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NON-INVASIVE ASSESSMENT OF WOUND-HEALING PATHOPHYSIOLOGY BY TRANSCUTANEOUS INDICATORS

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SUMMARY. The non-invasive assessment of post-burn wound recovery allows new insights into wound-healing pathophysiology. This pilot study enrolled thermal burn patients (n = 9) with autografted wounds. Grafted lesion (GL) and donor lesion (DL) areas were followed for 6 months by non-invasive measurement of local microcirculation, transcutaneous PO_2, and transepidermal water loss (TEWL); the contralateral intact areas were used as controls. The results show that local flow changes in GL were significantly different ($p < 0.05$) from normal until week 6, while in DL differences still persisted at week 8. No differences between GL and DL were found for transcutaneous PO_2 measurements. However, full functional recovery was achieved earlier in DL, while in GL statistically significant differences ($p < 0.05$) between the lesion and the control area were still present at week 26. TEWL evolution demonstrated that significant differences ($p < 0.05$) between lesions, compared with the respective controls, persisted in week 26, probably resulting from different recovery mechanisms. Globally, the present study helps to define the wound-healing functional profile of the lesions, highlighting the interest of the non-invasive assessment of wound pathophysiology in burn care and rehabilitation.

Introduction

Research on burn wound pathophysiology has evolved fast in the last decade, especially as regards the early post-burn period.[1,2] Knowledge of the biochemical response,[3] fluid resuscitation, and infection control[4,5] has definitively helped to increase the accuracy of the clinical intervention, reducing recognized complications and decreasing mortality rates.

Scarce information has been produced regarding the burn-healing pathophysiological mechanisms involved in the different stages of this long-term process, and this is particularly relevant to the prevention of healing abnormalities such as excessive scarring.[1,6]

The application of non-invasive diagnostic methods to this field, such as laser Doppler flowmetry (LDF), high-resolution sonography, and tissue oxymetry, has brought new possibilities. LDF[7-9] and, more recently, laser Doppler imaging[10,11] have helped to evaluate burn depth and have been regarded as powerful predictive tools for wound outcome.[11] High-resolution sonography has helped not only to assess the efficacy of therapeutic interventions during the scarring process[12-14] but also to approach the clinical severity of the wounds.[15,16] *In vivo* tissue monitoring was made possible by the local measurement of O_2 and CO_2, sometimes combined with local flow measurements.[17-19] The continuous search for more practical and precise assessment tools has motivated many other research strategies[20,21] but wound-healing quantitative description still faces many difficulties in the selection, definition, and assessment of the most useful descriptors for comparative purposes and documentation. This information is however critical, not only as a decision factor regarding excision and grafting but, simultaneously, in the post-trauma period, for a better understanding of the mechanisms contributing to a fast and effective healing process.

The present study helps to follow the time course of the wound-healing process, through several transcutaneous indicators obtained non-invasively, from the post-graft period until full recovery of such patients. By helping to identify the evolution of the wound's functional profile during the scarring process, both in the burn graft lesion and in the donor lesion areas, the study also aims to contribute to a better understanding of wound maintenance and rehabilitation.

Materials and methods

Experimental data were obtained from nine Caucasian patients (8 male, 1 female), aged between 19 and 68 yr (mean, 44.0 ± 14.8), all presenting full-thickness wounds as a consequence of a thermal burn trauma (*Table I*), with a specific indication for autograft surgery. The patients were selected in the hospital burns unit, according to previously defined inclusion criteria and after obtaining informed written consent. Depending on the clinical stabilization of each patient, the first experimental assessments occurred 2 to 4 weeks after the graft procedure.

