Acta Derm Venereol 2013; 93: 281–285

INVESTIGATIVE REPORT

Erythema Persists Longer than One Year in Split-thickness Skin Graft Donor Sites

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The recovery of skin function and appearance after harvest of split-thickness skin autografts is incompletely described. We followed the kinetics of skin restoration after a partial-thickness skin excision relative to adjacent normal skin over 12 months. Standardized donor site wounds were made on the thigh using a pneumatic dermatome in 19 consecutive Caucasian patients, median age 70 years, age range 44–86 years, who were undergoing skin graft surgery for leg ulcers. Transepidermal water loss (TEWL), erythema and pigmentation were measured quantitatively using non-invasive devices. The macroscopically healed wound was compared with adjacent normal skin at 1, 3 and 12 months. At 1 month postoperatively, TEWL was 108% ($p = 0.003$), erythema 145% ($p < 0.0005$) and pigmentation 24% ($p < 0.001$) higher in the wounds compared with adjacent uninjured skin. The corresponding values at 3 months were 48% ($p = 0.015$), 89% ($p < 0.0005$) and 15% ($p < 0.0005$). After 12 months, erythema was elevated by 36% ($p < 0.0005$), while TEWL ($p = 0.246$) and pigmentation ($p = 0.211$) had returned to same levels as in the surrounding normal skin. Diabetes mellitus ($p = 0.024$) and smoking ($p = 0.017$) were associated with increased TEWL of normal skin, and erythema decreased with age ($r_s = –0.53$, $p = 0.020$). In conclusion, erythema appears to be the significant component contributing to long-term postoperative donor site appearance. We hypothesize that this is due to increased microvasculature. *Key words: erythema; pigmentation; TEWL; scarring; wound healing.*

Accepted May 22, 2012; Epub ahead of print Sep 14, 2012

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Harvest of split-thickness skin grafts produces a skin defect that extends through the epidermal layer and into the papillary dermis. Healing of this type of injury is conventionally divided into 3 overlapping phases; inflammation, tissue formation and remodelling. The remodelling phase continues for years postoperatively and involves regression of blood vessels through apoptosis (1) in addition to remodelling of collagen molecules (2, 3). This remodelling phase eventually leads to a less visible scar. The appearance of scars is of great concern to patients (4).

Skin barrier function can be conveniently and reliably measured by the transepidermal water loss (TEWL) technique (5). Previous studies indicate that elevated TEWL levels from donor site wounds normalize after 6.5–13 months (6, 7). Scar erythema of small wounds on the inner aspect of the upper arms fades over a period of approximately 7 months when evaluated by the naked eye (8). However, only preliminary information is available on the evolution of pigmentation of scars in humans (9).

This study describes the natural changes in TEWL, erythema and pigmentation in donor site wounds and adjacent uninjured skin over a 12-month postoperative period in humans. The parameters were quantitated using non-invasive bioengineering devices (5, 10).

MATERIALS AND METHODS

Ethics

This clinical trial was approved by the local ethics committee (KF01286728), registered in the Current Controlled Trials (ISRCTN01737461) registry, and followed the guidelines of the Declaration of Helsinki.

Enrolment of participants

Patients 18 years or older, scheduled for elective skin grafting of chronic leg ulcers were included. Exclusion criteria were: non-Danish speaking, dementia, pregnant or lactating, ingesting more than 10 mg corticosteroid per day, thigh used previously for harvest of skin graft, or unsuitable for general/spinal anaesthesia (11). Patients were informed verbally and in writing of the study objective and their rights. They were enrolled once written informed consent was obtained.

The trial was conducted at Copenhagen Wound Healing Center at Bispebjerg Hospital in Copenhagen, which is a multidisciplinary expert national referral wound healing centre (12).

