Clinical effectiveness of dermal substitution in burns by topical negative pressure: a multicenter randomized controlled trial

Study group:
G.I.J.M. Beerthuizen
H. Boxma, J. Dokter
S.M.H.J. Scholten
F.R.H. Tempelman
A.F.P.M. Vloemans

Monica C.T. Bloemen
Martijn B.A. van der Wal
Pauline D.H.M. Verhaegen
Marianne K. Nieuwenhuis
Margriet E. van Baar
Paul P.M. van Zuijlen
Esther Middelkoop
Abstract

Background: Previous research has demonstrated clinical effectiveness of dermal substitution, however, in burn wounds only limited effect has been shown. A problem in burn wounds is the reduced take of the autograft, when the substitute and graft are applied in a one-step procedure. In other studies, application of topical negative pressure (TNP) was able to improve the take rate of an autograft.

Objective: Aim of this study was to investigate if application of a dermal substitute in combination with TNP improves scar quality after burns.

Methods: In a four-armed multicenter randomized controlled trial (RCT), a split-skin graft with or without dermal substitute Matriderm®, and with or without TNP were compared in adult patients with deep dermal or full-thickness burns which required skin transplantation. Graft take and rate of wound epithelialization were evaluated. Three and 12 months postoperatively, scar parameters were measured, including the primary outcome parameter scar elasticity.

Results: Eighty-six patients were included. Graft take and wound epithelialization did not reveal significant differences between the four groups. Significantly fewer wounds in the TNP group showed postoperative contamination, compared to other groups. Twelve months postoperatively, highest elasticity was measured in scars treated with substitute and TNP, which was significantly better compared to scars treated with the substitute alone.

Conclusion: In this RCT, we demonstrated the effectiveness of dermal substitution in combination with TNP in burns, based on an extensive array of wound and scar measurements, such as skin elasticity. Therefore, we recommend the use of TNP to optimize effectiveness of dermal substitution in acute burns.
Introduction

Full-thickness wounds usually heal with considerable scarring. Not only scar cosmesis is a problem, also skin function can cause difficulties, such as a diminished scar elasticity and scar contractures. Since several decades, it is known that a lack of dermal tissue in the healing wound is one of the factors that is causative for scarring. To improve scar outcome, the application of a dermal substitute has been investigated in different wounds. The common message of these reports is that dermal substitution improves scar function and cosmesis, however quantitative data and the number of treated patients in these studies are limited.

A well-known dermal substitute is Integra (Integra LifeSciences Corporation, Plainsboro, New Jersey, USA) which is used regularly for acute and reconstructive wounds. However, due to the slow vascular ingrowth into the dermal layer, Integra and the skin graft have to be applied in a two-step procedure with a larger risk for wound infection and loss of the graft. Furthermore, the high costs of the product and the demanding operation technique are reasons that Integra is not routinely used in every burn clinic today. Several other materials are or have been on the market, but have not demonstrated superior results, are only for specific indications outside of the burn wound area, or are only limited available. In summary, although the proof of principle has been reached for dermal substitution, the ideal product has not yet been found.

In previous work, our research group investigated the application of a dermal substitute in acute and reconstructive burn wounds. First, the application of the substitute and autograft in a one-stage procedure was shown to give a good graft take. Second, a significantly higher scar elasticity was seen three months after the operation for reconstructive wounds treated with the substitute. In addition, twelve years after surgery, reconstructive scars treated with the dermal substitute had a significantly smoother surface, which was measured objectively. For acute burn wounds, however, only a limited beneficial effect could be demonstrated. It was hypothesized that this difference could be explained by the fact that graft take was lower in substituted burn wounds versus burn wounds treated with the graft only, which consequently affected scar quality. The reduced and delayed outgrowth of the graft was probably caused by a slower diffusion of nutrients from the wound bed through the dermal substitute to the skin graft, whereas in the control wound, the skin graft was placed directly on the wound and thus diffusion distance was shorter. Burn wounds are characterized by unfavorable wound conditions, compared with reconstructive wounds. For this reason, the autograft in acute burn wounds may require more time to achieve complete revascularization. In other studies,
this problem was also demonstrated in one-stage grafting models\textsuperscript{13, 14}. The dermal substitute that has been tested in our previous work is now commercially available as Matriderm (Dr. Suwelack Skin & Health Care AG, Billerbeck, Germany). Some results of the application of Matriderm in burn wounds were published more recently. In these studies, a case report and patient series, a good graft take and an improved scar appearance and elasticity were reported, measured subjectively\textsuperscript{15-17}.

