Assessment of Hair Density and Sub-epidermal Tissue Thickness in Burn Scars Using High-Definition Ultrasound Imaging.

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Assessment of Hair Density and Sub-epidermal Tissue Thickness in Burn Scars Using High-Definition Ultrasound Imaging

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Burn scars show significant differences in structure, pigment, and hair density/sparsity from unburned skin, yet no formal documentation of these changes can be found in the literature. Evaluation of these differences is essential to assessing future intervention outcomes. The study was a prospective controlled clinical trial. Included were 19 adult burn survivors (18–63 years old, average age 47; 15 male, 4 female, 14 Caucasian, 2 African American, 1 Hispanic; 11 flame burns, 5 scald burns, 2 grease burns and 1 electrical burn, 2%–60% TBSA) with conspicuous, mature scars. All study subjects had either skin-grafted or nongrafted scars, as well as healthy skin in the same body area, to control for intraindividual variability. All scars were at least 9 months old and at a minimum 2 × 2 cm^2 in size. On each individual, at least one nongrafted scar or one grafted scar and healthy skin was imaged with a high-definition ultrasound device (Longport, Inc., Glen Mills, PA, 35MHz probe, 1500 m/s). Vancouver scar scale was assessed. Although scarred skin had significantly fewer follicles than healthy skin in both grafted ($P < .0001$) and un-grafted sites ($P = .0090$), there were even significantly fewer follicles in grafted scars than un-grafted scars ($P = .0095$). In thickness of the sub-epidermal layer, there was no difference between grafted and un-grafted scars ($P = .1900$). Both kinds of scars had a significantly thicker sub-epidermal layer than healthy skin ($P = .0010$). Vancouver scar scale was 7.4 for grafted and 4.6 for nongrafted scars with grafted flame burn scars ranging higher than all others (5–11). There was no discomfort during the imaging, and no adverse events occurred during the study period. Our study demonstrates two clear morphologic differences between scars and healthy skin: thickness of the sub-epidermal layer and hair follicle density. Grafted burn scars were shown to contain fewer hair follicles than un-grafted scars.

Scars are the most obvious and most common late effect of burn injuries. Due to overall improved survival of burn injuries, there is an increasing number of burn survivors, and their scars become a subject of increasing concern on several levels during reintegration in a burn survivor's life. Despite this, several aspects of scar physiology and morphology are virtually unknown and unstudied. A recent study by Engrav et al on the "state of the research" in burn care stated that scar research should be one of the areas prioritized in burn care and confirmed our finding of lack of literature in this field.[1]

As part of a larger project to study various aspects of scar physiology, hair follicle density and thickness of sub-epidermal deposits of collagen in burn scars were examined, using high-definition ultrasound. This now widely available imaging technique makes it possible to visualize hair follicles and other intradermal structures noninvasively in vivo, and with no risk to the study subject, as opposed to biopsies.[2] It is a noninvasive imaging technique which allows visualization of a real-time image through all layers of skin.[3,4]

The objective of our study was to evaluate hair density in burn scars of second (ungrafted) and third-degree (grafted) burns, as well as to evaluate sub-epidermal tissue thickness of various burn scars in adult burn survivors with obvious mature scars (at least 9 mo old; Figure 1). We hypothesized that: 1) burn scars have significantly less hair follicles than healthy skin of the same body area; 2) burn scars have a significantly thicker sub-epidermal layer than healthy skin of the same body area; 3) scars after split-thickness skin grafting (presumably third-degree burn scars) have significantly less hair follicles; and 4) thicker sub-epidermis than un-grafted scars (presumably second-degree burn scars).

The thickness of a scar ultimately determines the scar's flexibility and may influence the degree of sensation after scar maturation, whereas the amount of hair follicles in the scar determines some of the aesthetic appearance and its potential for regeneration in case of secondary injury. Therefore, an exploration of the morphology of burn scars could have a significant impact on reconstructive decisions, as well as disability evaluation. IRB approval was obtained.

