



AMERICAN SOCIETY OF  
PLASTIC SURGEONS

# Plastic and Reconstructive Surgery®

Journal of the American Society of Plastic Surgeons

Official Organ of the American Association of Plastic Surgeons

Official Organ of the American Society for Aesthetic Plastic Surgery, Inc.

Official Organ of the American Society of Maxillofacial Surgeons

## Cultured Autologous Keratinocytes in Suspension Accelerate Epithelial Maturation in an In Vivo Wound Model as Measured by Surface Electrical Capacitance

Mark Magnusson, F.R.A.C. S. (Plast.), Remo P. Papini, F.R.C.S (Plast.), Suzzane M. Rea, F.R.C.S.I (Past.),  
Chris C. Reed, B.Sc. (Eng.), Fiona M. Wood, F.R.A.C.S.



LIPPINCOTT  
WILLIAMS & WILKINS

# Cultured Autologous Keratinocytes in Suspension Accelerate Epithelial Maturation in an In Vivo Wound Model as Measured by Surface Electrical Capacitance

Mark Magnusson,  
F.R.A.C.S. (Plast.)

Remo P. Papini,  
F.R.C.S. (Plast.)

Suzzane M. Rea,  
F.R.C.S.I. (Plast.)

Chris C. Reed, B.Sc. (Eng.)

Fiona M. Wood, F.R.A.C.S.

Perth, Western Australia, Australia;  
and Birmingham, England

**Background:** Human epidermis serves as a protective covering against loss of endogenous fluids and exogenous microbial invasion. Cultured epithelial autograft suspensions have been used to enhance epithelialization to improve mortality, morbidity, and the overall quality of the scar. The measurement of surface electrical capacitance as an indicator of transepidermal water loss has been used in neonatology and dermatology to determine epidermal maturation. This tool has been used in a double-blind, randomized, controlled trial to compare the effects of cultured epithelial autograft in suspension on epidermal healing and maturation compared with a control in an in vivo partial thickness wound model.

**Methods:** In this double-blind, randomized, controlled trial ( $n = 8$ ), the authors assessed the effect of cultured epithelial autograft on epidermal healing and maturation in standardized partial thickness wounds. Surface electrical capacitance was compared on 16 split-thickness skin graft donor sites treated with cultured epithelial autograft ( $n = 8$ ) against controls treated with Dulbecco's Modified Eagle's Medium ( $n = 8$ ) using a NOVA Dermaphase Meter at 4, 5, 6, and 7 days postoperatively.

**Results:** A statistically significant difference on continuous readings at days 5 ( $p = 0.012$ ) and 7 ( $p = 0.036$ ) and instantaneously on days 5 ( $p = 0.025$ ) and 6 ( $p = 0.036$ ) in surface electrical capacitance was observed in the cultured epithelial autograft over the Dulbecco's Modified Eagle's Medium-treated wounds.

**Conclusions:** Measuring surface electrical capacitance provides an objective and repeatable method of assessing epidermal maturation. This study indicates that the rate of epithelialization and epidermal maturation is more rapid in partial thickness epidermal skin wounds treated with cultured epithelial autograft. (*Plast. Reconstr. Surg.* 119: 495, 2007.)

**H**uman epidermis is a complex structure that varies in thickness and in the amount of keratin produced. It is divided into several layers of progressively maturing cells from the basal layer, where the cells are being continually generated, to the stratum corneum, a network of fibrous protein, lipids, waxes, and sterols. Under

magnification, the stratum corneum presents a basket-like appearance, representing the keratinized outer layer of dead epidermal cells, filled with intracellular lipids derived from the sebaceous glands. This layer is responsible for the protective barrier function of the skin against bacterial invasion and evaporative water loss.<sup>1</sup>

The stratum corneum varies in thickness in different body sites and skin types.<sup>2-4</sup> In addition, diurnal variation exists in transepidermal water loss from human skin.<sup>5</sup> Breakdown or loss of this layer allows profuse water loss through the damaged skin.<sup>6,7</sup>

Epithelialization of wounds is followed by restoration of the stratum corneum by a mature stratified epithelium. This epithelial maturation is responsible for the reduction of evaporative water loss. Historical estimates of the rate of evaporation of water were made by recording the reduction in

From the Toowoomba Institute for Plastic Surgery, Queensland; Department of Medical Physics, Royal Perth Hospital; University of Western Australia; and West Midlands Regional Burns Unit.

Received for publication April 25, 2005; accepted June 16, 2005.

