Imposition of a Physiologic DC Electric Field Alters the Migratory Response of Human Keratinocytes on Extracellular Matrix Molecules

Diane M. Sheridan, R. Rivkah Isseroff, and Richard Nuccitelli*

Department of Dermatology and *Section of Molecular and Cellular Biology, University of California, Davis, California, U.S.A.

Outwardly directed ionic currents have been measured leaving skin wounds *in vivo*. These currents generate physiologic electric fields of approximately 100 mV/mm, which may function to direct keratinocyte migration toward the healing wound. We investigated whether the substrate on which the keratinocyte migrates modulates the galvanotactic response to an electric migratory signal. Cultured human keratinocytes were plated on different matrices: types I and IV collagen, fibronectin, laminin, and tissue culture plastic. The effect of an applied direct current (DC) electric field on directional migration was monitored by time-lapse video microscopy over a 2-h period. Directionality was quantitated by calculating the cosine of the angle of migration in relation to anodal-cathodal orientation. Migration toward the negative pole was observed on all matrices as compared with controls (no applied field), which displayed random migration. No significant increase in directional response occurred when the field strength was increased from 100 mV/mm (physiologic levels) to 400 mV/mm. The degree of directionality and the average net cell translocation, however, varied significantly with the substrate. The greatest cathodal migration in response to a DC electric field was observed with keratinocytes plated on types I and IV collagens and plastic. The directional migratory response was least on a laminin substrate, whereas cells on fibronectin demonstrated a response that was intermediate between those of collagen and laminin. These results suggest that physiologic ionic currents in concert with the underlying matrix may influence the rate of reepithelialization of skin wounds. ***Key words: galvanotaxis/motility/wound healing/collagen. J Invest Dermatol 106:642–646, 1996***

The migratory response of keratinocytes is known to be influenced by the composition of the underlying matrix (Woodley *et al*, 1985). Migration is enhanced by substrates of fibronectin and types I and IV collagens, yet is inhibited by laminin (O'Keefe *et al*, 1985; Woodley *et al*, 1988). The overall pattern of migration demonstrated in these cited phagokinetic studies appears to be random (nondirected). In the wounded environment, therefore, factors other than extracellular matrix (ECM) must be influencing the directed migration of keratinocytes to the center of the wound bed.

Direct current (DC) electric fields have been shown to modify the migratory pattern of several cell types. Early studies on embryonic neural crest cells and fibroblasts demonstrated directed translocation responses to fields as small as 10 mV/mm by exhibiting enhanced migratory activity in the direction of the negative pole (cathode) (Nuccitelli and Erickson, 1983; Erickson and Nuccitelli, 1984). Many other cell types studied display similar cathodal migration in response to imposed electric fields (Nuccitelli, 1988). This directed migratory response of motile cells in an electric field is termed "galvanotaxis." It is interesting that the electric field strength that produces the best galvanotaxis response *in vitro* is essentially identical to that measured near wounds in guinea pig skin *in vivo* (Barker *et al*, 1982). These wound currents are driven by the transcutaneous potentials of 10–100 mV (inside positive) across guinea pig and human skin. Current densities of 10–30 $\mu A/cm^2$ have been measured exiting fingertip wounds of children (Illingworth and Barker, 1980). Additionally, several investigators have demonstrated enhanced rates of healing in electrically treated skin wounds (Assimacopoulos, 1969; Carley and Wainapel, 1985; Chu *et al*, 1990).

Our studies of keratinocyte galvanotaxis suggest that physiologic currents from skin wounds may provide the initial guidance for keratinocyte migration (Nishimura *et al*, 1995). Because keratinocytes migrating in the wound bed will encounter a number of different ECM substrata during their travels, we investigated the effect of the superimposition of a DC electric field on keratinocytes migrating on ECM proteins ordinarily encountered in the healing wound.