METHODS

The study was designed as a prospective controlled clinical trial. Included were adult burn survivors, with an average

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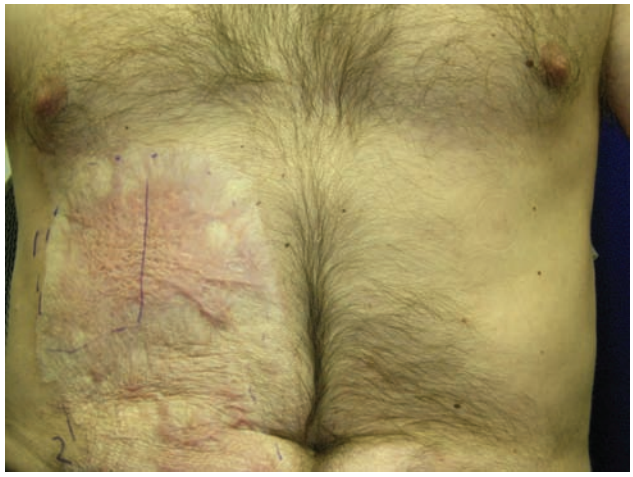

Figure 1. Absence of hair in burn scar (study patient).

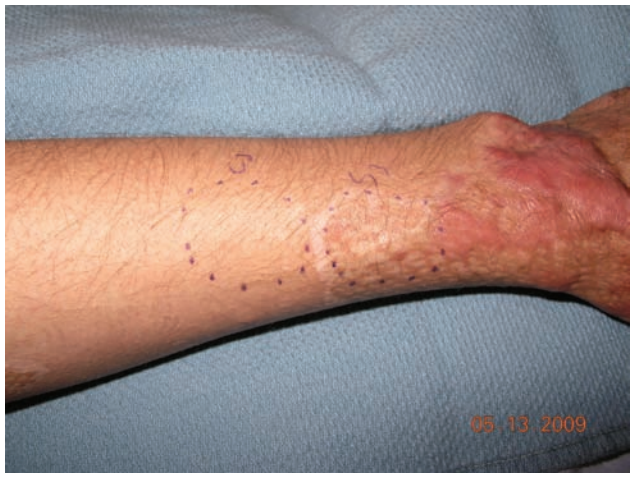

Figure 2. Healthy skin and grafted scar in the same body area (arm).

age of 47, showing conspicuous and mature scars from burn injuries. The prestudy power analysis suggested 15 patients for statistical significance. The scars themselves were required to be at least 9 months old for sufficient maturation[5] and at least 2 × 2 cm^2 in size. This size minimum was chosen because the ultrasound head measures 1.5 × 0.8 cm. Most scars were much larger. The same probe was used for all measurements and three measurements were taken per scar site. In addition, we required that study subjects had skin-grafted and/or non-skingrafted scars, as well as healthy skin of the same skin type (body-area, so for example dorsal forearm left with scars and dorsal forearm right without a scar) to control for intraindividual variability of hair density and dermal thickness (Figures 2 and 3). Excluded were burn survivors younger than 18 years old or those with newer or smaller scars than defined in the protocol.

Once selected, study subjects were asked to read and sign an informed consent document. On each individual, at least 1 scar, and healthy skin in the same body area, was imaged with the high-definition ultrasound device (Longport, Inc., Glen Mills, PA, 35MHz probe, 1500 m/s).[3] Three images per study site were taken (Figures 4–6). We were able to image skin adnexae, measure scar thickness in millimeters,

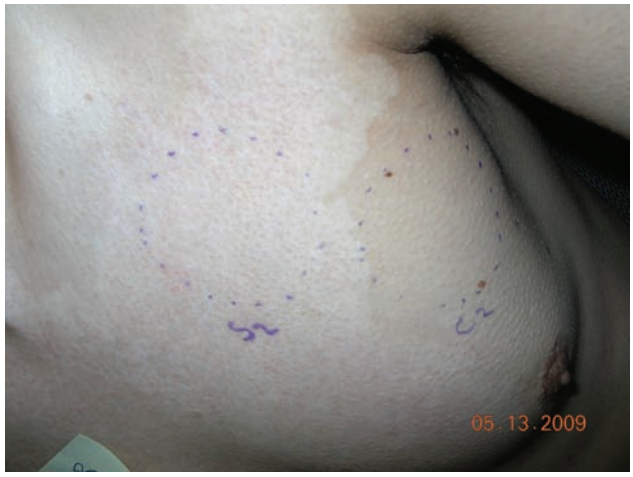

Figure 3. Healthy skin and un-grafted scar in the same body area (breast).

and distinguish hair follicles and glands. Each of these images were assessed for number of follicles and thickness of the subepidermal layer by the PI after de-identification and entered into statistical evaluation.

