Young investigator award

Surface electrical capacitance as an index of epidermal barrier properties of composite skin substitutes and skin autografts

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Restoration of the epidermal barrier is a requirement for burn wound closure. A rapid, reliable, and noninvasive measure of the rate of restoration of the epidermal barrier is not readily available. To monitor the reformation of the epidermal barrier, we measured surface electrical capacitance on cultured skin substitutes (human keratinocytes and fibroblasts attached to collagen-glycosaminoglycan substrates) and split-thickness skin autografts grafted to patients. Data were collected from four patients with burns and one pediatric patient with a congenital hairy nevus comprising > 60% total body surface area. Capacitance measurements were performed at days 7, 10, 12, 14, and 28 by direct contact of the capacitance probe for 10 seconds to the cultured skin substitutes or split-thickness autograft. On postoperative days 7, 10, 12, 14, 21, and 28, the surface electrical capacitance of cultured skin substitutes after 10 seconds of sampling was 2468 ± 268, 1443 ± 439, 129 ± 43, 200 ± 44, 88 ± 20, and 74 ± 19 picofarads (mean ± standard error of the mean), respectively. Surface electrical capacitance for split-thickness autograft on the same days was 1699 ± 371, 1914 ± 433, 125 ± 16, 175 ± 63, 110 ± 26, 271 ± 77 picofarads, respectively. Surface electrical capacitance in all of the grafts decreased with time. Cultured skin substitutes had approximately the same 10-second capacitance values as split-thickness autograft during 3 weeks of healing and approached values for uninjured skin (32 ± 5 picofarads) by 12 days. Measurement of surface electrical capacitance is a direct, inexpensive, and convenient index for noninvasive monitoring of epidermal barrier formation. **(WOUND REP REG 1995;3:419-25)**

Human epidermis serves as a protective covering against loss of endogenous fluids, as well as exogenous microbial invasion.[1] The epidermis includes the stratum corneum (SC) which protects the body from desiccation. The SC consists of 15 to 20 tightly stacked layers of corneocytes held together by a complex of intercellular lipids.[2] A fundamental requirement for closure and healing of partial- or full-thickness skin wounds is the restoration of the epidermal barrier.[3] Barrier properties of human skin have been attributed almost entirely to the SC of the epidermis and its intercellular lipid component.[4-6] The intercellular lipid component, while playing a primary role in barrier function, also plays a part in the water-holding capacity of the SC.[5,6] A water concentration gradient exists

CSS	Cultured skin substitute
pF	Picofarads
SC	Stratum corneum
SEC	Surface electrical capacitance
STAG	Split-thickness autografts
TBSA	Total body surface area
TEWL	Transepidermal water loss

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within the SC, in which hydration levels are highest in the most proximal layers and lowest in the most distal layer. The SC is the rate limiting barrier between the water saturated viable tissues and the dry outer environment; and diffusion of water takes place as a purely passive process through the SC.[2,7]

Electrical properties of the skin are related to the water content of the SC.[8] These electrical characteristics have allowed the use of biophysical analyses to evaluate the hydration state of the skin surface by measuring the skin impedance as an external voltage is applied across two surface sites, and determining the electrical current between the positions.[9] The relationship of capacitance to skin surface hydration has been well described in previous reports.[2,10-12]

Simple, noninvasive, and objective methods for the assessment of wound healing and graft viability are constantly being evaluated. Examples of instrumentation that are currently used at the bedside include laser Doppler monitoring, transcutaneous monitoring, and now surface electrical capacitance. The laser Doppler instrument has been used to evaluate scar vascularity[13] but does not appear to be useful for early graft evaluation or epithelial closure. Transcutaneous monitoring evaluates local skin perfusion. It has been shown that transcutaneous partial pressure of carbon dioxide of grafts provides a noninvasive and objective measure of skin graft vascularization and that graft transcutaneous partial pressure of oxygen values reflect graft maturity.[14] Transcutaneous monitoring, however, is dependent on local graft perfusion and does not measure the functional barrier properties of healing epidermis. Previous studies reported from this laboratory have shown that surface electrical capacitance (SEC) measured in vitro and on cultured skin substitutes (CSS) grafted to athymic mice is a reliable, noninvasive, and convenient technique for evaluation of epidermal barrier development.[15]

The purpose of this study was to (1) evaluate the clinical utility of SEC as an objective assessment of epidermal barrier function and (2) compare the development of a functional epidermal barrier in wounds grafted with CSS with those treated with split-thickness autografts (STAG).