MATERIALS AND METHODS

Cells and Cell Culture Normal human keratinocytes were derived from a single primary source of neonatal foreskin epidermis and were cultured using a modification (Isseroff *et al*, 1987) of the methods described by Rheinwald and Green (1975). All cells were cultured in serum-free keratinocyte growth medium (Shipley and Pittelkow, 1987) (Cascade Biologics Inc., Portland, OR) supplemented with 100 IU each of penicillin and streptomycin and 0.25 μg amphotericin, with a final calcium concentration of 0.2 mM. Cultures were kept at 37°C in a humidified atmosphere of 5% CO_2. Numerous aliquots of cells derived from a single donor were cryopreserved, and an aliquot was thawed and plated 4–8 d before

Manuscript received July 22, 1995; revised November 20, 1995; accepted for publication December 7, 1995.

Reprint requests to: Dr. Richard Nuccitelli, Section of Molecular and Cellular Biology, University of California, Davis, CA 95616.

Abbreviations: DC, direct current; KGM, keratinocyte growth medium.

0022-202X/96/$10.50

experimentation. Upon thawing, the freezing medium was removed after mild pelleting of cells by centrifugation, and the cells were resuspended and cultured in serum-free keratinocyte growth medium. Passage 2–4 cells were used for all experiments.

Matrix and Coverslip Preparation All matrix proteins were dissolved in Ca^{2+}/Mg^{2+}-free phosphate buffer solution at specified concentrations based on published studies by Woodley *et al* (1988), which identified matrix concentrations for optimal keratinocyte migration *in vitro*. Bovine type I collagen (Vitrogen; Celtrix Pharmaceuticals, Santa Clara, CA) and human placenta type IV collagen (Sigma Chemicals, St. Louis, MO) were plated at concentrations of 15 mg/ml each. Human plasma fibronectin (Gibco Laboratory, Grand Island, NY) was plated at a concentration of 60 mg/ml, and laminin (Engelbreath-Holm mouse sarcoma; Gibco) was plated at a concentration of 90 mg/ml. Tissue culture plastic coverslips were obtained from NUNC Inc. (Naperville, IL). Glass coverslips were soaked in 2 ml of matrix solution for 24 h in an incubator at 37°C. Excess matrix solution was then removed, and the coverslips were allowed to air dry for 5–10 min. Tissue culture plastic coverslips were not coated with matrix molecules; keratinocytes were plated directly onto them. When keratinocytes were plated on the prepared coverslips, the calcium concentration of the medium (Cascade) was raised to a physiologic level (1.8 mM). After 2–4 h to allow attachment and spreading of cells on the matrix, coverslips were rinsed with medium to remove unattached cells and placed in galvanotaxis chambers.

Galvanotaxis Chambers Chambers used for applying an electric field to keratinocytes in culture were identical to those described previously (Erickson and Nuccitelli, 1984). The average height of the fluid above the cells in the chambers ranged between 100 and 180 μm. The chambers were sealed with tape and silicone high-vacuum grease. Experimental medium consisted of serum-free, supplemented keratinocyte growth medium with added calcium (1.8 mM) and HEPES buffer (10 mM). Initially, medium was added to only one well of the chamber, and fluid was added to the remaining well only after flow into that well had occurred, to indicate a clear path for fluid flow over the cells. A constant DC voltage was applied across each chamber, measured by inserting Ag-AgCl electrodes into the well on both ends, and the current passing over the cells was monitored continuously with an ammeter. Agar-filled glass bridges separated the electrodes supplying the current from the wells of the chamber.

Filming Procedure Cells were observed with phase contrast optics on an inverted microscope, and their movements were recorded using a video camera and time-lapse recorder. Fields of cells to be studied were selected from those regions in which the cells were dispersed to reduce the influence of cell-cell interactions. Cells in groups were not used for subsequent analysis. Experiments were conducted for 2 h in the electric field. Cell positions were traced onto a plastic transparency at 15–min intervals throughout the experiment. Net cell displacement was measured from the initial position of each cell at the beginning of the observation period to its location at the end of 2 h. Some cells exited the field of view during the experiment. The elimination of these cells from the net translocation analysis would have caused a bias against the most responsive cells, so the final point of observation was taken as the point where they left the screen, even if this occurred before the standard 2–h period. These cells, however, were not included in the average velocity analysis.