Measurements were made in an environment with controlled temperature and humidity, in accordance with the usual recommendations involved in this type of technology.[22-24] Each patient was allowed to rest for at least 20 minutes in order to ensure full skin adaptation to room conditions (temperature, 21 ± 1 °C; humidity, 40-60%), remaining in the same postural position (seated) during the experiments, thus avoiding any haemodynamic modification influencing measurements.[23]

Anatomical areas were marked and mapped to ensure a consistent evolution analysis of results. Grafted lesions (GL) and donor lesions (DL) and the respective contralateral intact zones (cGL and cDL, respectively) were quantitatively described at weeks (W) 2, 4, 6, 8, 12, and 26, thus resulting in a regular follow-up in each patient. Results were always compared with the normal (contralateral) control areas in each individual. A normalization procedure was followed to better visualize the evolution of variables, also reducing inter- and intravariability, a well-known complication of *in vivo* studies. This was analysed as the ratio obtained after baseline results, meaning that the control reference should be close to one.

The transcutaneous (tc) variables considered adequate for the purposes of the present study were:

* local microcirculation assessed using LDF, expressed in arbitrary blood perfusion units or BPUs) with the Oxford Array system (Oxford Optronics, UK);
* tc PO_2 (in mm Hg) assessed using a TINA TCM3 system (Radiometer, Denmark); the probe consists of a sole combined electrode, which allows simultaneous recordings of tc PO_2 and tc PO_2;
* transepidermal water loss (TEWL, expressed in $g/h/m^2$) assessed using a Tewameter TM210/TM215 system (CK Electronics, Germany).

Photographic records were also made at each time of experiment. Descriptive statistics and com-

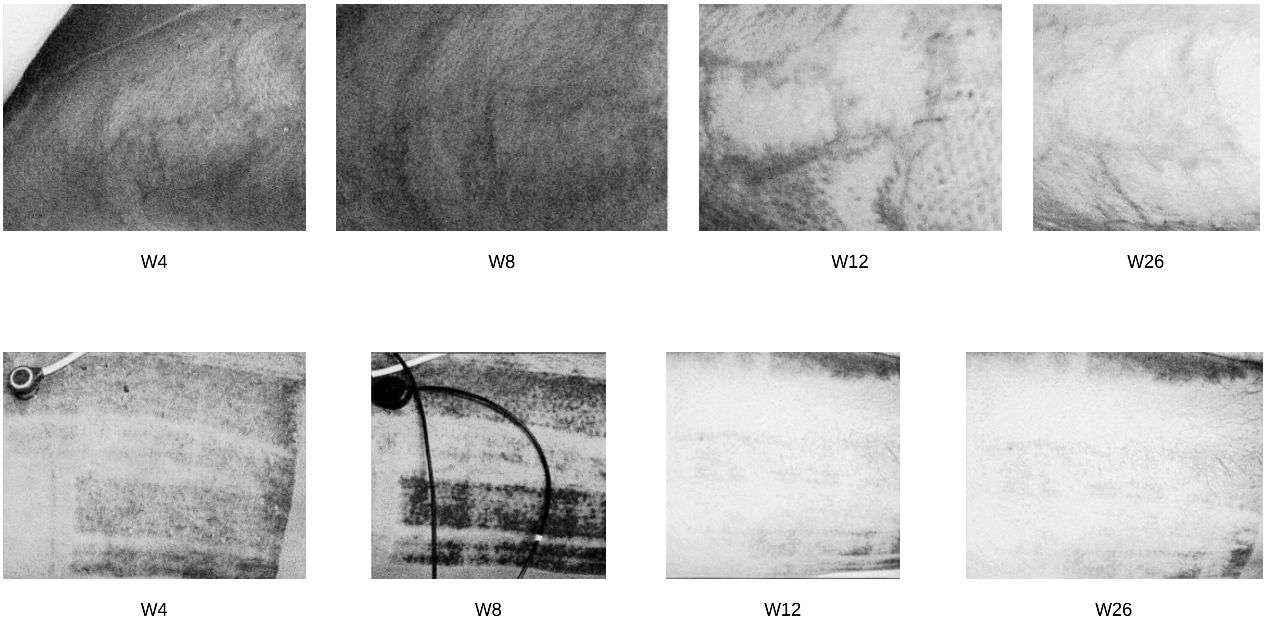
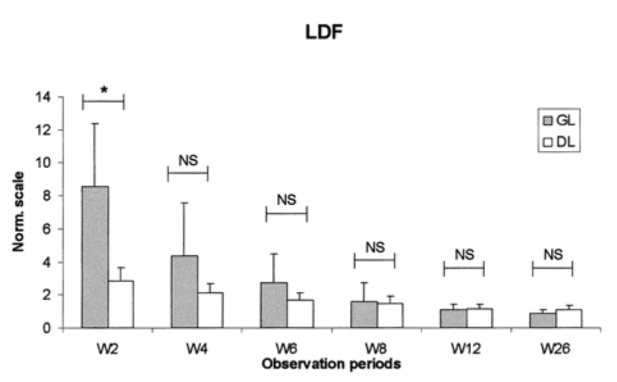