MATERIALS AND METHODS

Patients

Five patients hospitalized at the Shriners Burns Institute, Cincinnati Unit, or the University of Cincinnati Burn Special Care Unit were enrolled in this study. Informed consent was obtained, and the study was approved by the University of Cincinnati Institutional Review Board. All but one patient had a greater than 50% total body surface area (TBSA) burn, and they were studied in a paired site comparison of cultured cell collagen–glycosaminoglycan skin substitutes (CSS) and STAG for closure of full-thickness, excised burns. One patient had a congenital hairy nevus comprising greater than 60% TBSA that was excised and replaced with both CSS and STAG.

Preparation of cultured skin substitutes and wound treatment

CSS were prepared as described in a recent study.[16] In brief, skin biopsy samples were collected from each patient, and primary cultures of epidermal keratinocytes and fibroblasts were grown and expanded. Skin substitute grafts were made by inoculating these cells at a high density onto collagen-glycosaminoglycan biopolymer substrates.[17] CSS measured approximately 7 × 7 cm and were applied in the operating room the day after excision of the eschar or lesion. Wound dressings were made and graft care was performed as described in previous studies.[16,18] During the first 10 days of treatment, grafts were kept in topical nutrient ("wet") dressings. Dressings for CSS were soaked with solutions of noncytotoxic antimicrobial agents and nutrients, whereas STAG were irrigated with genital-urinary ("GU") solution (200 Units/ml polymyxin B and 40 μg/ml neomycin) alternating with 5% mafenide acetate every 2 hours.

STAG

Excluding the patient with the congenital nevus, greater than 90% of the total wound coverage was from STAG. In these patients, excision down to viable tissue (usually fat) was performed with grafting on the following day. Donor skin was taken at a thickness of 0.36 mm with a dermatome, meshed at 2:1 or 4:1, and grafted onto the patient. Subsequent graft care was performed as described previously.

Capacitance measurements

As a measure of surface hydration, skin SEC measurements were collected with a NOVA dermal phase meter (DPM 9003; NOVA Technology Corp., Gloucester, Mass.) connected to a portable lap top computer which recorded 10 serial readings at 1-second intervals. The probe used for data collection was a standard 6 mm DPM 9105 with a spring-loaded flat contact surface. The probe surface consists of concentric brass elec-

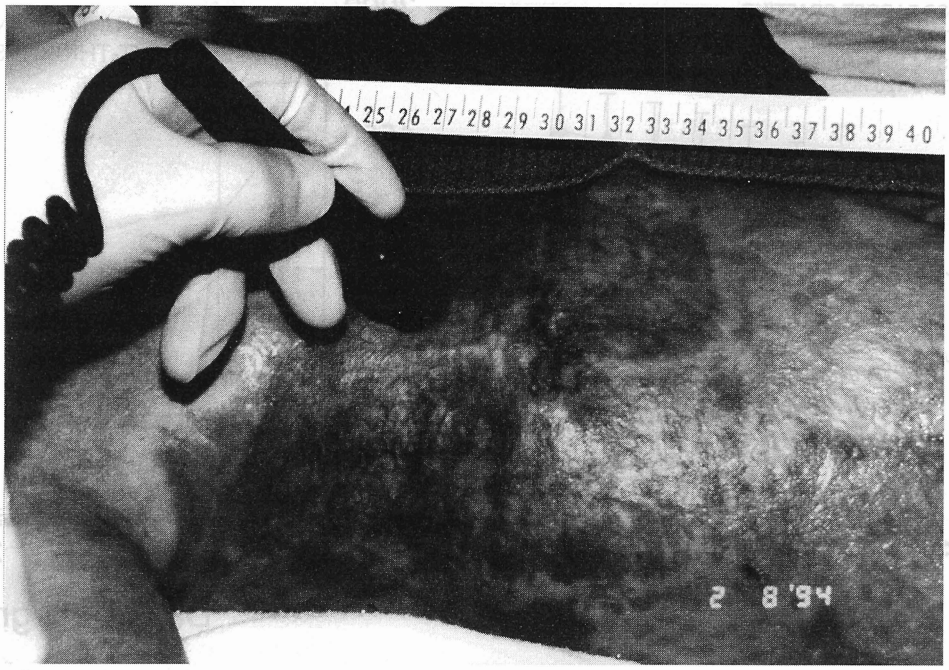

Figure 1 Surface electrical capacitance measurements at the bedside. The sensor probe is in direct contact with the surface of a piece of healing cultured skin substitute.