In 2003, Jeschke et al. demonstrated that a combined application of Integra and topical negative pressure (TNP) therapy in reconstructive wounds could significantly reduce the time necessary for vascular ingrowth into Integra. Additionally, the wounds treated with Integra and TNP showed a significantly improved Integra take rate compared to the group without TNP\textsuperscript{18}. In other reports, an improved graft take was seen after treatment with TNP therapy, compared with standard dressings in skin grafted wounds\textsuperscript{18, 20}. TNP was originally developed as a treatment for chronic wounds, however, it has become an accepted treatment also for acute, contaminated, and burn wounds\textsuperscript{19-22}. The exact working mechanism and the effect on wound healing is not fully understood. Nevertheless, several effects have been described, such as improved angiogenesis, reduction in bacterial count, and migration of endothelial cells\textsuperscript{22-28}.

In order to further improve graft take and scar quality after burns and to improve the effectiveness of the dermal substitute, we performed a prospective randomized controlled trial comparing the combinations of a dermal substitute, split-skin graft and TNP. Our hypothesis was that the combined application of a dermal substitute and a split-skin graft under TNP will lead to superior results in terms of graft take, rate of epithelialization and scar quality on the long term. Four groups were designed in which we investigated the effect of dermal substitution in combination with TNP on wound healing and scar quality using subjective and objective measurement techniques. The primary outcome parameter was scar elasticity measured objectively, representing clinical effectiveness in the long term. Secondary outcome parameters included graft take, rate of epithelialization, scar color, and surface roughness.

**Material and Methods**

*Study Design, Randomization, and Patients*

Patients were included from October 2007 until February 2010 in this four-armed multicenter randomized controlled trial conducted in the three burn centers of the Netherlands. The study protocol was approved by the medical ethics committee (M07-035) and registered at Clinical Trials (ID NCT00548314). In this parallel-group
trial, patients were recruited during their admission or at the consulting hours in the outpatient clinic. All patients were checked for eligibility according to the in- and exclusion criteria (Table 1). Before participation in the study, all patients gave written informed consent. Patients were randomly assigned to one of the following treatment groups:

1. dermal substitute (Matriderm®), split-skin graft (SSG) and TNP therapy (DS-TNP);
2. dermal substitute and SSG (DS);
3. SSG and TNP therapy (TNP);
4. standard treatment: SSG alone (ST).

An equal randomization ratio was performed (1:1:1:1) and allocation concealment was ensured by using sequentially numbered, opaque sealed envelopes. Carbon paper was used to transfer patient details from the outside of the envelope to the paper inside, containing the allocation. In each burn center, the first randomized patient received envelope number 1; the second patient number 2, etc. The envelope was opened peroperatively, after wound bed preparation and sufficient wound haemostasis, in order to avoid bias in wound bed preparation procedures. Blinding of clinician and patient was not possible, as it was obvious which treatment was allocated to the patient. Statistical analysis was performed according to the intention-to-treat analysis and randomization codes were kept secret until the analysis was completed.

Table 1: Study in- and exclusion criteria

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Deep dermal or full-thickness burn wounds requiring skin transplantation</td>
<td>1. Wounds without adequate possibility to apply TNP</td>
</tr>
<tr>
<td>2. Age ≥ 18 yrs</td>
<td>2. Infected wounds</td>
</tr>
<tr>
<td>3. TBSA third degree burns ≤ 15%</td>
<td>3. Severe cognitive dysfunction or psychiatric disorders</td>
</tr>
<tr>
<td>4. Study wound surface area min. 10 cm²</td>
<td>4. Immunocompromized patients</td>
</tr>
<tr>
<td>5. Study wound surface area max. 300 cm²</td>
<td>5. Pregnancy</td>
</tr>
<tr>
<td>6. Informed consent</td>
<td></td>
</tr>
</tbody>
</table>

TBSA, Total Body Surface Area; Min., Minimal; Max., Maximal; TNP, Topical Negative Pressure.

Materials

The dermal substitute used in this study was Matriderm (Dr. Suwelack Skin & Health Care AG, Billerbeck, Germany) which is a highly porous, 1mm-thick membrane consisting of a native bovine type I, III and V collagen fiber template coated with an elastin hydrolysate derived from the bovine nuchal ligament (GfNHerstellung
von Naturextracten GmbH, Michelbach, Germany) in a concentration of 3 weight-to-weight percent ratio. The substitute was treated with γ-irradiation (20-30kGy) and stored at room temperature. Substitutes were applied in a one-stage grafting procedure. TNP was provided by placement of white polyvinyl alcohol (PVA) foam (10x15x1cm) on top of the non-adhesive wound dressing and then sealed with accompanying foils (Kinetic Concepts International, San Antonio, Texas, USA). If the white PVA foam was too small in relation to the wound site, the black polyurethane (PU) foam was used. A suction tube was connected to the Vacuum-Assisted Closure (VAC) Advance Therapy System (Kinetic Concepts International, San Antonio, Texas, USA) which was set to deliver a negative pressure of 125mmHg, in a continuous mode.