Data Analysis A digitizing pad (Houston Instruments, Austin, TX) was used to transfer cell positions from the plastic transparencies to a computer for analysis. To quantitate the directedness of the average cellular translocation, we calculated the cosine of the angle at which each cell moved in relation to anodal-cathodal orientation. Specifically, a cosine value of 1 would indicate direct cellular movement toward the negative pole; 0 would indicate movement perpendicular to the field direction; and −1 would indicate direct cellular movement toward the positive pole. The average directedness, $\langle\cos\phi\rangle$, for each experiment was calculated from the formula: $\langle\cos\phi\rangle = \Sigma_i \cos N_i/N$, where Σ_i is the summation of cosine values obtained from individual cells, ϕ is the angle between the field axis and the cellular translocation direction, and N is the total number of cells observed at a given field strength. Average cell velocities (μm/min) were calculated by dividing the sum of each 15-min translocation distance for each cell by the total time (120 min). Significance was determined by Student's t test, with $p < 0.005$ considered significant.

RESULTS

Keratinocytes Exhibit Cathode-Directed Migration on All ECM Matrices Keratinocytes plated on substrates of type I collagen, type IV collagen, fibronectin, laminin, and plastic demonstrated active migration toward the negative pole of an applied DC electric field **(Fig 1)**. The magnitude of the directional response (galvanotaxis) varied with the matrix. The translocation response of keratinocytes on each matrix, as measured by the average cosine of the angle of migration, is depicted in **Fig 2**. An average cosine value of 1 indicates direct cathodal (negative pole) migration, −1 indicates direct anodal migration, and 0 indicates random migration. Keratinocytes migrating on type I and IV collagen demonstrated the strongest galvanotactic response at a field strength of 100 mV/mm (average cosine values of 0.74 ± 0.03 and 0.73 ± 0.04, respectively). Directional migration was significantly lower on laminin than on collagen or fibronectin ($p < 0.005$). The response on fibronectin (average cosine 0.62 ± 0.04) was less than that on type I and IV collagen; however, this difference did not reach significance ($p = 0.005$ and $p = 0.03$, respectively). Cells migrating on plastic demonstrated a directed migration (toward the cathode) equivalent in magnitude to that on collagen. Controls (no field applied) for each matrix demonstrated migration in a random pattern.

Experiments were performed at field strengths of 0 mV/mm (control), 100 mV/mm, and 400 mV/mm for each matrix. Cells exposed to the 400 mV/mm field strength did not demonstrate increased cathodal migration as compared with those exposed to