trodes (outer ring diameter = 5.08 mm, inner ring diameter = 2.54 mm) separated by a nonconducting insulator ring 0.6 mm wide.

SEC measurements were performed at the bedside on days 7, 10, 12, 14, 21, and 28 after grafting with minimal manual pressure (Figure 1). When possible, readings were collected from comparative sites of CSS grafts and autografts. Three to four readings per graft for each timepoint were recorded. Capacitance measurements were obtained only from areas of visible graft and not over the mesh interstices. During the first 10 days of treatment, while grafts were kept in wet dressings, measurements were obtained after irrigating the sites with sterile normal saline solution and allowing 20 minutes of air drying. After a blanching keratinized surface developed (7 to 10 days), wet dressings were discontinued and a nonadherent salve-impregnated dressing was administered daily. Antibiotic salve compositions were determined by the microbial specificity and sensitivity at each site. Capacitance measurements during this stage were performed after the patients' dressing change and bath, and, as previously described, the areas were allowed to air dry for 20 minutes. After a fully dry, keratinized surface developed, grafts were covered with a moisturizing cream and measurements were obtained as described previously for the salve dressing stage.

All SEC data are expressed as mean ± standard error of the mean in picofarads (pF). Capacitance values generated by the dermal phase meter were presented as an aggregate value calculated from the phase delay of an applied alternating current during a controlled scan of frequencies up to 1 MHz. The initial reading (t = 1 second) is defined as instantaneous capacitance. Subsequent readings (t= 10 seconds), taken after occlusion created by direct contact of the probe with the sampling site, are defined as continuous capacitance. Because the greatest degree of change in surface hydration occurs during the first 5 seconds of occlusion, a slope analysis of SEC $[(SEC_5 - SEC_1)/5 - 1]$ was performed. Instrument readings (C) were converted to pF by use of a constant formula:

$$pF = (8.38 \times 10^{-6} \times C^3) - (4.0 \times 10^{-3} \times C^2) + 1.668C - 122.1$$

developed from the calibration curve supplied by the manufacturer.[19] Normal skin values were obtained by measuring SEC on the volar forearm in 10 healthy volunteers (two female, eight male).

Results

Five patients had capacitance measurements collected from their grafts on days 7, 10, 12, 14, 21, and 28 after grafting (Table 1). All patients tolerated the procedures well, and there was no morbidity or subjective discomfort associated with the capacitance measurements. Initially, SEC was high because of greater surface hydration. As the epidermis differentiated to form

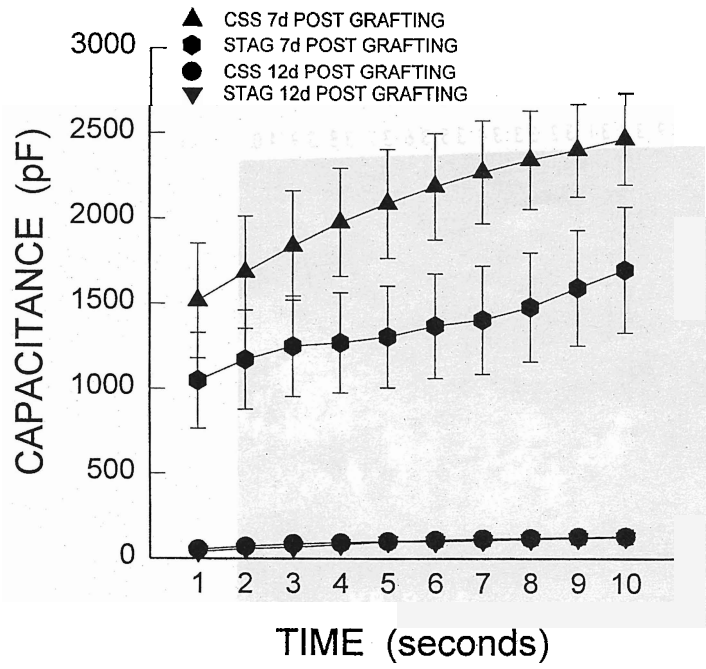

Figure 2 Serial surface electrical capacitance sampling of cultured skin substitutes and split-thickness skin grafts at day 7 and 12 after grafting.