Presented at the 33rd Annual Scientific Meeting of the British Burns Association, Truxeyan College, in Durham, 2000 (awarded prize for best presentation) and the Australian and New Zealand Burn Association Conference, in Hobart, Tasmania, 1999.

Copyright ©2007 by the American Society of Plastic Surgeons

DOI: 10.1097/01.prs.0000246315.80133.8d

body weight during a preselected time interval using a sensitive balance.<sup>8,9</sup> Tritium-labeled water has also been used to estimate changes in total body water content.<sup>10</sup>

Measurement of transepidermal water loss is a widely used bioengineering technique to determine epidermal barrier maturation in neonatology and dermatology.<sup>10,11</sup> The electrical properties of the skin are related to the water content of the stratum corneum.<sup>11</sup> These electrical characteristics have allowed the use of biophysical analyses to evaluate the hydration state of the skin surface by measuring skin impedance. An external voltage is applied to the skin, and the electrical current between two surface positions allows impedance and surface electrical capacitance to be determined.<sup>12</sup> The relationship of surface electrical capacitance to skin hydration has been well described.<sup>13-15</sup>

Goretsky et al. have shown that measurement of surface electrical capacitance is a direct, inexpensive, and convenient index for noninvasive monitoring of epidermal barrier formation. Epidermal maturation is required for stratum corneum hydration to decrease. This relationship has been well described in the literature and has been applied to cultured composite skin substitutes in animal and human studies.<sup>2,13</sup>

Several studies have demonstrated accelerated wound healing after grafting donor sites with allogenic cultured keratinocytes in confluent sheets using subjective methods.<sup>16,17</sup> This study used the principle of surface electrical capacitance to measure transepidermal water loss in a double-blind, randomized, controlled trial of skin graft donor-site healing augmented by cultured epithelial autograft in suspension. This study aimed to provide an objective assessment of the effect of autologous keratinocytes harvested in a subconfluent form as a suspension on epidermal healing and maturation and to validate transepidermal water loss as an objective measure of cultured epithelial autograft take.

## PATIENTS AND METHODS

### Patients

The patients in this double-blind, randomized, controlled trial ( $n = 8$ ) consisted of two women and six men with a mean age of 26 years (range, 23 to 56 years). All patients were admitted to the Adult Burns Unit of the Royal Perth Hospital and had acute burns of less than 20 percent total body surface area, mean total body surface area burn of 11.6 percent, and a total body surface

area burn range of 8 to 18 percent. Patients with any systemic illness were excluded. All patients gave consent for participation in the study, and the ethics committee of Royal Perth Hospital approved the study.

### Treatment

In accordance with standard practices in the Adult Burns Unit, these patients had a skin biopsy at admission and cultured epithelial autograft initiated, as the burn wounds were clinically considered to be deep partial thickness and therefore would not heal within 10 days. An initial pilot study was carried out on 16 patients to determine the optimum postoperative dressing regimen and time points for surface electrical capacitance measurement. These initial donor sites were measured on days 1 to 9 before it became apparent that transepidermal water loss was beyond the calibrated range of the instrument for the first 3 days.

Paired donor sites on each lateral thigh measuring  $8 \times 15$  cm were created at a depth of 6/1000 inch with an air dermatome (Zimmer Patient Care, Dover, Ohio) at the time of definitive surgery for the burn injury. One surgeon harvested all sites. All donor sites were dressed with OpSite (Smith & Nephew, London, United Kingdom) after application of tincture benzoin compound to the periphery of the donor sites to improve adherence.

The OpSite was then punctured with a 21-gauge needle and 1 ml of cultured autologous keratinocytes ( $0.25 \times 10^6$  cells/ml minimum concentration) or 1 ml of culture medium (Dulbecco's Modified Eagle's Medium) instilled. The puncture site was patched with a further piece of OpSite dressing until a seal was created. An outer protective dressing of plain dry gauze and a bandage were applied.

The donor-site dressing was removed on day 4 and the wound was washed with mild soap. If a resilient blood clot was present, saline-soaked gauze was applied for 5 to 15 minutes to soften the clot and then wiped gently to remove. The wound was allowed to dry in room air in the standard environmental conditions of a single room in the Burns Unit for 30 minutes before a reading was taken. All readings were taken in the morning to minimize variation attributable to diurnal rhythm.

On days 4, 5, and 6, the wound was dressed with Silon-TSR (Bio Med Sciences, Inc., Philadelphia, Pa.), and peripherally Fixomull (Beiersdorf, Hamburg, Germany) was used to secure the dressing so that it was not dislodged. A protective outer

dressing was applied for protection from trauma. At the completion of the study period on day 7, all donor-site wounds were dressed with Fixomull applied directly to the surface with no intermediate dressing.