Wound Treatment and Surgical Procedure

Until surgery, wounds were treated with topical silver sulfadiazine or cerium-silver sulpha-diazine cream. Usually, wounds were operated secondary between two to three weeks post burn in order for the superficial partial thickness wounds to heal conservatively. In case of extensive full-thickness burns, wounds were operated primarily (within the first week of burn injury). Burn wounds were scrubbed with chlorhexidine and cleaned with saline. Wound bed preparation was performed by avulsion, tangential excision, or scrubbing. In groups DS-TNP and DS, the substitute was soaked in sodium chloride 0.9% solution and placed on to the wound carefully, after adequate haemostasis. Thin split-thickness grafts were harvested with a Zimmer dermatome (Zimmer Inc., Dover, Ohio, USA) and expanded according to the fixed ratio 1:1.5 by means of the Mesh technique. Grafts were positioned on top of the substitute directly and fixated with staples. All transplanted wounds were covered with a non-adhesive (paraffin impregnated or silicone-based) wound dressing. In group DS-TNP and TNP, subsequently, the VAC system was applied and left in place for three to five days. Figure 1a-d shows an example of a patient randomized in group DS-TNP. In case of leakage of the VAC system, additional foils were applied to ensure negative pressure. In groups DS and ST, wounds were subsequently covered with gauzes. Dressings were supplemented with an antibacterial substance (e.g., povidone-iodine or fusidic acid) if determined necessary according to the treating surgeon. After four to seven days, staples and the non-adhesive wound dressing were removed. Following, wounds were dressed with non-adhesive wound dressings and supplemented with an adequate local antiseptic treatment, if necessary according to the burn specialists. Daily wound inspection was performed until complete healing (defined as >95% wound closure) was reached or until the patient was discharged from the hospital. In the latter case, weekly wound inspections were performed in the outpatient clinic until complete (>95%) wound closure.
Clinical effectiveness of dermal substitution in burns by topical negative pressure

Figure 1: Patient example peroperatively

A, Excised burn wound of a 56 years-old man after contact and steam burn of the foot; B, C, After wound bed preparation and adequate haemostasis, the Matriderm is applied according to the manufacturing instructions. Subsequently, the split-skin graft (mesh 1:1.5) is placed on top of the Matriderm; D, After fixation of the substitute and autograft with a non-adhesive wound dressing and staples, the foam and foils are applied before connection to the Vacuum Assisted Closure (VAC) system at 125mmHg, continuous mode.

Outcome Parameters

Wound Site Evaluation - Four to seven days after surgery, take rate of the autograft was assessed in a bedside procedure by an experienced burn clinician, not involved in the study. Wound parameter epithelialization was quantified by means of the reliable digital image analysis using NIS-Elements Ar (Nikon Instruments Europe B.V., Amstelveen, The Netherlands) [Bloemen et al. Digital image analysis versus clinical assessment of wound epithelialization: a validation study – manuscript in preparation]. The definition of take rate of the autograft was the percentage of the graft that appeared to be vital and showed good adherence to the wound bed. Epithelialization was defined as the percentage of the wound with a vital skin graft and healed graft interstices.

Microbiology, Pain and Complication Registration - Microbiology of the study wound was routinely monitored by swabs which were taken twice weekly. Based on these swabs, topical wound therapy was adapted if necessary. Pre- and postoperative pain scores were measured three times a day using the Visual Analogue Thermometer
(VAT), a reliable and valid measurement method for pain assessment\textsuperscript{29}. The VAT score ranges from 0 (no pain) to 10 (worst pain). In addition, the presence of complications, such as graft loss, haematoma and the necessity of regrafting was recorded.

\textit{Scar Quality} - Scar quality was examined 3 and 12 months postoperatively using various objective and subjective measurement methods. All measurements were performed by an experienced clinician and in each scar, the measuring probe was placed in the scar center, to prevent bias. A control measurement was performed on the contralateral side or on the adjacent healthy skin.

Scar elasticity was measured with the Cutometer Skin Elasticity Meter 575 (Courage and Khazaka GmbH, Cologne, Germany), a reliable and valid instrument for evaluation of skin elasticity\textsuperscript{30}. It measures the vertical deformation of skin in millimeters during a controlled vacuum and provides several elasticity parameters. In this study, parameter \textit{maximal skin extension} (Uf) (in mm) was used, as this was demonstrated to be the most reliable parameter\textsuperscript{30, 31}. To eliminate influence of different anatomic locations, elasticity was analyzed using the ratio of the scar and normal skin.

Scar color was measured by means of the DermaSpectrometer (Cortex Technology, Hadsund, Denmark), a reliable narrow-band spectrometer that computes an erythema and melanin index, based on the differences in light absorption of red and green by haemoglobin and melanin, respectively\textsuperscript{32}. For all measurement locations, the influence of sun exposure and the measuring error on the erythema and melanin scores was eliminated by analyzing the absolute difference between the scar and normal skin.