Statistical Methods

A mixed-models ANOVA with repeated measures was conducted, with repeated measures on the within-subject effect of skin type (scar vs healthy skin), a between-subject effect of graft (grafted vs not grafted), and on the interaction between them. The analytic model used restricted maximum likelihood estimation, an unstructured variance-covariance matrix, and modeled random intercept by subjects. To correctly model the nonindependence among scars, scars were nested within patients (1 patient had 3 scars). Outcomes were transformed with the natural log transformation to obtain normal distributions for each cell for analyses.

RESULTS

Nineteen burn survivors were included in the study, 15 male and 4 female. The mean age was 47 years (18–63 yr old, average age 47; 15 male, 4 female, 14 Caucasian, 2 African American, 1 Hispanic; 11 flame burns, 5 scald burns, 2 grease burns, and 1 electrical burn, 2%–60% TBSA), with an average time after wound healing of 26 months. Vancouver scar scale was 7.4 for grafted and 4.6 for nongrafted scars with grafted flame burn scars ranging higher than all others (5–11 vs 2–7). In assessing differences in number of hair follicles, the interaction between graft (grafted/nongrafted) and skin type (healthy skin vs scar of any kind) was significant ($P = .0090$). As shown in Table 1, the difference in number of hair follicles between healthy and scarred skin was significantly greater in grafted skin than in un-grafted skin. The results of post hoc tests indicated that, while scarred skin had significantly fewer follicles than healthy skin in both grafted ($P < .0001$) and un-grafted sites ($P = .009$), there were even fewer follicles in grafted scars than un-grafted scars ($P = .0095$; Tables 2 and 3).

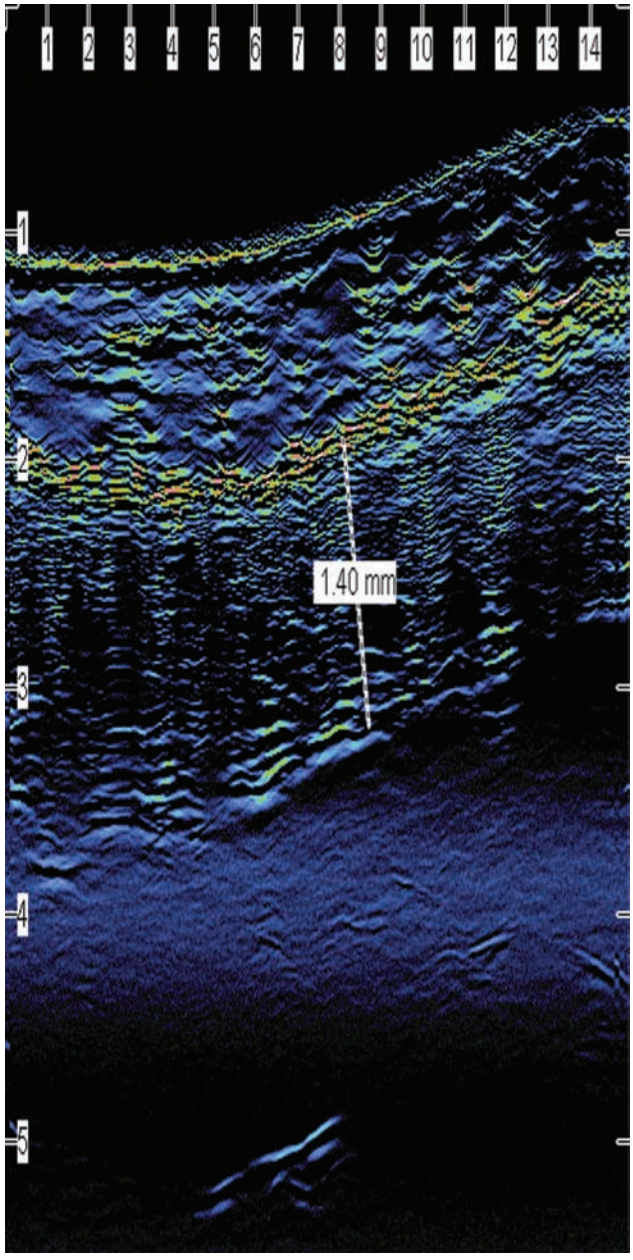

Figure 4. Ultrasound image of healthy skin.