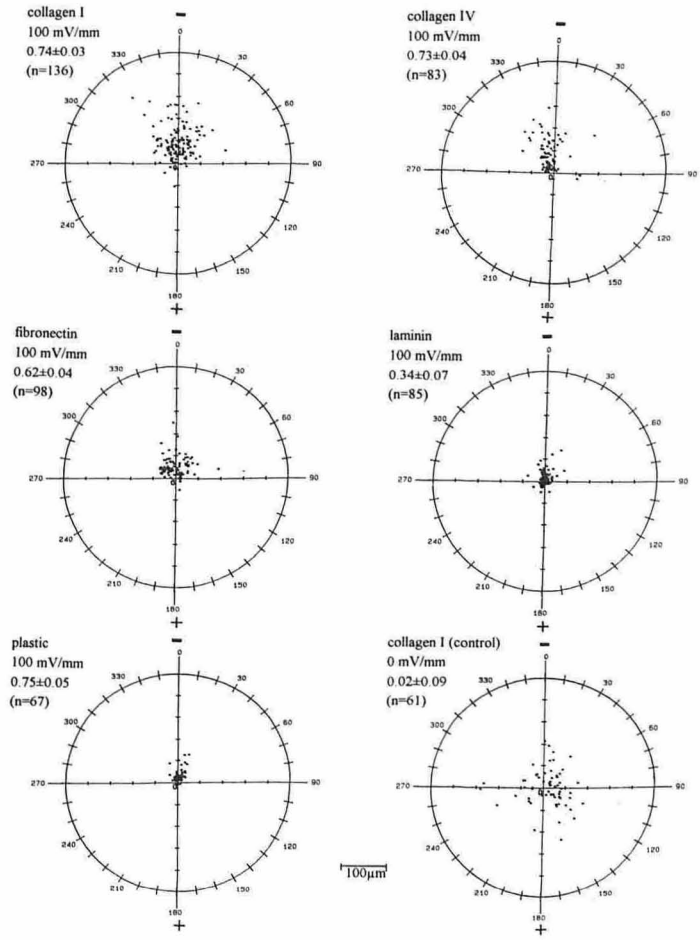

Figure 1. Cellular translocation distribution of human keratinocytes on ECM substrate after imposition of a DC electric field. Human keratinocytes were plated on different matrices, as described in *Materials and Methods*. Directed migration in an applied electric field was quantified by calculating the average cosine of the angles of migration in relation to the anodal-cathodal orientation. Migration paths were recorded and traced from a video screen. The starting position of each cell is represented by the center of the circle, and the final position was plotted as a single point on the graph (0° indicates cathodal direction; 180° indicates anodal direction). The average cosine of the translocation ± SEM is indicated in the text at the upper left corner of each plot. The number of cells is indicated by n.

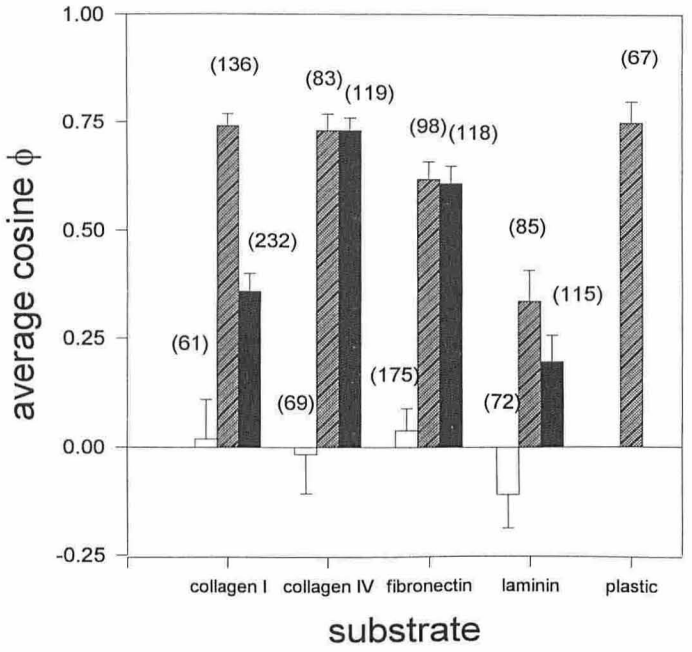

Figure 2. Average cosine of the translocation distribution. Cells were plated and exposed to a DC electric field as for **Fig 1**. Directed migration was quantified by calculating the average cosine of the angles of migration of each cell studied in relation to anodal-cathodal orientation, and is represented as a *bar* on the graph. An average cosine of 1 indicates direct cathodal migration, 0 indicates random migration, and −1 indicates anodal migration. *Error bars* indicate SEM. *Open bars*, 0 mV/mm; *striped bars*, 100 mV/mm; *solid bars*, 400 mV/mm. The number of cells studied for each condition is indicated in parentheses.