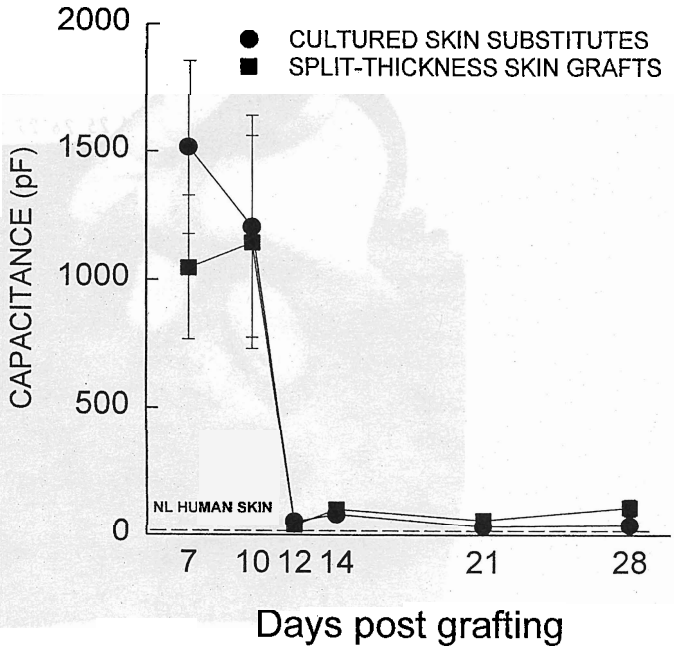

Figure 3 Surface electrical capacitance (t = 1 second, instantaneous capacitance) of cultured skin substitutes and split-thickness skin grafts. *NL*, Normal.

Table 1 Patient information

Patient No.	Age (yr)	Total body surface area (%)	Full-thickness (%)
1	7	65	65
2	3	74	74
3	16	75	75
4	13	66	66
5	50	82.5	74.5
Mean ± standard error of the mean	17.8 ± 7.5	72.5 ± 2.9	69.6 ± 2.6

more SC, SEC decreased as hydration decreased due to redevelopment of the epidermal barrier.

Serial SEC sampling during the 10 seconds of occlusion had a greater absolute increase at day 7 when compared with day 12 in both the CSS and STAG (Figure 2). At day 7, the slope for both graft types was steep (CSS, 143 ± 55 pF/sec; STAG, 64 ± 22 pF/sec), suggesting increased loss of water. By 12 days after grafting the slope for both graft types was essentially flat and equivalent (CSS, 11 ± 4 pF/sec; STAG, 14 ± 3 pF/sec), an indication of improved water barrier function.

The instantaneous capacitance (t = 1 second) measurements of both STAG and CSS approached normal values by day 12 after grafting (Figure 3). On day 7, the CSS mean values were higher when compared with STAG (1517 ± 338 versus 1047 ± 281 pF). At day 10 both grafts had similar values (CSS, 1206 ± 433 pF; STAG, 1145 ± 416 pF), and by day 12 both graft types approached baseline values and remained there throughout the remainder of the study period. Values for the open wound were off the scale, as expected, because there was no barrier to water loss.

Continuous capacitance (t = 10 seconds) measurements had a similar pattern (Figure 4) as seen with the instantaneous capacitance. At day 7, the CSS had higher capacitance values than those of the STAG (2468 ± 268 versus 1699 ± 371 pF). By day 10 the CSS had a lower value (1443 ± 439 pF), whereas the STAG had a slightly increased capacitance reading (1914 ± 433 pF). Again, by day 12 the STAG and CSS had similar capacitance measurements, both approaching baseline values (125 ± 16 versus 129 ± 43 pF).