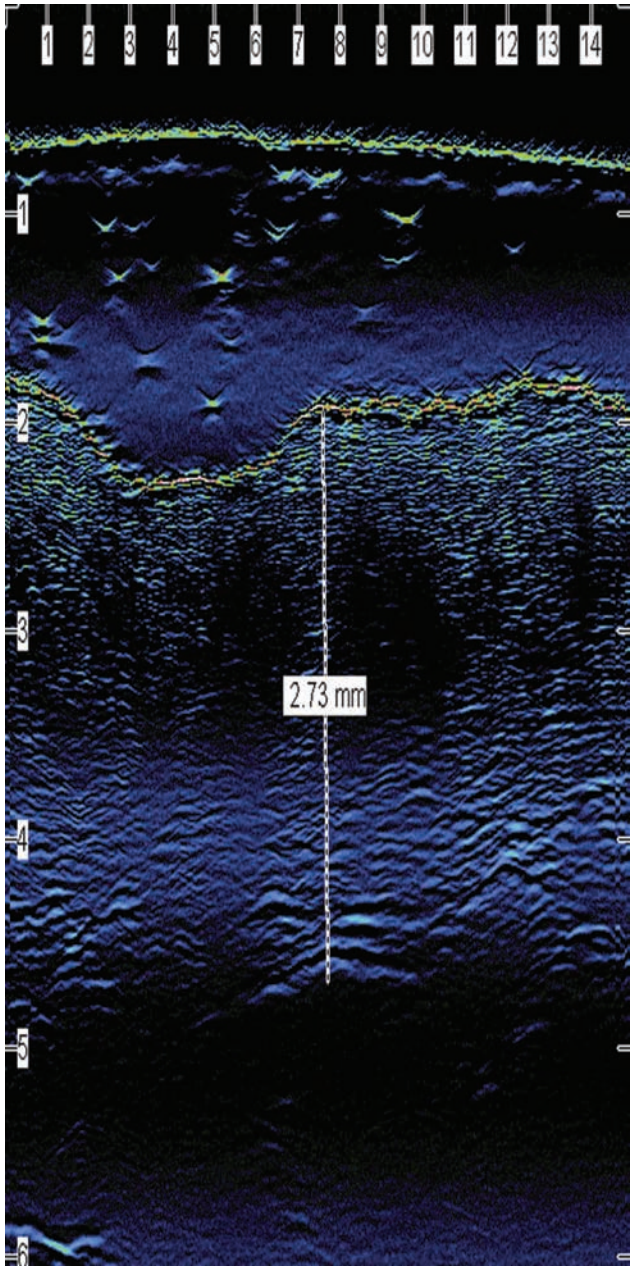

Figure 5. Ultrasound image of grafted scar.

In assessing thickness of the sub-epidermal layer, there was no difference between grafted and un-grafted scars ($P = .1900$). Instead, the main effect of "skin type" was significant ($P = .0010$), suggesting that scars, whether grafted or un-grafted, had a significantly thicker sub-epidermal layer than healthy skin (2.2 vs 1.71 mm; $P = .0010$). These findings supported three of our four hypotheses: 1) burn scars have significantly less hair follicles than healthy skin of the same body area; 2) burn scars have a significantly thicker sub-epidermal layer than healthy skin of the same body area; and 3) scars after split-thickness skin grafting have significantly less hair follicles than un-grafted scars. There was no statistically significant difference between the subepidermal thickness of grafted vs un-grafted scars on average.

There was no discomfort during the imaging and no adverse events occurred during the study period.