Scar roughness was measured using the PRIMOS (Phaseshift Rapid In-vivo Measurement of Skin) (GFMesstechnik GmbH, Berlin, Germany), a reliable and valid assessment tool that produces a three-dimensional image of the micro-topography of the skin\textsuperscript{33}. Calculation of scar surface roughness was carried out within an area of 30 by 40 mm, using the PRIMOS software 5.6. Surface roughness parameters Sa, Sz, and PC were used to determine scar roughness and are described as follows: Sa is the arithmetic mean of the surface roughness (\(\mu\)m), Sz is the mean of the five highest peaks and five deepest valleys from the measuring field (mm) and PC (Peak Count) demonstrates the number of peaks per unit length. The PRIMOS was only available in one burn center, therefore not all scars were evaluated on surface roughness.

Subjective scar assessment was performed by means of the Patient and Observer Scar Assessment Scale (POSAS), a reliable and valid scar assessment tool which consists of two parts, the patient and the observer scale\textsuperscript{34}. The patient scores scar
characteristics color, pliability, thickness, relief, itching and pain. The observer scale contains the items vascularization, pliability, pigmentation, thickness, and relief. All items are scored numerically on a 10-point rating scale, in which a score of 10 reflects the worst imaginable scar or sensation. The mean total observer score and patient score can be calculated by averaging the scores of the separate items of the POSAS. In addition to these items, the observer and the patient give a general opinion on the scar quality using the same rating scale of 1-10.

Finally, patient and burn-related characteristics were documented, including sex, age, TBSA, and skin type. Patient’s skin type was determined by means of a skin type scale discriminating between 1. very light, light, light - light brown and 2. light brown, deep brown, very dark skin.

Statistics
Power calculation was based on scar elasticity parameters found in the study by Van Zuijlen et al. on reconstructive wounds, 3 months postoperatively. Alfa was set at 0.05 and beta at 0.20 (power = 0.80). The calculated sample size was 16 per group, however to compensate for drop-outs, the number of patients required was set at 86 patients in total. Statistical analysis was performed using PASW statistics version 18.0 (SPSS Inc., Chicago, USA). Normal distribution was tested by applying the Kolmogorov–Smirnov test and by calculating the skewness and kurtosis. Differences within the treatment groups were tested using the Chi-Square test (categorical data), the one-way ANOVA and the independent samples Kruskal-Wallis test (numerical data). A factorial anova was used to examine the effect of treatment on the outcome parameters elasticity (primary endpoint), pigmentation, and erythema with adjusting for confounding by the multicenter setting, with burn center as independent variable. Post hoc analysis with Bonferroni correction was performed to compare the four treatment groups when the overall F-test was significant. The individual change of pain was investigated by linear mixed-effects model for repeated measures (with random intercept and slope). The VAT score was used as dependent variable, independent variables were time of the day, treatment group and days post burn. The standard deviation, 95% confidence interval, and the p-values are given where appropriate. The significance criterion was set at 0.05.

Results
In total 86 patients were included of which baseline characteristics are shown in Table 2. Figure 2 presents the study flowchart. Preoperatively, no significant difference was seen in contamination of wounds (Table 3) and in pain scores (data not shown). Postoperatively, group TNP showed significantly less contamination compared to the
other groups (Table 3). None of the wounds were infected, pre- or postoperatively. Postoperative pain scores were significantly lower in group DS compared to group ST. The estimated mean VAT scores (SD) were 1.69 (2.05), 1.48 (1.93), 2.53 (2.60), and 3.08 (2.53) for group DS-TNP, DS, TNP, and ST, respectively (with group ST as the reference category, p-values were 0.075, 0.036, and 0.106 for group DS-TNP, DS, and TNP, respectively; mixed-effects model). Furthermore, Table 3 shows the postoperative complications of the wounds. No significant differences were seen across the treatment groups. The assessment of the take rate of the autograft did not reveal significant differences across the four treatment groups, although take rate was lower in group DS. Wound epithelialization showed no significant difference between the study groups either (Table 4) (patient example Figure 3).

Figure 2: Study flowchart

† Other reasons were logistic problems, patients language, missed patients and patients expected to be lost to follow-up; * Reason: peroperative failure of TNP application; ** Reason: patient deceased due to coagulation disorders (unrelated cause); # Reason: wound was primarily closed; LTFU Lost to follow-up.
Table 2: Patient characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>DS-TNP n = 21</th>
<th>DS n = 23</th>
<th>TNP n = 22</th>
<th>ST n = 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>11/10</td>
<td>12/11</td>
<td>12/10</td>
<td>14/6</td>
</tr>
<tr>
<td>Age patient at admission (yrs)</td>
<td>44 (17.0)</td>
<td>48 (19.4)</td>
<td>49 (13.3)</td>
<td>53 (18.3)</td>
</tr>
<tr>
<td>TBSA burned (%)</td>
<td>8.0 (5.8)</td>
<td>9.6 (8.1)</td>
<td>10.0 (11.9)</td>
<td>7.7 (7.4)</td>
</tr>
<tr>
<td>No. of wounds full-thickness*</td>
<td>11 (52%)</td>
<td>10 (44%)</td>
<td>11 (50%)</td>
<td>13 (65%)</td>
</tr>
<tr>
<td>Skin type very light, light or light - light brown (n=79)</td>
<td>17 (81%)</td>
<td>14 (61%)</td>
<td>19 (86%)</td>
<td>15 (75%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2 (10%)</td>
<td>0</td>
<td>1 (5%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Other co-morbidities**</td>
<td>9 (43%)</td>
<td>11 (48%)</td>
<td>7 (32%)</td>
<td>9 (45%)</td>
</tr>
<tr>
<td>PBD operation</td>
<td>14.2 (6.6)</td>
<td>15.7 (5.0)</td>
<td>17.8 (10.9)</td>
<td>14.5 (6.4)</td>
</tr>
<tr>
<td>Etiology</td>
<td>Flame</td>
<td>13</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Scald</td>
<td>0</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Other***</td>
<td>8</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Location</td>
<td>Arm</td>
<td>10</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Leg</td>
<td>6</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Trunk</td>
<td>5</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