Surgical procedure and wound treatment

Two donor site wounds were made on the dorsal surface of the left or right thigh by a senior surgeon (BJ) using a pneumatic dermatome (Zimmer; Warsaw, IN, USA) set to a thickness of 0.03 cm (11). After haemostasis was achieved with compression alone, one donor wound was randomized to control treatment, and the other donor wound to platelet-rich fibrin (Vivostat A/S, Birkerød, Denmark). Control treatment comprised one petrolatum fabric dressing (Adaptic®; Johnson & Johnson, Skipton, UK), which was applied to the wound, and then covered with 4

layers of calcium alginate (Tegagen®; 3M, St Paul, MN, USA) and an outer adhesive high permeable polyurethane film dressing (Stabilon®; Coloplast, Humlebæk, Denmark). This study reports the results from the control-treated wounds. The early wound-healing effects of platelet-rich fibrin are reported elsewhere (11).

Postoperative treatment

On postoperative day 8, dressings were replaced with one layer of calcium alginate, which was applied to the wound and then covered with polyurethane film dressing. Subsequently, dressings were changed whenever judged necessary due to excessive exudation until the wounds were dry. Thereafter the wounds received no further treatment.

Non-invasive measurements

One, 3 and 12 months postoperatively, patients were acclimatized for 20 min in the test room with minimal air convection. Room temperature and humidity were not recorded. Single measurements were performed at each visit in the centre of the wounds and in adjacent normal skin 2 cm from the wound edge with the patient in a sitting position. TEWL was measured with the DermaLab® device (Cortex Technology, Hadsund, Denmark) to assess the epidermal barrier function (5) expressed in $g \cdot m^{-2} \cdot h^{-1}$. Erythema and pigmentation were measured with the DermaSpectrometer® device (Cortex Technology). This handheld reflectance spectrophotometer emits green (λ=568 nm) and red (λ=655 nm) light onto the skin. A photodetector measures the intensity of reflected light, which depends on the absorption by the skin chromophores haemoglobin and melanin (13, 14). The results are expressed in erythema (EI) and pigmentation (PI) indices.

Statistical analyses

The influence of gender, diabetes mellitus and smoking on TEWL, erythema and pigmentation of normal skin was assessed by a 2-factor analysis of variance (ANOVA) with mixed design for repeated measurements. The same test was applied for the comparison of wound and adjacent skin over time. If this test showed a statistically significant result, differences between 2 time-points or between wound and skin were assessed with the Wilcoxon test. Kruskal-Wallis test for unpaired data was applied for seasonal variation and Bonferroni correction was performed for multiple comparisons. Significance level was set to 5%. Numerical data are presented as median (25–75% percentile range). SPSS (Chicago, IL, USA) version 15.0 statistical software was used.

RESULTS

Nineteen Caucasian patients, 11 women and 8 men, age range 44–86 years (median 70 (IQR 53–88) years) were recruited over a 10-month period (from 1 April 2006 to 31 January 2007). No patients had a history of hypertrophic scarring or keloid formation. Five patients had non-insulin-dependent diabetes mellitus. Five patients were smokers and 8 ex-smokers. Seventeen patients underwent general and two patients spinal anaesthesia.

TEWL of normal skin was significantly higher in patients with diabetes mellitus and smokers (Table I). Age correlated negatively with erythema of adjacent normal skin (r_s=–0.53, p=0.020, n=19). For these analyses the median values of the 1, 3 and 12-month measurements were used.

The initial surface area of the donor wounds ranged from 12 to 151 cm^2 (median 65 (IQR 13–85) cm^2). The wound depth was determined indirectly in a portion of the skin graft that was formalin-fixed, paraffin-embedded and stained with haematoxylin-eosin (11). The median thickness of the graft determined by light microscopy was 0.035 (IQR 0.018–0.043) cm ranging from 0.016 to 0.064 cm.

All wounds were dry and macroscopically epithelialized within one month postoperatively. No wound infections or other complications occurred over the 12-month postoperative period.

Effect of wound area and depth

There were no significant correlations between wound area or depth for TEWL, erythema or pigmentation measurements at 1, 3 and 12 months postoperatively.

Overall temporal effect

In the donor wounds, TEWL (p<0.0005), erythema (p<0.0005) and pigmentation (p=0.001) decreased significantly between the 3 time-points except for TEWL, which did not differ significantly between 1 month and 3 months. In adjacent skin, TEWL, erythema and pigmentation levels remained constant throughout the experimental period, i.e. they did not display significant time-dependent changes (Fig. 1).