DISCUSSION

The objective evaluation of scars is in its infancy. No single reproducible and objective scar evaluation tool has been established that incorporates the overall physiology of scars. The only widely used assessment tool is the "Vancouver scar scale," a highly user-subjective assessment point system.[6] The Vancouver scale measures only four of the qualities that scars frequently exhibit pliability, pigmentation, height, and vascularity. It leaves out vital elements of physiological differences between healthy skin and scar: sensation, skin adnexae (hair,

sweat glands), durability, ability to regulate temperature, and depth or density of the actual scar tissue. Elasticity of scars has been measured and compared with the Vancouver scar scale in a previous study.[7] "Depth" measurement of the scar tissue may be far thicker than "height" (as defined in the Vancouver scar scale), which measures only the elevation of tissue over the surrounding skin plane. In order to document the difference in hair growth and thickness of sub-epidermal collagen, we chose the noninvasive high-definition ultrasound (HDU) technique. The frequency of this imaging ultrasound head is 35 MHz, 1500 m/s.[8]

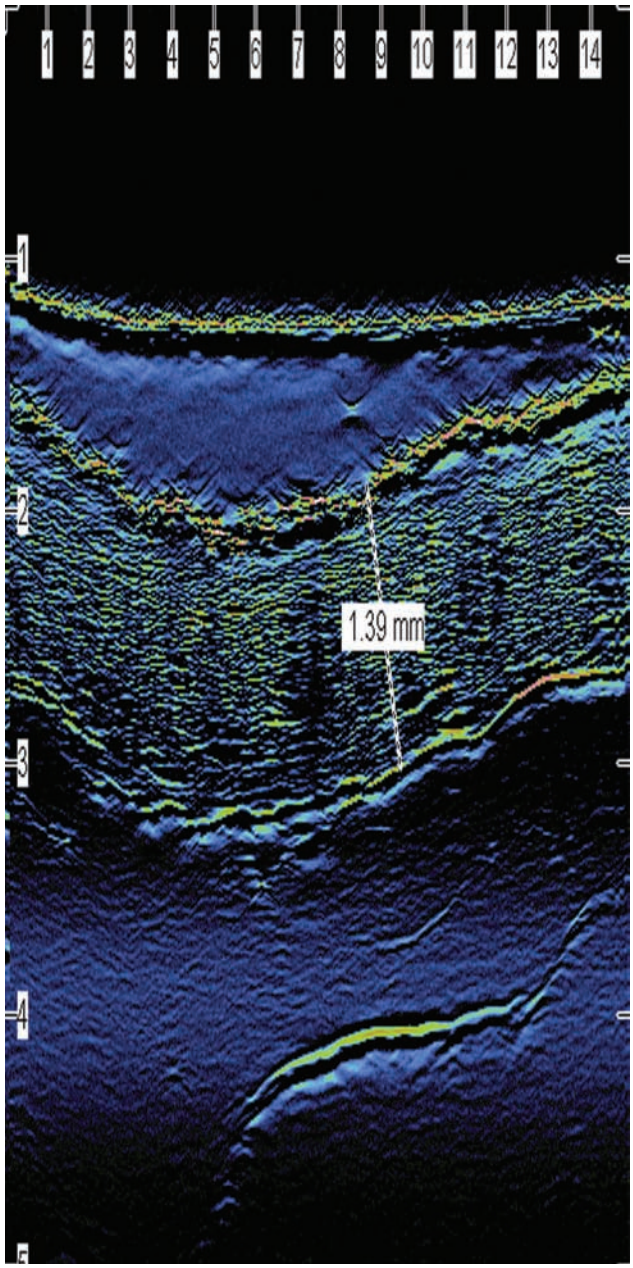

Figure 6. Sub-epidermal tissue thickness in un-grafted scar.

The same technique was employed by Wang et al, who correlated LDI (Laser Doppler imaging) of acute burns with thickness of scars after wound healing.[4] Although a recent study by Choi et al[9] comparing histology to high-definition ultrasound imaging questioned the reliability of the HDU measurements, they did not take into consideration that a formalin-fixed and paraffin-embedded tissue sample probably suffers a significant degree of dehydration and thereby loses thickness. We would argue that the in vivo measurement by HDU, albeit user dependent, is more accurate. Other than the two mentioned studies, histological evaluation of burn scars has never been systematically approached. Random studies of scar interventions mention scar histology,[9] but no specific discussion of hair follicle absence can be found (Figure 7).