W4 W8 W12 W26
W4 W8 W12 W26

Fig. 1 - Illustrative view (digital photo) of the graft lesion area (GL in the right lateral dorsum) and donor lesion area (DL in the anterointernal face of the left thigh) evolution during the study time course (vol. #9).

	#1	#2	#3	#4	#5	#6	#7	#8	#9
Age (yr)	49	46	44	19	25	53	68	40	52
Male (M); female (F)	M	M	M	F	M	M	M	M	M
Burn trauma	Thermal	Thermal	Thermal	Thermal	Thermal	Thermal	Thermal	Thermal	Thermal
Burn size %	5	25	5	10	15	10	10	15	5
Length of stay in ICU (days)	48	43	45	30	33	45	65	62	36

Table II - Descriptive statistics (mean ± standard deviation) of transcutaneous variables, laser-Doppler flow (LDF), transcutaneous oxygen partial pressure (tcPO$_2$) and transepidermal water loss (TEWL), obtained during the study time course in both lesion areas (GL-graft lesion; DL-donor lesion) and respective intact contralateral equivalents (cGL and cDL). Statistical significance of the differences found between the lesion and the respective control, in each evaluation

	LDF (BPU's)				tc pO$_2$ (mmHg)				TEWL (g/h.m^2)			
	GL	cGL	DL	cDL	GL	cGL	DL	cDL	GL	cGL	DL	cDL
W2 (n=4)	347.0 ±100.8	42.5 ±9.3 ns	142.2 ±50.4	53.0 ±17.3*	40.5 ±7.3	70.3 ±4.2 ns	26.8 ±10.6	54.0 ±6.2 ns	47.2 ±12.3	10.8 ±3.2 ns	28.1 ±4.1	8.0 ±2.1*
W4 (n=9)	191.5 ±105.3	48.2 ±10.9*	89.7 ±28	43.4 ±12.2*	32.6 ±8.1	63.5 ±7.5*	31.0 ±8.3	62.4 ±9.6*	42.8 ±14.8	10.5 ±2.5*	30.4 ±5.8	9.3 ±2.3*
W6 (n=9)	116.9 ±51.6	45.5 ±10.9*	71.4 ±21.3	41.8 ±8.4*	40.8 ±12.1	65.7 ±10.3*	41.4 ±9.9	66.2 ±13.9*	24.4 ±5.5	10.4 ±2.0*	24.5 ±6.2	9.4 ±1.0*
W8 (n=9)	70.1 ±33.2	48.3 ±12.7 ns	56.6 ±18.3	38.9 ±10.9*	44.6 ±14.7	63.1 ±12.3*	46.7 ±6.3	61.8 ±11.5*	17.5 ±3.5	10.8 ±2.1*	22.1 ±3.5	9.9 ±1.8*
W12 (n=9)	53.5 ±22.7	48.1 ±10.6 ns	45.4 ±19.6	39.4 ±8.2 ns	49.4 ±13.2	62.0 ±10.1*	46.9 ±5.3	61.6 ±10.8*	12.1 ±4.2	10.8 ±1.9 ns	19.2 ±2.6	9.6 ±2.2*
W26 (n=9)	41.8 ±13.4	46.9 ±11.7 ns	42.1 ±15.7	38.8 ±6.0 ns	51.7 ±13.8	64.1 ±11.1*	54.0 ±11.4	59.9 ±10.7 ns	8.2 ±1.0	11.0 ±2.7*	15.8 ±2.1	9.4 ±1.4*



(ns: non significant differences found; * : $p < 0.05$)

Fig. 2 - Graphic representation of the evolution of local microcirculation assessed by laser Doppler flowmetry in both graft lesion (GL) and donor lesion (DL) areas. Values were previously normalized (see text) so that "1" corresponds to the baseline reference value. Statistical significance of the diffe-

parative non-parametric analysis (Wilcoxon paired test) were applied. The Spearman non-parametric test was used to study the correlation of variables. A 95% confidence level was adopted ($p < 0.05$).