### Capacitance Measurements

Skin surface electrical capacitance, as a measure of surface hydration, was determined using the NOVA Dermaphase Meter (Model DPM9003; NOVA Technology Corp., Gloucester, Mass.) at the bedside with data downloaded directly onto a Toshiba 310CDS laptop computer (Toshiba, Tokyo, Japan) operating on Windows 95 (Microsoft Corp., Redmond, Wash.).

The machine was calibrated to room temperature and humidity. The same surgeon performed all measurements to minimize variability. Sixteen sites were measured on each donor site on each day. The measurements were made in a standard grid pattern with four rows of four readings to give a standardized method of recording sites, and probes were individualized and autoclaved to prevent cross-infection.

The Dermaphase Meter probe used for data collection was a standard 6-mm Dermaphase Meter 9105 with a spring-loaded flat contact surface. The probe surface consists of concentric brass electrodes (outer ring diameter, 5.08 mm; inner ring diameter, 2.54 mm) separated by a 0.6-mm-wide nonconducting insulator ring.

Room temperature and humidity were measured and the machine calibrated before readings. In addition, a measure of skin surface electrical capacitance was recorded from an uninjured site on the thigh. To minimize variation in pressure applied to the probe, the same surgeon performed all measurements.

Measurements were recorded on days 4, 5, 6, and 7 from each donor site. Recordings were obtained from 16 sites from each wound daily. Each site was recorded for 6 seconds, and there was an instantaneous reading and five further readings at each site. This generated an instantaneous record, a continuous reading, and a slope at each site for each reading. Data were downloaded directly onto a Toshiba 310CD laptop at the bedside and then

transferred to a CDM desktop running a Windows 98 operating system. Data were analyzed with Microsoft Excel 97 and SPSS version 12 (SPSS, Inc., Chicago, Ill.) using the Wilcoxon signed rank test for paired nonparametric data with a  $p < 0.05$  level of statistical significance.

### RESULTS

Donor sites treated with cultured epithelial autograft beneath the OpSite healed at a faster rate, as demonstrated in Table 1 and Figure 1 by lower continuous rates and in Table 2 and Figure 2 by instantaneous readings on all days.

### DISCUSSION

Keratinocyte migration proceeds across a wound until contact inhibition induces terminal differentiation into a stratified squamous epithelium, and culminates in the formation of a keratin layer at maturity. The latter reduces evaporative water loss once established. Epithelialization is always difficult to quantify, as it relies on the subjective assessment of an epithelial sheet, which may be only a few cells thick. A number of different mechanisms have been used previously, including clinical assessment, clinical photography, and laser Doppler imaging. We chose skin surface electrical capacitance as a measure of surface hydration, using the NOVA Dermaphase Meter, because it gives an objective and easily reproducible result that is not dependent on clinical judgment. The NOVA Dermal Phase Meter DPM9003 is a relatively cheap piece of equipment that is easy to use at the bedside. Further advantages include the ease and speed with which measurements can be taken. Hydration of the skin surface using the Dermaphase Meter is a measure of water that accumulates beneath the probe that occludes the surface of the skin during the sampling period. This must be distinguished from the measure of transepidermal water loss by an evaporimeter that measures water vapor flux above the skin surface with a nonocclusive probe and is therefore more subject to environmental difference.<sup>18,19</sup>

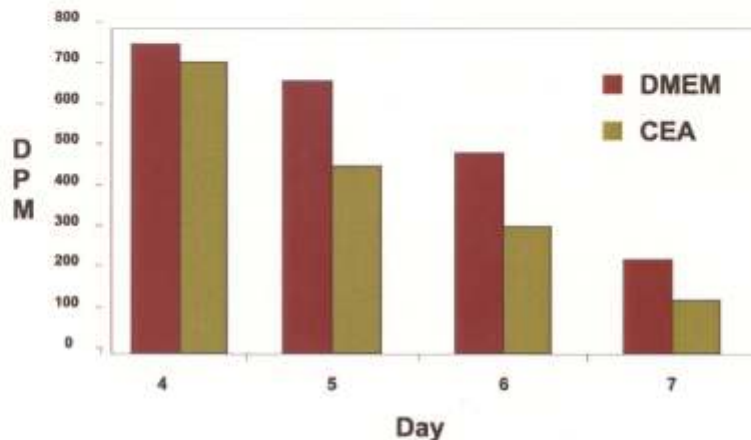
The instantaneous measurement represents the presence of epithelial cells, and in this study, we have shown that this is significantly greater in

**Table 1. Average Continuous Reading ( $n = 8$ ) Using the Wilcoxon Signed Rank Test**

Test Statistic	Day 4	Day 5	Day 6	Day 7
Z value	-1.540*	-2.521*	-1.820*	-2.100*
Asymptotic significance (two-tailed) ( $p > 0.05$ )	0.123	0.012†	0.069	0.036†

\*Based on negative ranks.