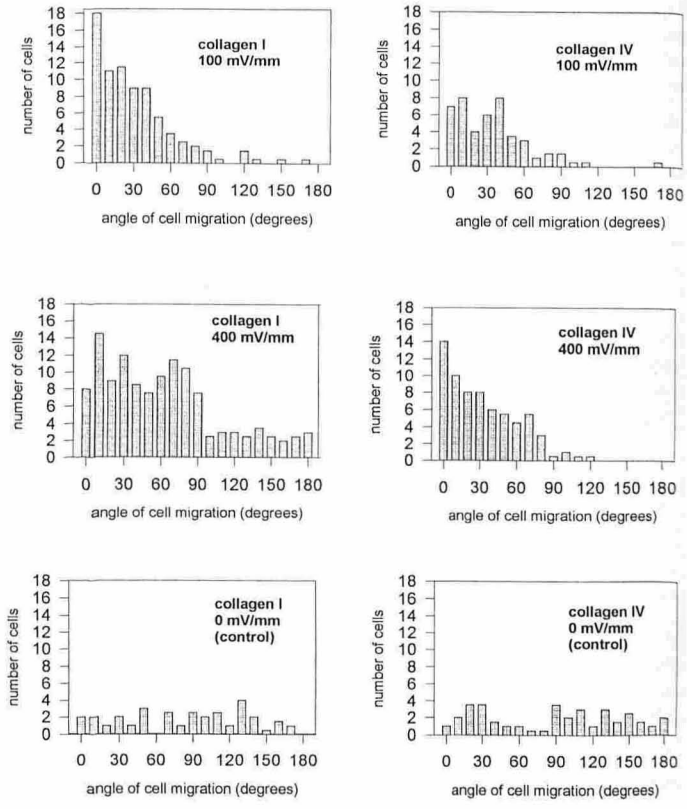

Figure 3. Translocation distribution of human keratinocytes on types I and IV collagens. Cells were plated on different matrices and exposed to a DC electric field as in **Fig 1**. Each *bar* represents the number of cells that migrated in the DC field to a final position lying within the indicated 10° sector (0° indicates cathodal direction; 180° indicates anodal direction). Because this response is symmetric about the 0°–180° axis, the number of cells in each 10° sector between 5° and 175° was averaged with the number of cells in the mirror-image sector on the opposite side of the *y* axis, and this number was plotted. The *bars* at 0° and 180° represent the actual number of cells that migrated within their respective 10° sectors.

the 100 mV/mm field strength. In fact, the directional response (as measured by the average cosine of the angle of migration) was less at 400 mV/mm than at 100 mV/mm for cells on type I collagen, fibronectin, and laminin. Cells on type IV collagen demonstrated equal galvanotactic responses for both field strengths. A comparison of the directional response for cells on each matrix and at each field strength is presented in **Fig 2**. Cells exposed to 400 mV/mm assumed an elongated morphology perpendicular to the direction of the field. Although movement was generally in a cathodal direction, more lateral migration (perpendicular to field direction) was observed at the higher field strength.

The Greatest Cathodal Migration Is Seen in Cells Plated on Type I and IV Collagen Another method of quantifying the results is depicted in **Figs 3** and **4**. The plane of migration was divided into 10° sectors between 0° and 180° (negative pole = 0°; positive pole = 180°). The number of cells traveling in each 10° sector was averaged with the number in the corresponding symmetric sector on the opposite side of the *y* axis, and plotted for each matrix and field strength. The most significant cathodal translocation was noted for cells on type I and IV collagen at a field strength of 100 mV/mm. In controls (no field), cells migrated randomly in all sectors.

The average net cell translocation after 2 h was greatest on type I and IV collagen, less on fibronectin, and least on laminin and plastic in both the experimental (applied field) and control (no field) groups. These results are depicted graphically in **Fig 5**. For each matrix, cells exposed to a field demonstrated a net cell translocation slightly greater than that of controls. It is important to note that net cell translocation is a measure of the linear distance from a cell's initial position to its location after 2 h, and not a measure of the total distance traversed. Thus, cells that traveled in circular migratory paths or underwent changes of direction during their migration (characteristic of cells not exposed to a field) had a decreased net cell translocation as compared with those that displayed directed, linear migration. The major effect of the field, therefore, was not to enhance migratory distance or velocity, but rather to influence the directedness of migration, resulting in more linear movement toward the negative pole.