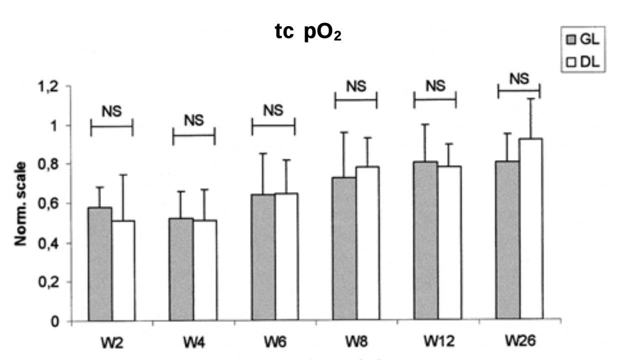
Results

Fig. 1 illustrates a typical evolution of one patient's lesions during the study.

Individual data and global results obtained for the different variables are summarized in *Tables I* and *II* respectively. Only four patients were clinically able to be included from W2, for safety reasons, but these results are nevertheless also presented since they basically illustrate the same functional situation detected in W4, considering their clinical status.

The evolution of the different variables after "normalization" is shown in *Figs. 2-4*. This procedure, relating each absolute value to the respective contralateral control, helps to reduce variability and facilitates readings; 1 is taken as the reference (normal) level of function.

Local flow changes (*Fig. 2* and *Table II*) were especially pronounced in the GL area, although higher readings were consistently present in the first weeks after intervention. For GL, significant differences ($p < 0.05$) between the lesion and the normal contralateral area were detected only at W4 and W6. For the



(ns: non significant differences found; * : $p < 0.05$)

Fig. 3 - Graphic representation of the evolution of local tc pO_2 in both graft lesion (GL) and donor lesion (DL) areas. Values were previously normalized (see text) so that "1" corresponds to the baseline reference value. Statistical significance of the

DL area, significant differences ($p < 0.05$) were present until W8. However, differences between these areas could not be demonstrated statistically.

Regarding transcutaneous gas measurements, *Fig. 3* and *Table II* show that tc PO_2 was consistently reduced in both lesion areas with regard to the reference value, progressively increasing during these experimental periods. Regarding the respective control, significant differences were detected from W4 to W26 for GL, while for DL full functional recovery was present at W26, with no significant differences between the lesion and the respective contralateral control. Comparison between both lesions confirmed the absence of statistically significant differences.

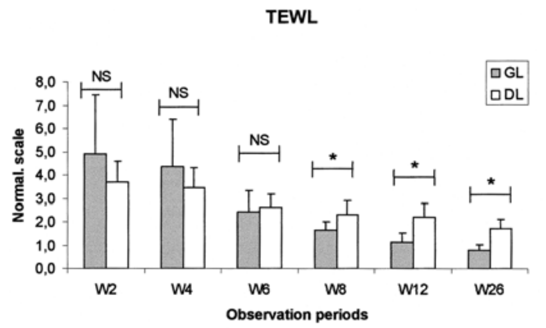
TEWL (*Fig. 3* and *Table II*) show a consistent evolution in both areas, with significant differences detected between lesion areas and the respective controls from W4 on. A particularity was detected for graft comparison at W12, when a shift in the variable tendency was noted. Up to this point, GL values were always higher than cGL, while from W12 on cGL values were significantly higher than GL. This trend was not present in the donor lesion, where DL values were significantly higher than the respective control from W2 to W26. Statistically significant differences between both lesions were seen from W8 on ($p < 0.05$).