TBSA, Total Body Surface Area; * At least 50% of the total wound area was full-thickness; ** Other co-morbidities included: cardiovascular disease, alcohol or nicotine abuse, light psychiatric disorder, chronic obstructive pulmonary disease, malignancy, epilepsy; *** Other included steam, fat, and contact burns.

Table 3: Contamination and complications

<table>
<thead>
<tr>
<th></th>
<th>DS-TNP</th>
<th>DS</th>
<th>TNP</th>
<th>ST</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contaminated wounds preop. (n,%)</td>
<td>10/21 (48%)</td>
<td>10/22 (45%)</td>
<td>5/21 (24%)</td>
<td>8/18 (44%)</td>
<td>0.360</td>
</tr>
<tr>
<td>Contaminated wounds postop. (n,%) *</td>
<td>10/14 (71%)</td>
<td>13/17 (76%)</td>
<td>6/17 (35%)</td>
<td>7/9 (78%)</td>
<td>0.042 **</td>
</tr>
<tr>
<td>Pts with &gt; 1 operation of study wound (n,%)</td>
<td>2 (10%)</td>
<td>3 (13%)</td>
<td>1 (5%)</td>
<td>1 (5%)</td>
<td>0.697</td>
</tr>
<tr>
<td>Pts with complications (n,%)</td>
<td>7 (33%)</td>
<td>7 (30%)</td>
<td>5 (23%)</td>
<td>2 (10%)</td>
<td>0.303</td>
</tr>
<tr>
<td>Hematoma and graft loss</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0.279</td>
</tr>
<tr>
<td>Graft shift</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Graft loss ($5 to 100%)</td>
<td>2</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Postoperative bleeding</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis was performed using the Pearson Chi-square test; Contamination was defined as the presence of one or more of the following pathogens: Staphylococcus aureus, Pseudomonas aeruginosa, group G Streptococcus, Escherichia coli; Pts Patients; * Preoperatively and postoperatively respectively, 82/86 and 57/86 wounds were cultured. Postoperatively, fewer wounds were cultured because closed wounds were not cultured. Furthermore, out-patients were not cultured until their first visit: at that time wounds were often closed; ** A statistically significant difference was found across the four groups, further analysis (Pearson Chi-square test) showed that the number of contaminated wounds in group TNP was significantly lower compared to groups DS-TNP, DS, and ST.
Table 4: Graft take and wound epithelialization

<table>
<thead>
<tr>
<th>Wound evaluation</th>
<th>DS-TNP (95% CI)</th>
<th>DS (95% CI)</th>
<th>TNP (95% CI)</th>
<th>ST (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graft take (%)</td>
<td>n = 21</td>
<td>n = 23</td>
<td>n = 21</td>
<td>n = 20</td>
<td></td>
</tr>
<tr>
<td>Clinical assessment</td>
<td>94.8 (88.3 - 101.2)</td>
<td>92.4 (88.3 - 99.5)</td>
<td>94.2 (88.5 - 100.0)</td>
<td>96.1 (94.2 - 97.9)</td>
<td>0.552</td>
</tr>
<tr>
<td>Wound epithelial (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digital image analysis</td>
<td>91.7 (88.8 - 94.5)</td>
<td>85.3 (78.1 - 92.4)</td>
<td>88.3 (82.1 - 94.6)</td>
<td>91.3 (86.5 - 96.1)</td>
<td>0.433</td>
</tr>
<tr>
<td>Evaluation day (PO)</td>
<td>5.3 (4.8 - 5.7)</td>
<td>4.8 (4.5 - 5.0)</td>
<td>5.1 (4.8 - 5.4)</td>
<td>4.9 (4.5 – 5.2)</td>
<td>0.145</td>
</tr>
</tbody>
</table>

Statistical analysis was performed using the independent samples Kruskal-Wallis test; epithelial., epithelialization; PO, postoperatively; CI, Confidence interval.

Figure 3: Patient example postoperatively

Wound 5 days postoperatively (patient shown in Figure 1).