†Statistically significant.



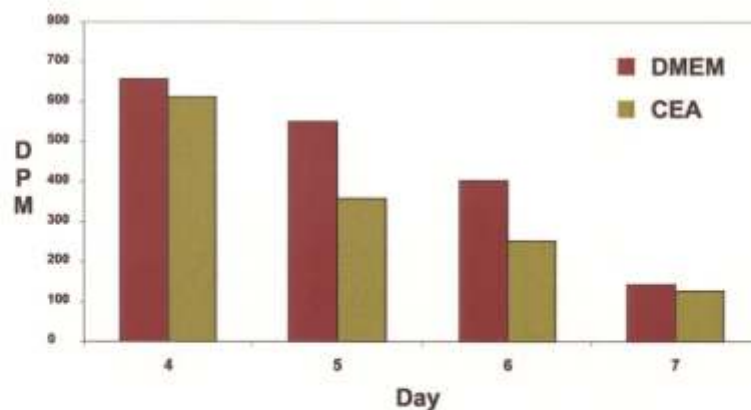
**Fig. 1.** Average continuous transepidermal water loss readings for all patients ( $n = 8$ ). *DPM*, Dermaphase Meter reading; *DMEM*, Dulbecco's Modified Eagle's Medium; *CEA*, cultured epithelial autograft.

**Table 2. Average Instantaneous Readings ( $n = 8$ ) Using the Wilcoxon Signed Rank Test**

Test Statistic	Day 4	Day 5	Day 6	Day 7
Z value	-0.980*	-2.240*	-2.100*	-1.703*
Asymptotic significance (two-tailed) ( $p > 0.05$ )	0.327	0.025†	0.036†	0.089†

\*Based on negative ranks.

†Statistically significant.



**Fig. 2.** Average instantaneous transepidermal water loss readings for all patients ( $n = 8$ ). *DPM*, Dermaphase Meter reading; *DMEM*, Dulbecco's Modified Eagle's Medium; *CEA*, cultured epithelial autograft.

the cultured epithelial autograft group at days 5 and 6. Continuous readings represent water loss over a sustained time period, thus representing epithelial maturity because of epidermal stratification and development of the keratin layer. This was statistically significant at days 5 and 7 in the cultured epithelial autograft group.

The study has a limited sample size ( $n = 8$ ); however, each patient had two donor wounds (a treatment and a control), and 16 sites were recorded on each donor site (32 sites per patient) on

each day. Because the validity of the test is well established, the reliability of the findings is increased. The results demonstrate statistically significant differences in the rates of epithelialization and the maturation of epithelialized partial thickness wounds treated with autologous cultured keratinocytes in a suspension when compared with control wounds.

These cells could be acting by means of biological or cytokine activity or by cell adhesion. One could argue that the introduced cells solely stim-

ulate epidermal remnants within the wound to facilitate cell proliferation, migration, and epithelialization from native elements in the wound bed. Alternatively, the introduced keratinocytes may adhere at the treatment site, producing these effects because of the growth and incorporation of the cultured cells. Which of these is the primary factor has not been demonstrated within the scope of this study. The long-term persistence of cultured autologous keratinocytes has been demonstrated previously using the same methods of cell culture and delivery in a preconfluent suspension using immunofluorescent cytokeratin 9 markers.<sup>20</sup>

### CONCLUSIONS

We have confirmed the observations of others that surface electrical capacitance as a measure of transepidermal water loss is a reproducible and objective measure of assessing wound healing. We have also demonstrated the accelerating influence on healing achieved by introducing a preconfluent suspension of cultured epithelial autograft onto a standardized partial thickness wound.

Fiona Wood, F.R.A.C.S.

Burns Unit  
Royal Perth Hospital  
G.P.O. Box X2213  
Perth, Western Australia 6847, Australia  
fionaw@mccomb.org.au

### ACKNOWLEDGMENT

The contribution of Bess Fowler in the statistical analysis and editing of this article is acknowledged.

