR photocamera (Polaroid UK Ltd., Vale of Leven, United Kingdom), photos were taken at a fixed distance perpendicular to the surface resulting in full-size images (1:1 enlargement). The margins of the drawings were then traced on a plastic sheet, scanned and exported to computer-software (Adobe Photoshop, Adobe Systems Inc., San Jose, California, USA). The margins of the lesion were digitally traced. The surface area of a lesion was calculated from the number of pixels. In this paper the surface areas are expressed as percentage of the surface area at surgery.

**Histopathology**

Biopsies were taken three months after surgery from the centre of the study area. After disinfecting the area, and local infiltration with lidocaine (1% solution with epinephrine), a punch biopsy was obtained with a diameter of three millimetres. Finally, 30 biopsies were obtained three months after surgery. The biopsy was transported in formalin and processed into histological slides of approximately 5 μm. The slides were stained for α-smooth-muscle-actin by routine immunohistochemical procedures. Biopsies were analysed by light microscopy for the presence of myofibroblasts. The myofibroblasts were scored following a four step semi-quantitative scale from ‘absent’ to ‘abundant’.
Statistical analysis

Statistical analysis was performed by SPSS for Windows 8.0 (SPSS Inc. Chicago IL USA). After being tested for normality by means of the Kolmogorov-Smirnov test, a paired sample t test could be applied for the planimetry data. Both a 95% confidence interval (CI) and a p-value are given. The two tailed significance criterion was set at 0.05. Multiple linear regression was performed to study the outcome of the remaining scar surface area after one year and the parameters that hypothetically influenced the extent of contraction at this time. Before the regression analysis, all independent parameters were tested for a linear relation with the parameter ‘remaining scar surface area after one year’. The following parameters were considered as independent variables to study their potential influence on the remaining scar surface area after one year: age, location of treatment, the extent of contraction after three months, and the presence of myofibroblasts after three months.

The continuous variable ‘age’ was not linearly related to the contraction after one year. As suggested by literature\textsuperscript{10}, a binary variable was created that could fit into the regression. Two groups ‘old’ and ‘young’ were determined as being older or younger than the median of 37.4 years. Also, the location of treatment was converted into a binary variable. Two groups were formed: ‘treatment at flexion areas’, which are prone to develop a flexion contracture’ and ‘remaining locations’. As flexion areas were considered: neck, axilla, elbow, wrist, groin, and knee (n=32). The treatments not directly located at ‘flexion areas’ of a joint were performed on the thorax, arm, hand, leg, and foot (n=16). A dummy variable was created for myofibroblasts: absent or present, the latter being the combination of the categories few, frequent and abundant. Backward stepwise elimination was performed to refine the model. For the backward elimination the inclusion criterion was set at p<0.05 and for exclusion at p>0.10.

Results

The planimetry results indicate a statistically significant reduction of the original wound area to a remaining 62.8 percent (SD:28.2, p<0.001, CI: 25.8 - 48.6) after three months. After one year, the average scar sur-
Figure 1 The relation between scar contraction after three and twelve months

The remaining scar surface area after three versus twelve months is given in percentages. The correlation is presented by the equation line and 95% prediction interval for new observations. Equation for regression line: 'remaining scar surface area after one year' = 23.7 + (0.935 x 'remaining scar surface area after three months'), the adjusted $R^2$ = 0.736 and the residual standard deviation = 20.0.
Figure 2 Scar contraction and relaxation

A clinical example of scar contraction and relaxation of the same surface area at the day of surgery (a), after three weeks (b) and after approximately eighteen months (c) of a scar reconstruction of the presternal region. Figure 2d shows the contraction-time curve. The day of measurement (after surgery) is displayed on the horizontal axis and remaining surface area in percentage (compared to the surface area at surgery) is shown on the vertical axis.

Figure 3 Scar contraction by myofibroblasts category

Remaining scar surface area after one year versus presence of myofibroblasts. The line indicates the average remaining scar surface area for all scars that were analysed for the presence of myofibroblasts. These data show no correlation between the presence of myofibroblasts and contraction.
face area was increased to 75.4 percent (SD:36.8) of the original area. Although this area was still significantly smaller in comparison with the original wound area (p<0.001, CI: 12.3 - 36.9), the average scar area at one year was significantly increased compared to the planimetry at three months of follow-up (p=0.004, CI: -25.2 - -5.4).

The multiple linear regression was performed with the parameter 'remaining scar surface area after one year' as dependent variable. The independent variables of this model were: 'age', 'location of treatment', 'remaining surface area after three months', and 'presence of myofibroblasts after three months'. The outcome of the regression analysis is given in Table 1.