Scal Quality
Scar quality was evaluated at 3 and 12 months postoperative (patient example Figure 4a and b). Assessment times were not statistically different between treatment groups (data not shown).

Scal Quality - Elasticity
At 3 months postoperative, elasticity ratio (scar/normal skin) was highest in group DS-TNP, although no statistical significant difference was seen (Table 5). Twelve months postoperatively, highest elasticity was found in group DS-TNP. After post hoc testing, it was demonstrated that elasticity of group DS-TNP was significantly higher compared to group DS (Table 5).
Clinical effectiveness of dermal substitution in burns by topical negative pressure

Figure 4a and b: Patient example at short and long-term follow-up

Scar outcome at 3 and 12 months postoperative (patient shown in Figure 1).

Table 5: Scar elasticity 3 and 12 months postoperatively

<table>
<thead>
<tr>
<th>Means, adjusted*</th>
<th>DS-TNP (95% CI)</th>
<th>DS (95% CI)</th>
<th>TNP (95% CI)</th>
<th>ST (95% CI)</th>
<th>p-value F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio Uf 3 months PO</td>
<td>0.57 (0.40 - 0.73)</td>
<td>0.46 (0.31 - 0.60)</td>
<td>0.52 (0.37 - 0.66)</td>
<td>0.46 (0.31 - 0.62)</td>
<td>0.632</td>
</tr>
<tr>
<td>Ratio Uf 12 months PO</td>
<td>0.80 (0.62 - 0.98)</td>
<td>0.51 (0.33 - 0.68)</td>
<td>0.66 (0.49 - 0.84)</td>
<td>0.69 (0.53 - 0.86)</td>
<td>0.027**</td>
</tr>
</tbody>
</table>

Statistical analysis was performed using factorial anova with post hoc testing with Bonferroni correction; CI, Confidence Interval; Uf, maximal skin extension (mm); PO, postoperatively; * Adjusted for the confounding variable burn center; ** Post hoc test with Bonferroni correction shows a significant difference (p = 0.012) between group DS-TNP and DS.
Chapter 8

Scar Quality - Color
At 3 and 12 months postoperative, the difference between scar and normal skin erythema was shown to be lower in group TNP compared to the other groups, although not statistically significant (Table 6). The difference between melanin of scar and normal skin was also lowest in group TNP compared to the other treatment groups, which was significant at 12 months postoperative (Table 6).

Table 6: Scar erythema and melanin 3 and 12 months postoperatively

<table>
<thead>
<tr>
<th></th>
<th>Means, adjusted *</th>
<th>DS-TNP (95% CI)</th>
<th>DS (95% CI)</th>
<th>TNP (95% CI)</th>
<th>ST (95% CI)</th>
<th>p-value F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythema**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scar</td>
<td>9.7 (6.2 - 13.3)</td>
<td>9.8 (6.6 - 12.9)</td>
<td>7.4 (4.2 - 10.7)</td>
<td>11.2 (7.8 - 14.5)</td>
<td>0.330</td>
<td></td>
</tr>
<tr>
<td>12 months</td>
<td>7.5 (3.8 - 11.1)</td>
<td>6.2 (2.7 - 9.8)</td>
<td>4.5 (1.2 - 7.9)</td>
<td>5.7 (2.2 - 9.3)</td>
<td>0.540</td>
<td></td>
</tr>
<tr>
<td>Melanin**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>12.5 (8.6 - 16.4)</td>
<td>12.6 (9.1 - 16.0)</td>
<td>8.1 (4.5 - 11.6)</td>
<td>12.2 (8.5 - 15.9)</td>
<td>0.114</td>
<td></td>
</tr>
<tr>
<td>12 months</td>
<td>6.8 (3.0 - 10.5)</td>
<td>5.7 (2.0 - 9.3)</td>
<td>1.5 (-1.9 - 5.0)</td>
<td>6.3 (2.7 - 10.0)</td>
<td>0.048***</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis was performed using factorial anova with post hoc testing with Bonferroni correction; CI, Confidence interval; * Adjusted for the confounding variable burn center; ** Absolute difference between scar and normal skin; *** Significant difference between group TNP and the other treatment groups.

Scar Quality - Surface Roughness
Three months postoperatively, no significant difference in scar surface roughness was seen between the four treatment groups (data not shown). At 12 months postoperative, surface roughness scores were lower in scars treated with the substitute (indicating a smoother surface), however no significant differences were seen (Table 7).