One month postoperatively

Wound borders were sharply demarcated against normal skin due to intense wound erythema. The erythema

Table I. *Effect of gender, non-insulin-dependent diabetes mellitus and smoking on transepidermal water loss (TEWL), erythema and pigmentation of adjacent skin*

	Gender			Diabetes mellitus			Smoker		
	Female	Male		No	Yes		No	Yes	
	n=11	n=8		n=14	n=5		n=14	n=5	
Parameter	Median (IQR)	Median (IQR)	p	Median (IQR)	Median (IQR)	p	Median (IQR)	Median (IQR)	p
TEWL[a]	6.7 (4.9–9.2)	8.3 (5.9–14.0)	0.065	6.9 (5.4–9.0)	7.3 (5.9–13.8)	0.024	6.8 (5.6–9.0)	8.3 (5.9–14.3)	0.017
Erythema Index	10.0 (8.0–15.0)	11.5 (7.3–16.0)	0.902	12.0 (9.8–14.3)	8.0 (7.0–10.0)	0.098	11.0 (8.8–12.4)	13.0 (8.0–16.0)	0.339
Pigmentation Index	23.0 (20.0–27.0)	25.0 (19.0–26.8)	0.790	26.0 (23.0–29.0)	22.0 (20.0–26.0)	0.114	25.0 (20.8–27.0)	27.0 (22.0–31.0)	0.169

[a] $g \cdot m^{-2} \cdot h^{-1}$.
IQR: interquartile range.

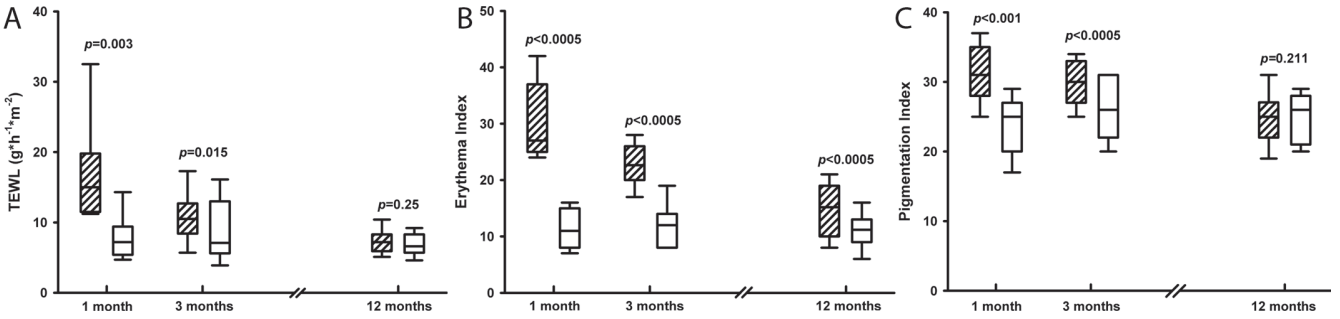


Fig. 1. Transepidermal water loss (TEWL) (A), erythema (B) and pigmentation (C) in donor partial-thickness wounds (*hatched boxes*) and adjacent normal skin (*open boxes*) 1, 3 and 12 months postoperatively. Boxes represent 25th to 75th percentile, whiskers 5th to 95th percentile and horizontal lines within the boxes median values. *p*-values were calculated using Wilcoxon test.

had a follicular appearance with darker nuances (Fig. 2A).

The median TEWL was 108% higher in wounds compared with normal skin (7.2 (IQR 5.4–9.4) $g \cdot m^{-2} \cdot h^{-1}$) (Fig. 1A). The median wound erythema was almost 3 times (145%) higher than normal skin (11.0 (IQR 8.0–15.0) EI) (Fig. 1B). Median pigmentation was increased in wounds by 24% compared with normal skin (25.0 (IQR 20.0–27.0) PI) (Fig. 1C). There was no significant correlation between erythema and pigmentation measurements in the donor wounds ($r_s = 0.388$, $p = 0.101$).