DC Field Exposure Does Not Significantly Enhance Keratinocyte Velocity The average velocities of migration for cells on each matrix are presented in **Table I**. Exposure to the electric field did not significantly affect the velocity of translocation. A slight but nonsignificant enhancement of velocity was noted for cells migrating on types I and IV collagens ($p = 0.007$ and $p = 0.008$, respectively) at high field strength exposure (400 mV/mm). Migratory speeds were greatest on types I and IV collagens, least on laminin and plastic, and intermediate on fibronectin.

Cross-Flow Control To investigate the possible influence of field-induced fluid flow or concentration gradients of charged molecules on the galvanotactic response of keratinocytes, we performed experiments in which a continuous cross flow of medium (0.7 ml/min) was passed over the cells perpendicular to the direction of the electric field. These cells responded normally with an average cosine of 0.67 ± 0.06, suggesting that the directed migratory response is a cellular response to the field itself and not to the establishment of a charged gradient by the electric field.

DISCUSSION

This study reports on the galvanotactic response of a population of cultured keratinocytes (approximately 1,000 total cells) from a

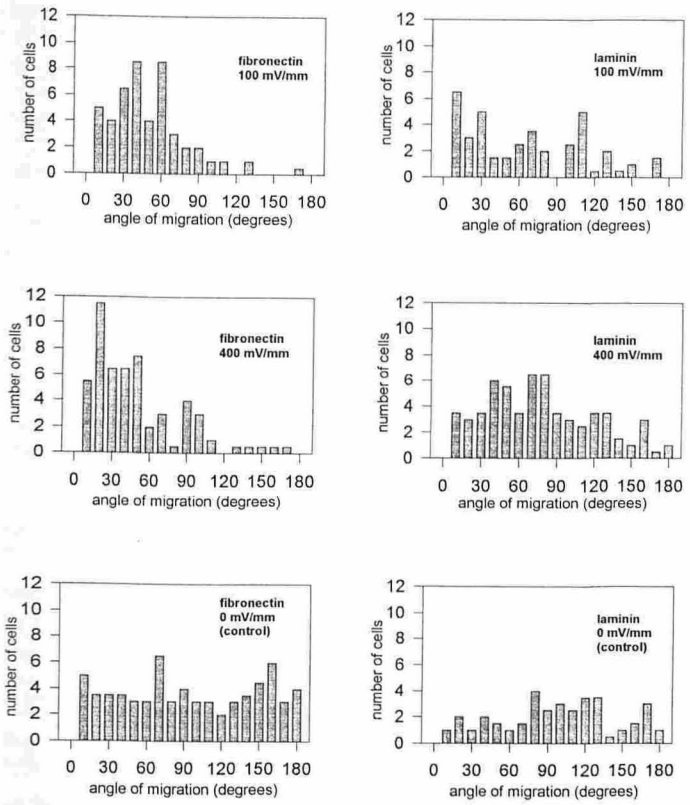

Figure 4. Translocation distribution of human keratinocytes on fibronectin and laminin. Cells were plated on fibronectin- or laminin-coated coverslips as described in *Materials and Methods* and exposed to DC electric fields as in Fig 1. Data were collected and analyzed as for Fig 3.