The change in capacitance values correlated with the changing hydration of the skin surface, and this value reflected the maturity and efficiency of the epidermal barrier. The slope value was an indirect measure of transepidermal water loss.[20] An analysis of the change in capacitance values, measured as the initial slope $[(SEC_5 - SEC_1)/5 - 1]$ during the first 5 seconds of occlusion (Figure 5) had a similar pattern for both the STAG and CSS as seen in the continuous capacitance readings. The capacitance values correlated with the clinical healing and reepithelialization of both graft types. As values decreased, the grafts showed greater vascularization, a more visible epidermis, and a better ability to repel water.

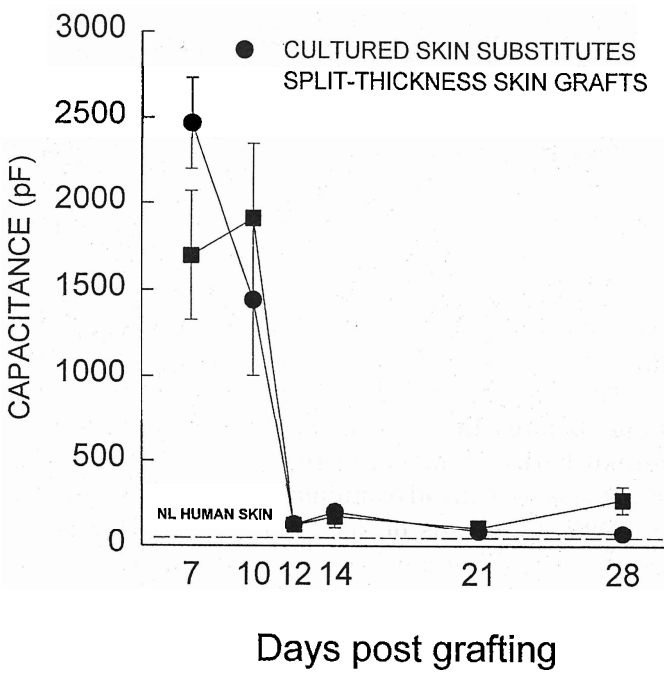

Figure 4 Surface electrical capacitance (t = 10 seconds, continuous capacitance) of cultured skin substitutes and split-thickness skin grafts. *NL*, Normal.

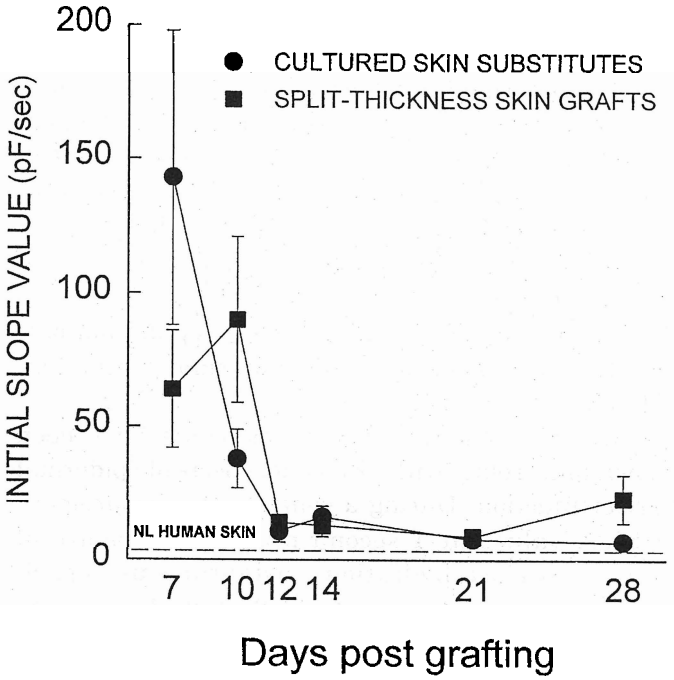

Figure 5 Surface electrical capacitance (initial slope values from t = 1 to t = 5 seconds) of cultured skin substitutes and split-thickness skin grafts. *NL*, Normal.