We were able to demonstrate significant differences between grafted scars, un-grafted scars, and healthy skin. The healthy skin image was taken in the same body area as that of the scar to control for differences in hair density and dermal thickness in different body areas and different skin types (including gender,age and race) that occur naturally. As expected, the hair follicle density (exemplary for other adnexae) was significantly lower in the imaged scars, whether grafted or un-grafted, than in the surrounding healthy skin. (Table 1). The fact that hair follicle density was even lower in the grafted scar group suggests that the initial injury was deeper in the grafted scars (prompting the initial team to graft in the first place, i.e., third-degree wounds) and also that no hair follicles were transplanted within the graft skin in this population. (There is possible variability in assessing hair density in grafted burn wounds because hair follicles can actually be transplanted within the skin graft.)

Surprisingly, there was no statistical difference of sub-epidermal tissue thickness between grafted and un-grafted scars. We expected the grafted scars to be thicker because of their presumed greater depth of initial injury. Physiologically, the less dermal structure is left in the wound, the less organized the collagen deposition. Deep second-degree wounds therefore may heal with more hypertrophy than grafted third-degree wounds because of the dermal structure introduced to the wound by skin grafting. Wang et al

Table 1. Results: mean, Standard deviation (SD), and number of data sets (N) (least square adjusted) for measurements of hair follicle number and sub-epidermal tissue thickness, total scars evaluated were 21

		Not grafted (N = 10)		Grafted (N = 11)	
		Mean	**SD**	**Mean**	**SD**
Number of hair follicles	Healthy skin	7.17	3.63	7.47	2.22
Number of hair follicles	Scar	3.62	2.46	1.18	1.50
Sub-epidermal tissue thickness (cm)	Healthy skin	1.56	0.46	1.75	0.55
Sub-epidermal tissue thickness (cm)	Scar	1.78	0.64	2.31	1.13

Table 2. Paired sample *T*-Test of raw scores of scars compared with healthy skin of the same body area on the same individual shows significant difference between scars and healthy skin in hair follicle number and sub-epidermal tissue thickness (N = number of data sets, df = degree of freedom, *t* = relative error difference in contrast to the null hypothesis, *P* = statistical significance of a measurement)

		Healthy skin control			Scar			Results		
		Mean	SD	N	Mean	SD	N	*t*	df	*P*
Not grafted	Number of hair follicles	7.50	3,63	10	4.20	2.46	10	3.96	9	.003
Not grafted	Sub-epidermal tissue thickness	1.61	0.46	10	1.87	0.64	10	–2.55	9	.03
grafted	Number of hair follicles	7.73	2.22	11	1.51	1.50	11	6.72	10	.000
grafted	Sub-epidermal tissue thickness	1.81	0.55	11	2.51	1.13	11	–2.58	10	.03

Table 3. *T*-Test for independent groups (raw scores) shows significant difference between grafted vs not grafted scars in hair follicle number, but not in sub-epidermal tissue thickness (N = number of data sets, df = degree of freedom, *t* = relative error difference in contrast to the null hypothesis, *P* = statistical significance of a measurement)

	Not grafted			Grafted			Results		
	N	Mean	SD	N	Mean	SD	*t*	df	*P*
Scar hair follicle number	10	4.20	2.46	11	1.51-	1.50	3.06	19	.006
Scar sub-epidermal tissue thickness	10	1.87	.64	11	2.51	1.13	–1.56	19	.14
Healthy skin hair follicle number	10	7.50	3.63	11	7.73	2.22	–.17	19	.86
Healthy skin sub-epidermal tissue thickness	10	1.61	.46	11	1.81	.55	–.87	19	.40

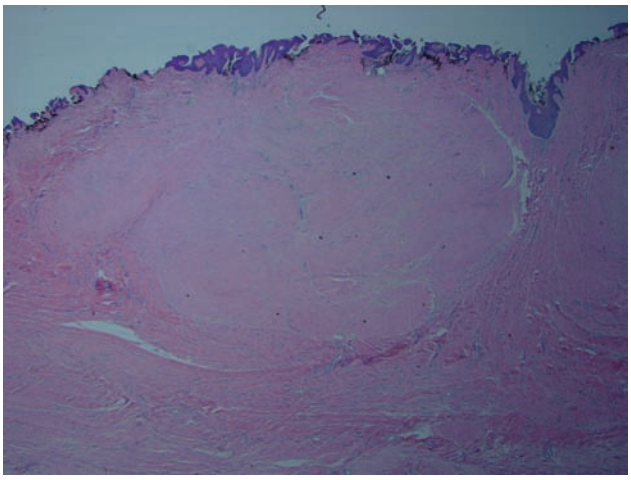

Figure 7. Histology of hypertrophic burn scar.