The analysis demonstrates that only one predicting factor remains

<table>
<thead>
<tr>
<th>Regression models &amp; variables</th>
<th>Variable codes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regression model:</strong></td>
<td></td>
</tr>
<tr>
<td>Age at surgery</td>
<td>0= &lt; 37.4 years, 1= &gt; 37.4 years</td>
</tr>
<tr>
<td>Location</td>
<td>0= not at joints, 1= at joints</td>
</tr>
<tr>
<td>Myofibroblasts - 3 months after surgery</td>
<td>0= absent, 1= present</td>
</tr>
<tr>
<td>Remaining surface area - 3 months after surgery (Constant)</td>
<td>Percentage</td>
</tr>
</tbody>
</table>

**Model after Backward elimination:**

| Remaining surface area - 3 months after surgery (Constant) |

*Table 1: Regression model for parameters affecting the remaining surface area one year after surgery*

Regression model for parameters affecting the remaining surface area one year after surgery. The regression before and after backward stepwise elimination is given. The surface area after three months was established as the only predictor for the surface area after one year.
after backward removal of not statistically significant variables: 'remaining surface area after three months' (Standardised Coefficient Beta = 0.870, p<0.001). The relation between the remaining scar surface area after three and after twelve months is demonstrated in Figure 1 and can be expressed as the following equation; 'remaining scar surface area after one year' = 23.7 + (0.935 x 'remaining scar surface area after three months').

The linear regression analysis was repeated in the same way for 'remaining surface area after three months'. The following parameters were selected for this analysis: 'age', 'location of treatment', and 'presence of myofibroblasts after three months'. In this model no significant parameters were established.

<table>
<thead>
<tr>
<th>Unstandardised Coefficients</th>
<th>Standardised Coefficient</th>
<th>Significance</th>
<th>95% Confidence Interval for B</th>
</tr>
</thead>
<tbody>
<tr>
<td>B</td>
<td>Standard Error</td>
<td>Beta</td>
<td>p-value</td>
</tr>
<tr>
<td>-18.666</td>
<td>11.811</td>
<td>-0.246</td>
<td>0.149</td>
</tr>
<tr>
<td>1.496</td>
<td>13.516</td>
<td>0.019</td>
<td>0.914</td>
</tr>
<tr>
<td>7.184</td>
<td>14.175</td>
<td>0.087</td>
<td>0.624</td>
</tr>
<tr>
<td>0.899</td>
<td>0.162</td>
<td>0.836</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30.573</td>
<td>21.083</td>
<td>0.870</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0.935</td>
<td>0.153</td>
<td>0.654</td>
<td>0.054</td>
</tr>
</tbody>
</table>
Discussion

Scar contraction is common practice after many surgical interventions. Nevertheless, literature on long term follow-up of scars in a 'human scar model' is lacking. This study provides scientific evidence for the clinical experience that scar contraction within the first three to four months is followed by expansion of the surface area. The average remaining surface area was reduced to 62.8 percent during the first period and followed by a relaxation to 75.4 percent. The differences between the surface area at both time points (three and twelve months after surgery) were significant despite the large standard deviations of the data. An example of these phases is illustrated by the photographs in Figure 2 that were taken of one wound at different time points and the contraction-time curve of this scar surface area.

The regression analysis enabled us to describe the relation between the single outcome value 'remaining scar surface area after one year' and several variables that were hypothesised to predict the remaining scar surface area after one year. Ideally, our regression model should explain why we encountered the large differences (standard deviations) in-between different scars. Apparently, only one significant prognostic factor could be identified for the remaining scar surface area over a long term, namely the remaining scar surface area in the short term (three months). This means that the extent of contraction on the short term predicts the long-term outcome and shows that a severely contracted scar is unlikely to become widely stretched after one year and vice versa.

Other parameters that were hypothesised to be related to the remaining scar surface area after one year such as the presence of myofibroblasts, age, and location showed no statistically significant correlation with remaining scar surface area.

After the discovery of the myofibroblast in the beginning of the seventies, many papers implied an important role for the myofibroblast in contraction. Since then, the role of the myofibroblast in relation to scar contraction has also come under debate. For example, when rats received a substance that blocked the expression of \( \alpha \)-smooth-muscle-actin in myofibroblasts (Vanadate, an inhibitor
of tyrosine phosphate phosphatases), a normal contraction rate occurred in the absence of myofibroblasts.