DISCUSSION

In this study, SEC measurements were used to assess skin hydration as an index for epidermal barrier formation in experimental skin grafts (CSS) and conventional skin grafts (STAG). SEC provided a quantitative assessment of epidermal healing which correlated well with clinical observations. Both graft types approached SEC values of uninjured skin by 12 days after grafting.

In a semiquantitative comparative analysis between CSS, prepared from autologous skin cells and collagen glycosaminoglycan membranes, and autografts, it has been shown that CSS may provide a potential alternative to skin autografting for burns and other major skin defects.[16] During the first 2 weeks of engraftment with the CSS, it has been noted that slower vascularization, slower keratinization, greater graft loss from microbial contamination, and greater mechanical fragility occurs than with STAG.[16] Analyses for this comparative study were primarily from subjective clinical assessments of graft "take." In comparison, SEC provided a noninvasive, objective index for measurement of the biophysical properties of the epidermal surface during early and late healing of skin grafts. In the present study, both CSS and STAG had similar rates of epidermal barrier formation, which coincided with the subjective clinical findings. For both grafts, epidermal barrier function approached normal levels by 12 days.

Although previous studies have used instruments to assess wound healing,[13,14] none have specifically addressed the restoration of epidermal barrier function which is definitive to the healing of a surface wound or skin graft. Most biophysical techniques used to assess skin hydration are based on the efficiency of water binding in the SC, of which conductance and capacitance measurements are the parameters most commonly used.[11,21] Capacitance measurements are performed by occlusion of the surface with the probe. The probe detects a phase shift created in an alternating current as it is passed through the skin. This phase shift is caused by water which accumulates between the skin surface and the probe during the sampling period. A greater degree of surface hydration generates a larger dielectric constant which is detected as a greater capacitance.

The SC defines and regulates barrier function of the skin. Therefore, measurements of the changes in skin surface hydration under occlusion will be indicative of the relative barrier function of the SC.[22] With a more mature epidermal barrier, less surface hydration accumulates during occlusion by the probe. As the outer layers of the SC are removed in serial tape-stripping experiments, capacitance increases.[11] Thus, these electrical relationships allow an indirect evaluation of the epidermal barrier function as indicated by changes in the skin surface hydration.[21,23] As an index of epidermal barrier formation and skin hydra-

tion, capacitance measurements have been shown to be reliable with minimal variability and easily reproducible in measuring the hydration state of the skin surface between different groups of patients.[9,24,25] Capacitance measurements are more sensitive and accurate at low hydration levels than at high hydration levels,[24,25] and they are less affected by environmental factors (temperature and humidity) than other previously reported methods for assessing epidermal barrier function, such as transepidermal water loss (TEWL).[9,24]

SEC readings at various timepoints have been shown to correlate with differing aspects of epidermal barrier function. During a continuous measurement of SEC, values at 1 second provide a measure of baseline surface hydration (instantaneous capacitance).[26] This measurement is reflective of a baseline epidermal water content. Values obtained after 10 seconds of continuous occlusion allow time for water to move across the epidermis and accumulate beneath the probe head (continuous capacitance). The change in capacitance during the initial 5 seconds of occlusion generates a slope which correlates with the changing hydration of the skin surface, and provides an indirect measure of TEWL.[20] A flatter slope would correlate with a restriction of water movement to the surface—therefore the lower the initial slope value, the more competent the barrier. This slope value reflects the maturity and efficiency of the epidermal barrier and its ability to restrict transepidermal water movement. Although a baseline value may be low, if the barrier is immature this would be reflected in a steeper slope during the 10 seconds of occlusion.

In this study, a greater degree of change was seen in serial SEC sampling in both graft types at day 7. By 2 weeks, minimal change was seen during the 10 seconds of occlusion. Previous analyses performed in this laboratory have shown that the greatest degree of change occurs during the first 5 seconds of occlusion (unpublished data). A steeper slope during this time period correlates with the greater degree of change which is indicative of an immature epidermal barrier. At 2 weeks the slope of both graft types is fairly flat, implying a more mature and effective epidermal barrier, along with low baseline readings.