Table 7: Scar surface roughness 12 months postoperatively

<table>
<thead>
<tr>
<th></th>
<th>DS-TNP (95% CI)</th>
<th>DS (95% CI)</th>
<th>TNP (95% CI)</th>
<th>ST (95% CI)</th>
<th>p-value</th>
<th>Normal skin n = 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sa</td>
<td>32 (22 - 41)</td>
<td>30 (23 - 37)</td>
<td>37 (28 - 47)</td>
<td>35 (19 - 51)</td>
<td>0.608</td>
<td>22 (19 - 26)</td>
</tr>
<tr>
<td>Sz</td>
<td>464 (244 - 685)</td>
<td>416 (312 - 519)</td>
<td>470 (367 - 572)</td>
<td>516 (152 - 879)</td>
<td>0.837</td>
<td>271 (230 - 312)</td>
</tr>
<tr>
<td>PC</td>
<td>36 (16 - 56)</td>
<td>29 (8 - 51)</td>
<td>39 (23 - 55)</td>
<td>40 (-9 - 89)</td>
<td>0.865</td>
<td>11 (5 - 17)</td>
</tr>
</tbody>
</table>

Statistical analysis was performed using independent samples Kruskal-Wallis test; CI, Confidence interval; Sa, Arithmetic mean of the surface roughness (μm); Sz, Mean of the five highest peaks and five deepest valleys from the measuring field (mm); PC, Peak Count - number of peaks per unit length.

Scar Quality - Clinician’s and Patient’s Impression
In the separate items of the POSAS at 3 months postoperative, no significant differences were found between the groups, except for color in the patient scale (group DS-TNP, DS, TNP, and ST, respectively 5.4 (2.0); 7.4 (2.5); 5.6 (2.5) and 6.9
Clinical effectiveness of dermal substitution in burns by topical negative pressure

Twelve months postoperatively, thickness evaluated by the clinician was significantly higher in group DS compared to group TNP (group DS-TNP, DS, TNP, and ST respectively, 2.1 (1.3); 2.9 (1.2); 1.8 (0.7); 2.1 (1.0), p = 0.041, ANOVA). Table 8 shows the total scores of both the patient and the clinician.

Table 8: Mean total POSAS score, provided by the patient and the clinician

<table>
<thead>
<tr>
<th></th>
<th>DS-TNP (95% CI)</th>
<th>DS (95% CI)</th>
<th>TNP (95% CI)</th>
<th>ST (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient total score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>4.0 (3.3 - 4.7)</td>
<td>4.9 (4.0 - 5.9)</td>
<td>3.7 (2.9 - 4.4)</td>
<td>4.5 (3.4 - 5.6)</td>
<td>0.147</td>
</tr>
<tr>
<td>12 months</td>
<td>3.2 (2.5 - 3.8)</td>
<td>3.7 (2.8 - 4.6)</td>
<td>2.9 (2.2 - 3.6)</td>
<td>3.4 (2.6 - 4.2)</td>
<td>0.457</td>
</tr>
<tr>
<td><strong>Clinician total score</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 months</td>
<td>3.5 (3.0 - 4.1)</td>
<td>3.7 (3.3 - 4.0)</td>
<td>3.7 (3.2 - 4.2)</td>
<td>3.6 (3.1 - 4.1)</td>
<td>0.964</td>
</tr>
<tr>
<td>12 months</td>
<td>2.7 (2.1 - 3.2)</td>
<td>3.2 (2.7 - 3.6)</td>
<td>2.6 (2.2 - 3.0)</td>
<td>2.8 (2.3 - 3.4)</td>
<td>0.308</td>
</tr>
</tbody>
</table>

Statistical analysis was performed using the one-way ANOVA. The mean total score is calculated by adding the scores of the separate items of the POSAS and dividing this by the number of items; POSAS, Patient and Observer Scar Assessment Scale; CI, Confidence interval.

**Discussion**

For the first time, a multicenter randomized controlled trial was carried out that investigated the long-term effects of dermal substitution combined with TNP in acute burn wounds. Most importantly, it was shown that scars treated with a dermal substitute and TNP were significantly more elastic compared to scars treated with the substitute alone. In addition, we demonstrated the successful application of a dermal substitute in a one-stage grafting procedure: both graft take of the autograft and wound epithelialization were comparable in the four treatment groups. Furthermore, postoperative pain scores were significantly lower in patients treated with the substitute, compared to the standard group and significantly lower postoperative wound contamination was demonstrated in wounds treated with TNP alone, compared to the other groups. Finally, scar pigmentation was significantly more similar to normal skin in scars treated with TNP alone, compared to the other groups at 12 months postoperative.

Previously, we found only a limited beneficial effect of dermal substitution in acute burns. This was thought to be due to the reduced and delayed take of the autograft. We hypothesized that the vascular ingrowth of the autograft on top of a dermal substitute could be improved by applying TNP. As expected, we found that...
graft take in group DS-TNP was higher compared to graft take in group DS, although not statistically significant. Additionally, partial graft loss occurred more frequently in group DS which was also not significantly different. Nevertheless, in this RCT the successful application of dermal substitution in one procedure was characterized by a high take rate and rate of epithelialization. Previously, several reports demonstrated that TNP is a good method for securing the skin graft and improving the graft take\textsuperscript{19, 20, 35-38}, which was indeed reflected by a high graft take in group DS-TNP and TNP in our study. The application of TNP in combination with a dermal substitute and a SSG has only been described in two case reports and a patient series\textsuperscript{39-41}. There are reports of the successful application of negative pressure therapy in combination with Integra, preceding the application of an autograft, however data on the long-term effects of this therapy, such as scar outcome, were not described\textsuperscript{18, 42-46}.