also found a statistically significant difference between grafted and ungrafted scars within their deep second degree burn group (light blue on LDI), with grafted scars having less sub-epidermal tissue thickness.[4]

As is well known, burn scars, whether grafted or un-grafted, show several obvious differences from normal skin, making them very visible, and often seemingly disfiguring to the burn survivor. Depending on the depth of the original injury, the age of the individual, their genetic predisposition, and several other factors, burn scars can be conspicuous due to a slight structural difference, pigmentation differences, vascularity differences, lack or abundance of hair, or an overshooting collagen deposition leading to hypertrophy and even keloid formation. In addition, the lack of elasticity in these scars can impose significant restrictions on range of motion in joints, and may even cause bone deformities in growing children. Although the medical community is working on improving the scar physiology and appearance, it is imperative that these differences are clearly defined, documented and, ideally, objectively measurable. Although it would be interesting to compare scar formation and characteristics of female and male survivors as well as survivors with diabetes and on steroid medications or with hormonal diseases, that was not the goal of this study. Again, the baseline skin quality in all subjects was used as baseline for the measurements in the same subject, thereby correcting for these differences as much as possible. There were only two NIDDM survivors and none with steroid hormone abnormalities or taking steroids in this series. This study was designed to show morphological qualities of grafted and spontaneously healed burn scars and compare them to healthy skin. Two morphological differences between scars and healthy skin were clearly demonstrated, noninvasively, using high-definition ultrasound imaging: the thickness of the sub-epidermal layer and the hair follicle density. Furthermore, differences between grafted and un-grafted burn scars were also significant, with un-grafted scars having more hair follicles than grafted scars. A significant weakness of this study was that the original burn wound depth was not documented by laser doppler imaging. Further research should focus on objective measurements of other scar morphology and physiology like elasticity, sensory nerve endings (causing pruritus), and pigmentation.

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REFERENCES

1. Engrav LH, Garner WL, Tredget EE. Hypertrophic scar, wound contraction and hyper-hypopigmentation. J Burn Care Res 2007;28: 593–7.
2. Reinholz M, Schwaiger H, Poetschke J, et al. Objective and subjective treatment evaluation of scars using optical coherence tomography, sonography, photography, and standardised questionnaires. Eur J Dermatol 2016;26:599–608.
3. Suliman YA, Kafaja S, Fitzgerald J, et al. Ultrasound characterization of cutaneous ulcers in systemic sclerosis. Clin Rheumatol 2018;37: 1555–61.
4. Wang XQ, Mill J, Kravchuk O, Kimble RM. Ultrasound assessed thickness of burn scars in association with laser Doppler imaging determined depth of burns in paediatric patients. Burns 2010;36:1254–62.
5. Schwanholt CA, Ridgway CL, Greenhalgh DG, et al. A prospective study of burn scar maturation in pediatrics: does age matter? J Burn Care Rehabil 1994;15:416–20.
6. Baryza MJ, Baryza GA. The Vancouver scar scale: an administration tool and its interrater reliability. J Burn Care Rehabil 1995;16:535–8.
7. Rennekampff HO, Rabbels J, Reinhard V, Becker ST, Schaller HE. Comparing the Vancouver Scar Scale with the cutometer in the assessment of donor site wounds treated with various dressings in a randomized trial. J Burn Care Res 2006;27:345–51.
8. Fong SS, Hung LK, Cheng JC. The cutometer and ultrasonography in the assessment of postburn hypertrophic scar–a preliminary study. Burns 1997;23 (Suppl 1):S12–8.
9. Choi YH, Kim KM, Kim HO, Jang YC, Kwak IS. Clinical and histological correlation in post-burn hypertrophic scar for pain and itching sensation. Ann Dermatol 2013;25:428–33.