Impedance-based capacitance meters have primarily been used in dermato-cosmetic research in measuring surface hydration of normal and pathologic skin and as a quantitative assessment of the efficiency of various moisturizing products.[24] More recent studies have used these capacitance meters to measure skin hydration in vivo in both clinical and research protocols.[21,27] Prior clinical use of capacitance measurements have involved analyses of fetal and newborn skin physiologic characteristics.[19,21,26,28,29] These studies have shown that electrical resistance in skin increases with gestational and natal age in neonates.[28] SEC has shown that mature newborn mammal skin is hydrophobic.[19,21] This condition permits easy elimination of exogenous water from the skin surface and therefore reduces the amount of heat loss by evaporation. The ability to minimize heat loss is also vital in the patient with burns.

There is potential clinical relevance for this technique. Before SEC, the only methods to evaluate epidermal barrier function were by histologic analysis from biopsies, clinical examination, and measurement of TEWL by means of an evaporimeter. Histologic analysis is neither quick nor noninvasive. TEWL is a good evaluation for the skin's barrier properties; however, it is highly dependent on external variables.[30] For obtaining accurate readings with TEWL, it is recommended that humidity and temperature be kept relatively constant and that the patient be allowed to acclimate from 10 to 60 minutes during the measurement.[30] For routine clinical assessment, these conditions are not optimal. SEC solves both limitations from the previously mentioned procedures. Until now it has been used only on intact epidermis to evaluate conditions that effect the barrier properties of the skin. We have shown the usefulness of this modality in evaluating barrier formation in healing wounds by evaluating CSS and STAG. Further potential uses are the evaluation of various wounds in a variety of clinical settings. One example is the objective evaluation of epidermal barrier formation in split-thickness donor sites and partial-thickness burns. SEC has the potential to objectively evaluate donor site healing. The ability to definitively determine restoration of the epidermal barrier in the donor site may make it possible to harvest and graft sooner, thereby reducing the total hospitalization time.

It is apparent that SEC provides a reliable, rapid, accurate, and convenient technique for evaluation of epidermal healing in experimental (CSS) and conventional grafts (STAG). This method is also noninvasive and does not appear to cause any discomfort to the patient or mechanical stress to an immature graft. Although early epidermal function appears to be greater in STAG, there is a rapid decrease in capacitance measurements during the first 2 weeks for both CSS and STAG, both approaching baseline normal values by day 12. SEC can be used to evaluate healing in both experimental and conventional skin grafts, and, as a biophysical assessment, it can provide objec-

tive, quantitative, and kinetic data for experimental skin substitutes to validate their usefulness in clinical and preclinical studies.