Three months postoperatively

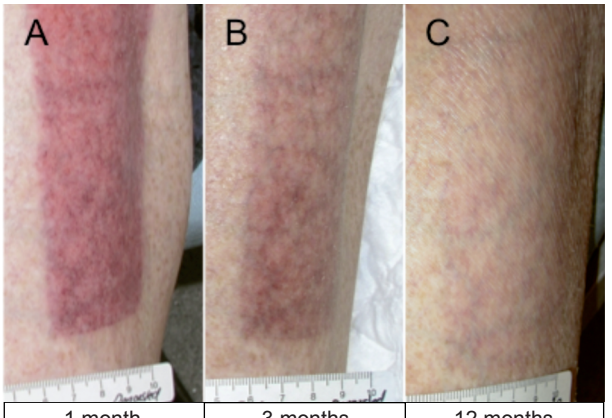
The original wound outline was discernible from normal skin although the borders were indistinct and irregular due to patchy clearings of normally appearing skin. These clearings were distributed regularly in the entire wound area (Fig. 2B).

Median TEWL from the wounds remained higher (48%) than normal skin (7.1 (IQR 5.6–13.0) $g \cdot m^{-2} \cdot h^{-1}$) (Fig. 1A). Median donor wound erythema was elevated by 88% compared with normal skin (12.0 (IQR 8.0–14.0) EI) (Fig. 1B). Median pigmentation of the wounds was 15% higher than adjacent normal skin (26.0 (IQR 22.0–31.0) PI) (Fig. 1C). There was no significant correlation between erythema and pigmentation measurements in the donor wounds ($r_s = 0.020$, $p = 0.935$).

Twelve months postoperatively

Wound areas were clinically discrete but still evident (Fig. 2C).

TEWL from wounds had normalized (7.2 (IQR 5.9–8.3) $g \cdot m^{-2} \cdot h^{-1}$) compared with skin (6.6 (IQR 5.7–8.3) $g \cdot m^{-2} \cdot h^{-1}$) (Fig. 1A). Erythema was elevated by 36% ($p < 0.0005$) at 12 months compared with normal skin (11.2 (IQR 9.0–13.0) EI)) (Fig. 1B). There was no statistically significant difference in pigmentation of the wounds (25.0 (IQR 22.0–27.0) PI) and normal skin (26.0 (IQR 21.0–28.0) PI) at this stage of remodelling (Fig. 1C).



	1 month		3 months		12 months	
	Wound	Skin	Wound	Skin	Wound	Skin
TEWL	38.0	14.3	10.4	3.9	10.5	7.2
Erythema	39.0	17.0	21.0	13.0	18.0	13.0
Pigmentation	35.0	37.0	34.0	31.0	27.0	28.0

Fig. 2. Appearance of one donor wound (A) 1, (B) 3 and (C) 12 months postoperatively. Corresponding measurements for transepidermal water loss (TEWL) ($g \cdot m^{-2} \cdot h^{-1}$), erythema (Erythema Index) and pigmentation (Pigmentation Index) are given below the 3 images.

Effect of season on the TEWL, erythema and pigmentation measurements

Because measurements were performed over the entire year we analysed the influence of season on TEWL, erythema and pigmentation outcome. For these analyses, the measurements carried out during the 4 seasons winter (December to February), spring (March to May), summer (June to August) and fall (September to November) were combined and then compared using the Kruskal-Wallis test. This analysis showed no statistically significant variations due to season.

DISCUSSION

This study describes the process of remodelling of standardized excisional partial-thickness wounds in human skin. Of the 3

non-invasive parameters studied here, erythema was the most striking.

Wound healing can be valued by clinical observation of epithelialization. However, complete epithelialization assessed macroscopically does not equate to functional and physiological recovery of injured skin. TEWL is a reliable predictor of recovery of functional skin barrier residing in stratum corneum after wounding (6, 7, 15–17). TEWL from healing skin remains elevated beyond complete epithelial resurfacing over varying periods depending on the depth of the injury (6). In pure epidermal wounds, TEWL is normalized in approximately 1 month (16, 17). In contrast, Suetake et al. (6) found that normalization of TEWL in human partial-thickness wounds took 6.5–13 months. In accordance with these findings, Kunii et al. (7) observed higher TEWL at 6 months in 0.033–0.042 cm deep donor wounds but found no significant difference compared with normal skin at 12 months. Our data also indicate that normalization of TEWL occurred before or at 12 months postoperatively. We were unable to show a correlation between TEWL and wound depth. We hypothesize that this is due to the fairly uniform and narrow range of perioperative wound depths.