The immunohistochemical findings of our clinical study could also not provide substantial evidence for the effect of myofibroblasts on scar contraction in humans. Although some of the myofibroblast positive scars could have been missed as the myofibroblast may have disappeared before biopsies were taken, we also established that non-contracting scars were positive for myofibroblasts. For example, of three scars that showed no contraction after three months, two were positive for myofibroblasts. After one year, six scars were larger than the surface area at surgery of which three had been positive for myofibroblasts, as illustrated in Figure 3. This implies that scars that are positive for myofibroblasts do not necessarily contract. This human scar model therefore could not give a scientific basis for a prognostic value and causative role of the myofibroblast in contraction.

Others emphasized the role of the fibroblast in contraction, instead of the myofibroblasts. Fibroblasts have been shown to exert forces much larger than needed for locomotion, which may be used for contraction forces. The role of the fibroblast in contraction during our study was established as we repeated exactly the same regression model as shown in Table 1 with the inclusion of the number of fibroblasts (data not shown). The fibroblasts were scored following a four step semi-quantitative scale from ‘absent’ to ‘abundant’. The concentration of fibroblasts in scars was not correlated to the extent of contraction. On the other hand, this finding cannot exclude that fibroblasts are involved in contraction as all sections at least demonstrated the presence of some fibroblasts, so no comparison could be made between scars with and without fibroblasts.

Another issue of studies on contraction has been the influence of the anatomic region. An enormous range of the extent of contraction for separate wounds has been found during this clinical study. Are these differences the result of the application in different anatomic regions? By the regression analysis we compared the scars on flexion surfaces of joints with reconstructions performed in other regions (thorax, arm, hand, leg, and foot). Flexion surfaces are clinically defined problematic areas as contraction in those areas...
may result in function disability of the joint (contracture). It explains the high incidence of burn scars in our population that required surgical reconstruction on flexion areas of joints (32 of 48 included scars), whereas no reconstructions were performed on the extensor surface of a joint.

We could not establish a different extent of contraction for scars at flexion surfaces compared to scars at other areas. This, however, does not directly show that contraction is not site dependent since we were not able to study scars at every body region. By evaluating the largest subcategory of this study, consisting of neck reconstructions (n=17), we found an enormous range from 6.5 to 144.6 percent (SD: 42.2) of the initial surface area one year after surgery. It signifies that parameters other than the location of the graft have a major influence in contraction. It has been hypothesised that mucopolysaccharides function as glue that binds the collagen bundles when the joint is in flexion. In this model mucopolysaccharides explain the contraction on flexion surfaces. Contraction has been believed to increase with age, however no significant influence was found for age on contraction in this study.

Also parameters which could not be evaluated in this study model should be considered as prognostic factors in future studies so that finally causative factors might be discriminated. Mainly experimental studies indicated a role for systemic factors that were related to scar formation such as immunological and genetic factors. These studies are not concerned with scar contraction but evaluate the pathology of hypertrophic scarring and keloid. Although these factors seem to be relevant from a clinical perspective (not only wounds but also patients appear to have a 'contraction habit') it is yet unclear whether these factors contribute in the same extent to contraction as they do to hypertrophy. Local factors, in contrast to systemic factors, are studied more specifically towards contraction. Mainly in vitro studies revealed influence of TGF β, interferon α, tension, free fatty acids, serum factors, mast cells and photolysis on contraction. Despite the clear study design and the accurateness of the data of an in vitro study, the clinical relevance of such studies remains questionable.

A mathematical model has been developed to predict the extent of
Scar contraction in humans: long term evaluation and prognostic factors

Although the authors appeared unacquainted with scar relaxation, their numerical simulations nevertheless predicted that the scar is not in a permanently contracted state. This impressive work gives interesting new perspectives on contraction, and these findings proclaim innovative future developments of computer models. On the other hand, it remains a mathematical and not a biological model. Another mathematical model was developed to calculate the amount of additional tissue needed to regain full range of motion of joints. This model does not, and can not, correct for the extent of scar contraction if a split skin graft should be the treatment of choice. As long as all relevant parameters are not fully understood the outcome for practical purposes of both models remains questionable.

Knowledge of the aetiology and pathophysiology involved in scar formation is of great help for the plastic surgeon who uses split skin grafts. 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 on the short term appeared to be the only predictive parameter for the long-term outcome of contraction. No evidence was obtained of an explanatory relation between age, location of treatment, and presence of myofibroblasts with the long term outcome. Essential data continue to remain elusive.

Acknowledgements

We acknowledge Dr Wim Tuinebreijer for his advice concerning statistical issues and Dr Adam Angeles for his valuable comments. This study was supported by the Technology Foundation STW, The Netherlands Organisation for Scientific Research (NWO) and the Dutch Burns Foundation.
References