Discussion

Wound management is a critical aspect of the full-thickness burn lesion healing process, especially after the shock phase and wound closure.[1,24] Abnormal evolution of this multifactorial process compromises the barrier recovery, and may alter the scarring mechanisms, affecting wound cosmesis and the patient's quality of life.[21,26]

The scarring process and wound outcome are normally assessed by conventional clinical evaluation, although a few attempts have been made to establish correlations with objective parameters obtained by non-invasive biophysical methods.[9-11,14-16,21] Burn healing outcome was frequently considered to be a function of local blood flow, and the potential use of the laser Doppler technique was explored as a powerful predictive tool.[8,9,27] Some limitations are however recognized. It is known that the LDF signal depends on light scattering by the tissue, which is particularly complex at the skin surface owing to the general variability and structural specificity of the cutaneous vascular architecture, from the superficial to the deeper structures. LDF signals always include specific artefacts such as the pulsatile nature of the signal, which is mostly related to the heart-synchronous rhythm determined at the capillaries, and to some degree of laser instability, eventually impairing analysis.[27,28] Nevertheless, LDF has been extensively studied, including its use in the assessment of skin burns, skin flaps, and skin grafts[8,9,29] and a variable degree of LDF correlation with clinical assessment has been found, depending on the depth of wounds and the (high) value of variation obtained in each measurement site. This high variability was also present in our results, being more marked in the first follow-up weeks (*Table II*). The LDF profile (*Fig. 2*) exhibited a coherent evolution in both lesions that might be compatible with progressive regularization of the local vascular status. However, this process seemed to happen earlier in GL, where the absence of functional differences between the lesion area and the respective control was seen until W6, probably reflecting different revascularization mechanisms in these different lesion approaches. The extraordinary variation of readings during W2 and W4, particularly considering the grafted region, was probably emphasized by the intense oedema affecting these areas, also explaining the absence of statistically significant differences between both lesions.

As we knowing that blood flow alone is not an absolute indicator of the healing process,[8,27] the evolution of other potentially related transcutaneous variables was also analysed. Transcutaneous PO_2 measurements is accepted as representing local arterial blood flow and oxygenation.[24,30] However, few studies explore this parameter, probably reflecting the known theoretical and experimental complexities affecting the method.[17,19,30] Gas diffusion through the skin critically depends on the cardiorespiratory function and requires previous pre-heating of the skin (43-45 °C), since at 37 °C recorded values are near zero.[24] The PO_2 in the capillary bed is higher than in the arterial blood owing to the temperature effect that contributes to increase the local flow and oxygen dissociation from haemoglobin (Hb), displacing the Hb-dissociation curve to the right.[24,30] Stratum corneum thickness and cohesion affect tc PO_2, offering a considerable resistance to oxygen diffusion; epidermal consumption should also be considered since it contributes to decrease the oxygen partial pressure detected at the skin surface.[19,24] As shown (*Table I* and *Fig. 3*), tc PO_2 readings were consistently reduced in both lesion areas, compared with the respective normal contralateral controls. This reduction, which occurred with similar magnitudes until W6, may have been primarily related to



(ns: non significant differences found; * : p <

Fig. 4 - Graphic representation of the local evolution of transepidermal water loss (TEWL) in both graft lesion (GL) and donor lesion (DL) areas. Values were previously normalized (see text) so that "1" corresponds to the baseline reference value. Statistical significance of the differences found between

the physical barrier created by the maturation process in both areas. From W8 on, a clear trend to higher values seems to have been established in the donor area, although no significant differences between GL and DL could be found. This may have resulted from differences in the maturation processes occurring, rather than from local revascularization processes. Maturation probably involved higher levels of collagen and extracellular matrix accumulation in the grafted area, providing a thicker and more resistant barrier to local oxygen diffusion.