### DISCLOSURE

The cultured epithelial autograft CellSpray is produced by Clinical Cell Culture Ltd. (C3), in which Fiona Wood has a financial interest.

### REFERENCES

1. Jarret, A. The epidermis and its relationship with the dermis. In A. Jarrett (Ed.), *The Physiology and Pathophysiology of the Skin*. New York: Academic Press, 1972. Pp. 3-5.
2. Schwandt, D., Wilhelm, K. P., and Maibach, H. Water diffusion characteristics of human stratum corneum at different anatomical sites in vivo. *J. Invest. Dermatol.* 111: 385, 1998.
3. Berardesca, E., and Maibach, H. Racial differences in skin pathophysiology. *J. Am. Acad. Dermatol.* 34: 667, 1996.

4. Reed, J. T., Ghadially, R., and Elias, P. M. Skin type, but neither race nor gender, influence epidermal permeability barrier function. *Arch. Dermatol.* 131: 1134, 1995.
5. Yosipovitch, G., Xiong G. L., Haus, E., Sackett-Lundeen, L., Ashkenazi, I., and Maibach, H. Time dependent variations of the skin barrier function in humans: Transepidermal water loss, stratum corneum hydration, skin surface pH, and skin temperature. *J. Invest. Dermatol.* 110: 20, 1998.
6. Moyer, C. A., and Butcher, H. R. *Burns, Shock, and Plasma Regulation*. St. Louis: Mosby, 1967.
7. Jelenko, C. Studies in burns: VII. The water retaining lipids of eschars: Their presence and potential usefulness. *Am. Surg.* 5: 709, 1969.
8. Moncrief, J. A., and Mason, A. D. Water vapour loss in the burned patient. *Surg. Forum* 13: 38, 1962.
9. Harrison, H. N., Moncrief, J. A., and Duckett, J. W. The relationship between energy metabolism and water loss from vapourisation in severely burned patients. *Surgery* 56: 203, 1964.
10. Birke, G., Liljedahl, S. O., Olsson, K. E., and Plantin, L. O. Total exchange and insensible loss of water in severe burns during the first two weeks. *Acta Chir. Scand.* 136: 125, 1970.
11. Hammarlund, K., and Sedin, G. Transepidermal water loss in newborn infants: III. Relation to gestational age. *Acta Paediatr. Scand.* 68: 795, 1979.
12. Sedin, G., Hammarlund, K., Nilsson, G. E., Stromberg, B., and Oberg, P. A. Measurement of transepidermal water loss in newborn infants. *Clin. Perinatol.* 12: 79, 1985.
13. Leveque, J. L., and De Rigal, J. Impedance methods for studying skin moisturization. *J. Soc. Cosmet. Chem.* 29: 419, 1983.
14. Gabard, B., and Treffel, P. Hardware and measure principle: The Nova DPM 9003. In P. Elsner, E. Berardesca, and H. I. Maibach (Eds.), *Bioengineering of the Skin: Water and the Stratum Corneum*. Boca Raton, Fla.: CRC Press, 1994. P. 177.
15. Jemec, G. B., and Serup, J. Epidermal hydration and skin mechanics: The relationship between electrical capacitance and the mechanical properties of the human skin in vivo. *Acta Derm. Venereol.* 70: 245, 1990.
16. Boyce, S. T., Supp, A. P., Harriger, M. D., Pickens, W. L., Wickett, R. R., and Hoath, S. B. Surface electrical capacitance as a noninvasive index of epidermal barrier in cultured skin substitutes in athymic mice. *J. Invest. Dermatol.* 107: 82, 1996.
17. Goretsky, M. J., Supp, A. P., Greenhalgh, D. J., Warden, G. D., and Boyce, S. T. Surface electrical capacitance as an index of epidermal barrier properties of composite skin substitutes and skin autografts. *Wound Repair Regen.* 3: 419, 1995.
18. Fratiante, R., Papay, F., Housini, I., Lang, C., and Schafer, A. J. Keratinocyte allografts accelerate healing of split-thickness donor sites: Applications for improved treatment of burns. *Burn Care Rehabil.* 14: 54, 1993.
19. Tagami, H., Ohi, M., Iwatsuki, K., Kanamaru, Y., Yamada, M., and Ichijo, B. Evaluation of the skin surface hydration in vivo by electrical measurement. *J. Invest. Dermatol.* 75: 500, 1980.
20. Stoner, M. L., and Wood, F. M. Cultured epithelial autograft "take" confirmed by the presence of cytokeratin 9. *J. Invest. Dermatol.* 112: 391, 1999.