In this study, scars were expected to have a relatively good quality, as wounds were treated with SSG’s with a small expansion (mesh 1:1.5). This small graft expansion was chosen for ethical reasons, as in some wound areas, the use of large expansions would not be desirable. Twelve months postoperatively, our expectation was confirmed by the mean total POSAS scores, which were < 4 for all treatment groups (Table 8). The use of a small graft expansion could also explain why no significant difference was found in scar surface roughness, although treatment with the substitute showed a tendency towards a smoother surface. Earlier we showed that especially in wounds treated with a dermal substitute and a largely expanded skin graft, a smoother scar was obtained\textsuperscript{10}. We expect more improvement in scar quality of wounds treated with a dermal substitute and a large graft expansion, as the hypertrophy usually occurs in the interstices of the meshed autograft.

The overall good scar quality could have hampered demonstrating a clinical variation between the different treatments. Nevertheless, it was shown that scars treated with dermal substitution and TNP were more pliable compared to scars of the other treatment groups. Previous studies on dermal substitution used the parameter scar elasticity as outcome measure, indicating that this is an important parameter for scar quality. For this reason, we chose scar elasticity as primary outcome measure. An improved scar elasticity in burns treated with Matriderm has been reported elsewhere in the literature\textsuperscript{17, 47}. Unfortunately, in these reported studies, scars were evaluated in a subjective manner and RCT’s are lacking. Therefore, our present study is the first RCT in which an improved elasticity, measured objectively, was demonstrated in a direct comparison of four groups.

In our previously published paper, we did demonstrate effectiveness of dermal substitution in reconstructive wounds, but not in acute burn wounds (without TNP.
Clinical effectiveness of dermal substitution in burns by topical negative pressure

The presence of potentially toxic products from bacteria and lysosomal enzymes from the dead autologous cells in burn wounds, may degrade the collagen present in the substitute. Therefore, dermal substitutes in burns might degrade faster, which will reduce their effectiveness. The differences in effectiveness of the dermal substitute on skin elasticity between group DS-TNP and group DS could be explained by the removal of these toxic products through the action of TNP, causing a slower degradation of the substitute. Another working mechanism could be an improved vascularization of the substitute and autograft in wounds treated with the substitute and TNP therapy. Overall, these results demonstrate that dermal substitution in acute burns can improve functional scar outcome, however TNP is needed to optimize the effect. We believe, the combination of these therapies is especially important for burned areas in which an optimal scar quality is required for adequate function, such as the hands, elbows and knees. The use of these techniques may provide the maximum gain in scar quality after burns in these areas.

In this study, TNP was applied according to the designed study protocol. First, negative pressure was set at 125 mmHg, which was reported to produce the most significant increase in blood supply in the wound. However, more recently, it has been described that the same effect can be reached with lower pressures. The results of the present study, therefore, might also have been obtained using lower negative pressures. Secondly, we applied the white polyvinyl alcohol (PVA) foam on the majority of the wounds, as growth of granulation tissue into the foam surface and patients pain scores seemed to be lower, compared to the PU foam. In the present study, patients treated with TNP did not have higher postoperative pain scores compared to the standard group, i.e. split-skin graft alone. It is noteworthy that patients treated with the substitute alone had significantly less postoperative pain compared to patients in the other groups. To our knowledge, this effect of dermal substitution has not been reported before.

There are some limitations to this study. First, due to the multicenter setting of this trial, site specific adjustment for the measurement tools used in each burn center was proved necessary in the analysis. Secondly, the clinicians and patients in this study could not be blinded. Nevertheless, we believe effectiveness of the different treatments was investigated reliably due to the objective measurement tools.

Concluding, the effectiveness of dermal substitution in reconstructive wounds was previously reported. In this study, we demonstrated the effectiveness of dermal substitution in acute burn wounds, based on an extensive array of reliable and valid wound and scar measurements. In a randomized prospective design, we showed the successful application of dermal substitution in a one-stage grafting model with a
good take of the substitute and autograft and the beneficial effect of the dermal substitute in combination with TNP on long-term scar elasticity.

Acknowledgements

We would like to acknowledge W. Tuinebreijer, MD PhD for his statistical advice and G. Beerthuizen, MD PhD, H. Boxma, MD PhD, J. Dokter, MD, S. Scholten, MD, F. Tempelman, MD, and A. Vloemans, MD for their dedication to this study. In addition, we thank H. Eshuis, J. Hiddingh, and H. Hofland for collecting part of the data. This research was supported by a grant from the Dutch Burns Foundation (grant number 07.109).
References


Clinical effectiveness of dermal substitution in burns by topical negative pressure