Finally, measurement of TEWL, a principal indicator of the cutaneous "barrier" function,[21,31,32] clearly suggested that grafting and skin harvesting dramatically changed this basic property of *in vivo* skin (*Table I* and *Fig. 4*). Progressive recovery of this variable was observed from W2 on, the GL values being higher than DL values until W4 (although not statistically different). From W6 on, however, TEWL in GL was progressively reduced, while DL values were more sustained, meaning that DL readings were significantly higher than GL values from that period on. It should be stressed that TEWL registered in both lesions in W26 still showed higher values than expected, when compared with the normal reference (*Fig. 4*). GL values were however nearer to the expected "normality". Although these differences may have been affected by the variability determined by different anatomical sites (the donor site was the anterior face of the thigh in all the volunteers), these may also have been related to the different nature

RÉSUMÉ. L'évaluation non-invasive de la guérison des brûlures permet des nouvelles intuitions sur la pathophysiologie de la guérison des lésions. Dans cette étude pilote, les Auteurs ont inclus neuf patients atteints de lésions thermiques soumis à l'autogreffe des lésions. Les zones de la lésion greffée (LG) et de la lésion donatrice (LD) ont été suivies pendant 6 mois moyennant l'évaluation non-invasive de la microcirculation locale, le PO_2 transcutané et la perte hydrique transepidermique (PHTE); les zones contralatérales intactes ont été employées comme témoins. Les résultats démontrent que les variations de flux local dans la LG étaient significativement différentes (p < 0,05) de la condition normale jusqu'à la 6ème semaine, tandis que dans la LD les différences continuent jusqu'à la 8ème semaine. Les Auteurs n'ont pas observé aucune différence entre la LG et la LD pour ce qui concerne l'évaluation du PO_2 transcutané. Cependant, la guérison fonctionnelle complète a été obtenue plus tôt dans la LD, tandis que dans la LG des différences statistiquement significatives (p < 0,05) entre la lésion et la zone témoin étaient encore présentes à la 26ème semaine. L'évolution da PHTE démontrait que des différences significatives (p < 0.05) entre les lésions, par rapport aux témoins respectifs, persistaient dans la 26ème semaine, probablement à cause de mécanismes différents de guérison. Globalement, cette étude aide à définir le profil fonctionnel de la guérison des lésions, soulignant l'intérêt de l'évaluation non-invasive de la pathophysiologie des lésions dans les soins des brûlures et pendant la réhabilitation.

BIBLIOGRAPHY

1. Rose J.K., Herndon D.N.: Advances in the treatment of burn patients. Burns, 23: 1, S19-S26, 1997.
2. Herndon D.N.: "Total Burn Care" (2nd ed.), W.B. Saunders, London, 2002.
3. Young Y.K., Lalonde C., Demling R.: The role of mediators in the response of thermal injury. World J. Surg., 16: 30-35, 1992.
4. Baxter C.R.: Fluid, volume and electrolyte changes in the early post-burn period. Surg. Clin. North Am., 1: 693-6, 1974.
5. Tokyay R., Zeigler S.T., Traber D.L. et al.: Post-burn gastrointestinal vasoconstriction increases bacterial and endotoxin translocation. J. Appl. Physiol, 74: 1521-9, 1993.
6. Herndon D.N., Rutan R.L.: Comparison of cultured epidermal autograft and massive excision with serial autografting plus homograft overlay. J. Burn Care Rehabil., 13: 154-62, 1992.
7. Michaels J., Alsbjorn B., Sorensen B.: Clinical use of laser Doppler flowmetry in a burn unit. Scand. J. Plast. Reconstr. Surg., 18: 65-73, 1984.