Interestingly, TEWL of normal skin was significantly elevated in diabetic patients and in smokers. In contrast, Seirafi et al. (18) found no difference in TEWL at various sites in non-insulin-dependent diabetic patients compared with healthy volunteers. The discrepancy could be due to regional and ethnic factors (18). Sandby-Møller et al. (19) found an inverse correlation between years of smoking and the thickness of stratum corneum, which could explain the slightly impaired skin barrier function observed here in the smokers.

Scar appearance is particularly governed by colour, notably erythema. The exact time-point at which the erythema of a scar returns to normal is unknown. Bond et al. (8) showed that erythema of full-thickness incisional scars matched that of normal skin in the inner part of the upper arm 7 months postoperatively. In full-thickness excisional scars at the same location the time-horizon was somewhat longer (8). The excisional, partial-thickness wounds in the present investigation were undoubtedly more extensive than those examined by Bond et al. (8). Also, our measurements were objective, while Bond et al. (8) evaluated clinical photographs. Accordingly, in our study erythema remained elevated 12 months after wounding when quantitated with reflectance spectroscopy, which is more sensitive than macroscopic assessment of erythema and measures the blood content of capillaries in upper dermis (13). This is also consistent with the elevated microvessel density found in human surgical scars at 12 months determined invasively by immunohistochemistry (1). We found a negative correlation between age and erythema in normal skin. In corroboration with this finding earlier studies found an age-dependent reduction in the number of dermal capillary loops using video capillaroscopy (20, 21).

Evidence from mouse models suggests that the melanocytes that re-pigment a regenerated epidermis are of follicular rather than epidermal origin (22–24). This supports clinical observations that epidermal repigmentation typically occurs in a regularly spaced, spotted (follicular) pattern in regenerated epidermis (25). The wounds in our study were follicular in appearance, with darker nuances after one month, but after 3 months clearings were distributed regularly in the entire wound area. Furthermore, we observed increased pigmentation during the 1–3-month postoperative period. This may be explained by increased density of melanocytes, which has been found for experimental wounds of this age (26). In contrast, mature scars are normally paler than the surrounding skin at later stages. According to our data hypopigmentation develops later than 12 months. Notably, Velangi & Rees (27) concluded that melanocyte number and melanogenic activity of long-standing pale scars are similar to normal skin. Thus, the reason for hypopigmentation of old scars remains elusive.

It should be emphasized that the results of the present study are limited to the same thickness (0.03 cm) of skin grafts harvested here. In burn surgery the skin grafts are typically 0.02 cm thick. We have shown previously that the thicker the skin graft, the less epithelialized are the donor wounds in the patient cohort examined here (11). In theory, therefore, restoration of skin appearance and function after harvesting thinner skin grafts would display a different kinetic behaviour and occur earlier than found here. On the other hand, the age of the patients had no influence on the rate of epithelialization of this wound type (11). Admittedly, the impact of intrinsic aging on the biomechanical properties of skin and wounds was not studied here.

We cannot exclude that treatment of adjacent donor site wounds in this study with platelet-rich fibrin on day 0 influenced the 3 parameters of the control wounds after 1, 3 and 12 months. However, in an earlier study we failed to demonstrate a systemic effect of platelet-rich fibrin administered subcutaneously, at least on early wound healing (28).

In conclusion, scar remodelling was not completed 1 year after split-thickness skin graft harvest from the thigh, as shown by increased erythema, probably due to cutaneous hypervascularity.

ACKNOWLEDGEMENTS

The authors would like to thank Elsa Justinussen, Hanne Vogensen, Lone Haase, Rebekka Nielsen and Ulla Hemmingsen for their help with this study. The manuscript was proofread by Emily O'Halloran.

Financial disclosure: Patricia Danielsen was funded by a grant from Vivostat A/S, Denmark. Vivostat A/S covered the extra

expenses for the nursing staff. None of the authors have financial interests in Vivostat A/S.

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