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REFERENCES

1. Kligman AM. The biology of the stratum corneum. In: Montagna W, Lobitz WC, editors. The epidermis. New York: Academic Press, 1964:387-433.
2. Tagami H. Measurement of electrical conductance and impedance. In: Serup J, Jemec GBE, editors. Handbook of non-invasive methods and the skin. Boca Raton: CRC Press, 1995:159-64.
3. Peacock EE. Wound repair. Third edition. Philadelphia: WB Saunders, 1984:141-86.
4. Elias PM. Epidermal lipids, barrier function, and desquamation. J Invest Dermatol 1983;80:44s-9s.
5. Elias PM. Lipids and the epidermal permeability barrier. Arch Dermatol Res 1981;270:95-117.
6. Imokawa G, Akasaki S, Hattori M, Yoshizuka N. Selective recovery of deranged water-holding properties by stratum corneum lipids. J Invest Dermatol 1986;87:758-61.
7. Warner RR, Myers MC, Taylor DA. Electron probe analysis of human skin: determination of the water concentration profile. J Invest Dermatol 1988;90:218-24.
8. Leveque JL, DeRigal J. Impedance methods for studying skin moisturization. J Soc Cosmet Chem 1983;29:419-28.
9. Gabard B, Treffel P. Hardware and measuring principle: the Nova™ DPM 9003. In: Elsner P, Berardesca E, Maibach HI, editors. Bioengineering of the skin: water and the stratum corneum. Boca Raton: CRC Press, 1994:177-95.
10. Jemec GB, Serup J. Epidermal hydration and skin mechanics: the relationship between electrical capacitance and the mechanical properties of human skin *In Vivo*. Acta Derm Venereol (Stockh) 1990;70:245-7.
11. Tagami H. Impedance measurement for evaluation of the hydration state of the skin surface. In: Leveque JL, editor. Cutaneous investigation In health and disease. New York: Marcel Dekker, 1989:79-111.
12. Boyce ST, Medrano EE, Abdel-Malek ZA, Supp AP, Dodick JM, Nordlund JJ, Warden GD. Pigmentation and inhibition of wound contraction by cultured skin substitutes with adult melanocytes after transplantation to athymic mice. J Invest Dermatol 1993;100:360-5.
13. Leung KS, Sher A, Clark JA, Cheng JCY, Leung PC. Microcirculation in hypertrophic scars after burn injury. J Burn Care Rehabil 1989;10:436-44.
14. Greenhalgh DG, Warden GD. Transcutaneous oxygen and carbon dioxide measurements for determination of skin graft "take". J Burn Care Rehabil 1992;13:334-9.
15. Boyce ST, Supp AP, Pickens WL, Hoath SB. Surface electrical capacitance as a non-invasive measure of epidermal barrier of cultured skin substitutes in vitro and in vivo [Abstract]. J Invest Dermatol 1994;102:537.
16. Boyce ST, Goretsky MJ, Greenhalgh DG, Kagan RJ, Rieman MT, Warden GD. Comparative assessment of cultured skin substitutes and native autograft for treatment of full-thickness burns. Ann Surg 1995;222:743-52.
17. Hansbrough JF, Boyce ST, Cooper ML, Foreman TJ. Burn wound closure with cultured autologous keratinocytes and fibroblasts attached to a collagen-glycosaminoglycan substrate. JAMA 1989;262:2125-30.
18. Boyce ST, Greenhalgh DG, Kagan RJ, Housinger T, Sorrell JM, Childress CP, Reiman M, Warden GD. Skin anatomy and antigen expression after burn wound closure with composite grafts of cultured skin cells and biopolymers. Plast Reconstr Surg 1993;91:632-41.
19. Okah FA, Wickett RR, Pompa K, Hoath S. Human newborn skin: the effect of isopropanol on skin surface hydrophobicity. Pediatr Res 1994;35:443-6.
20. Wickett RR, Nath V, Tanaka R, Hoath SB. Use of continuous electrical capacitance and TEWL measurement for assessing barrier function in neonatal rat skin. Skin Pharmacol 1995;8:179-85.
21. Wickett RR, Mutschelknaus JL, Hoath SB. Ontogeny of water sorption-desorption in the perinatal rat. J Invest Dermatol 1993;100:407-11.
22. Obata M, Tagami H. Electrical determination of water content and concentration profile in a simulation model of in vivo stratum corneum. J Invest Dermatol 1989;92:854-9.
23. Tagami H, Ohi M, Iwatsuki K, Kanamura Y, Yamada M, Ichijo B. Evaluation of the skin surface hydration *in vivo* by electrical measurement. J Invest Dermatol 1980;75:500-7.
24. Barel AO, Clarys P. Measurement of epidermal capacitance. In: Serup J, Jemec GBE, editors. Handbook of non-invasive methods and the skin. Boca Raton: CRC Press, 1995:165-70.
25. Blichman CW, Serup J. Assessment of skin moisture: measurement of electrical conductance, capacitance, and transepidermal water loss. Acta Derm Venereol(Stockh) 1988;68:284-90.
26. Okah FA, Pickens WL, Hoath SB. Effect of prenatal steroids on skin surface hydrophobicity in the premature rat. Pediatr Res 1995;37:402-8.
27. Wolf R, Tur E, Wolf D, Landau M. The effect of smoking on skin moisture and surface lipids. Int J Cos Sci 1992;14:83-8.
28. Muramatsu K, Hirose S, Yukitake K, Ogata H, Mitsudome A, Oda T. Relationship between maturation of the skin and electrical skin resistance. Pediatr Res 1987;21:21-4.
29. Masuda Y, Nishikawa M, Ichijo B. New methods of measuring capacitance and resistance of very high loss materials at high frequencies. IEEE Trans Instr Meas IM 1980;29:28-36.
30. Kajs TM, Gartstein V. Review of the instrumental assessment of skin: effects of cleansing products. J Soc Cosmet Chem 1991;42:249-71.