8. Yeong E.K., Mann R., Goldberg M., Engrav L., Heimbach D.: Improved accuracy of burn wound assessment using laser Doppler. J. Trauma, 40: 956-2, 1996.
9. Atiles L., Mileski W., Purdue G., Hunt J., Baxter C.: Laser Doppler flowmetry in burn wounds. J. Burn Care Rehabil., 16: 388-93, 1995.
10. Pape A.S., Skouras A.C., Byrne O.P.: An audit of the use of laser Doppler imaging (LDI) in the assessment of burns of intermediate depth. Burns, 27: 233-9, 2001.
11. Jeng J.C., Bridgeman A., Shivnan L., Thornton P.M., Alam H., Clarke T.J., Jablonski K., Jordan M.H.: Laser Doppler imaging determines need for excision and grafting in advance of clinical judgment: A prospective blinded trial. Burns, 29: 665-70, 2003.
12. Serup J., Keinding J., Fullerton A., Gniadecka M., Gniadecki R.: High-frequency ultrasound examination of skin. Introduction and Guide. In “Handbook of Non-Invasive Methods and the Skin”, Serup J., Jemec G. (eds), CRC Press Inc., Boca Raton, 1995.
13. Kaulas A.M., Aindow J., Caulfield M.R.: Application of ultrasound in assessing burn depth. Lancet, 1: 188, 1979.
14. Wohlrab J., Wohlrab R., Fisher M., Marsch W.C.: Ultrasonographic characterization of burn scars in children. Unfallchirurg., 103: 754-60, 2000.
15. Iraniha S., Cinat M.E., Vanderkam V.M.: Determination of burn depth with non-contact ultrasonography. J. Burn Care Rehabil., 21: 333-8, 2000.
16. Dyson M., Moodley S., Verjee L., Verling W., Weinman J., Wilson P.: Wound healing assessment using 20 MHz ultrasound and photography. Skin Res. & Technol., 9: 116-21, 2003.
17. Venkatesh B., Meacher R., Muller M.J., Morgan T.J., Fraser J.: Monitoring tissue oxygenation during resuscitation of major burns. J. Trauma, 50: 485-94, 2001.
18. Boyko E.J., Ahroni J.H., Stensel V.L.: Tissue oxygenation and skin blood flow in the diabetic foot: Responses to cutaneous warming. Foot Ankle Int., 22: 711-4, 2001.
19. Rodrigues L.M., Pinto P.C., Leal A.: Transcutaneous flow related variables measured *in vivo*: The effects of gender. BMC Dermatology, 1: 4-15, 2001.
20. Baryza M.J., Baryza G.A.: The Vancouver Scar Scale: An administration tool and its interrater reliability. J. Burn Care Rehabil., 16: 535-8, 1995.
21. Timar-Banu O., Beauregard H., Tousignant J., Lassonde M., Harris P., Viau G.: Development of non-invasive and quantitative methodologies for the assessment of chronic ulcers and scars in humans. Wound Repair Regen., 9: 123-32, 2001.
22. Pinnagoda J., Tupker R.A., Agner T., Serup J.: Guidelines for transepidermal water loss (TEWL) measurement. A report from the standardization group of the Europ. Soc. Contact Dermatitis. Cont. Derm., 22: 164-78, 1990.
23. Bircher A.J., de Boer E.M., Agner T., Wahlberg J.E., Serup J.: Guidelines for measurement of cutaneous blood flow by laser Doppler flowmetry: A report from the Standardization Group of the European Society of Contact Dermatitis. Cont. Derm., 30: 65-72, 1994.
24. Takiwaki H.: Measurement of transcutaneous oxygen tension. In “Handbook of Non-Invasive Methods and the Skin” Serup J., Jemec G. (eds), CRC Press, Boca Raton, 185-95, 1995.
25. Ramakrishnan K.M., Jyaraman V.: Management of partial-thickness burn wounds by amniotic membrane: A cost-effective treatment in developing countries. Burns, 23: 1, S33-S36, 1997.
26. Atiyeh B.S., El-Musa K.A., Dham R.: Scar quality and physiological barrier function restoration after moist and moist-exposed dressings of partial-thickness wounds. Dermatol. Surg., 29: 14-20, 2003.
27. Choi C., Bennett R.: Laser Dopplers to determine cutaneous blood flow. Dermatol. Surg., 29: 272-9, 2003.
28. Berardesca E., Leveque J.L., Masson Ph., and the EEMCO Group: EEMCO guidance for the measurement of skin microcirculation. Skin Pharmacol. Appl. Skin Physiol., 15: 442-56, 2002.
29. Schliephake H., Schmelzeisen R., Nenkam F.W.: Long-term results on blood flow and cutaneous sensibility of flaps used for reconstruction of facial soft tissues. J. Oral Maxillofac. Surg., 52: 1247-52, 1994.
30. Jaszczak P., Poulsen J.: TC PO_2 dependence on a sufficient

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