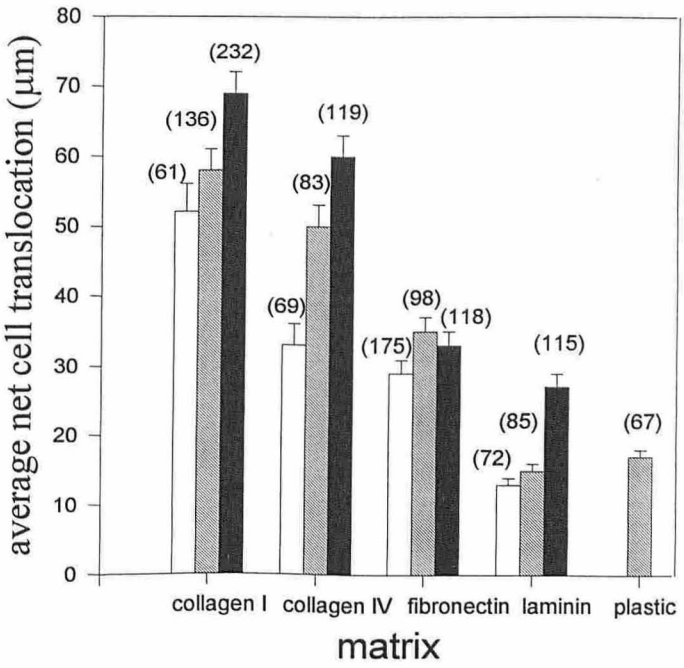

Figure 5. Average net cell translocation of keratinocytes plated on ECM substrates. Cells were plated and exposed to DC electric fields as in **Fig 1**. The average linear distance in μm ± SEM traversed after 2 h is presented for the cells on each matrix. The number of cells averaged for each field strength is indicated by *n* above each *bar*. *Open bars*, 0 mV/mm; *striped bars*, 100 mV/mm; *solid bars*, 400 mV/mm. Number of cells for each condition is indicated in parentheses.

single human foreskin. Equivalent results were noted with keratinocytes derived from three additional foreskin sources (data not shown). The results indicate that human keratinocyte migration on ECM is modified by the imposition of a DC electric field. Keratinocytes exposed to electric field strengths normally found near wounds in skin demonstrated directed migration toward the negative pole. Not only was the direction of travel altered, but the individual migratory paths also were significantly more linear. The interaction of the keratinocytes with the underlying matrix is an important component of the galvanotactic response, indicated by the varying response levels on different substrates. The response was maximal on plastic and type I and IV collagen, minimal on laminin, and intermediate on fibronectin. This observed sensitivity of keratinocytes to imposed electric fields, regardless of the underlying substrate, suggests that endogenous wound fields may function to guide keratinocyte migration in a healing wound until current leakage ceases when reepithelialization is complete.

The process of reepithelialization of a skin wound can best be understood if divided into independent, but synergistic components. For example, keratinocyte locomotion and keratinocyte proliferation are two components that are influenced by separate mechanisms (Sarret *et al*, 1992; Woodley *et al*, 1993). In this study, we have proposed yet another component of reepithelialization: directed migration of keratinocytes in response to an endogenous wound electric stimulus (galvanotaxis). We have demonstrated that keratinocytes are guided toward the negative pole of an electric field *in vitro* at strengths equivalent to those that have been measured at mammalian wound edges *in vivo* (Barker *et al*, 1982). It is important to note, however, that these measurements of lateral fields near wounds were made between the epidermis and the stratum corneum, where the wound is positive with respect to the lateral regions. The region in which the fields will guide keratinocytes toward the wound is beneath the epidermis, where the field exhibits the opposite polarity, with the wound negative with respect to the lateral regions. Here the resistivity and, consequently, the electric field generated by the wound current is likely to be lower than it is beneath the stratum corneum. It is likely, however, that the voltage gradient in this region will still be in the physiologic response range of 10–100 mV/mm.

Epithelial cell interactions with the underlying matrix are complex. Grinnell (1990) proposed the idea of "activated" keratinocytes to describe the specialization of keratinocytes for migration induced by a wounded environment. The ability of keratinocytes to migrate on dermal collagen is considerably greater than that on laminin (Woodley *et al*, 1985), implying that cell-matrix interaction signals the activation of keratinocytes. The electric field may then function to localize the components of the signaling cascade for migration, so that "activated" keratinocytes move directionally. Fibronectin is an insoluble glycoprotein synthesized by both mesenchymal and epithelial cells that is found diffusely throughout the

Table I. Average Cellular Velocities on ECM Substrates[a]

Matrix Type	Mean Cellular Velocity at Indicated Electric Field Strength		
	0 mV/mm	100 mV/mm	400 mV/mm
Collagen I	0.7 ± 0.04 (61)	0.7 ± 0.03 (136)	0.9 ± 0.04 (232)[b]
Collagen IV	0.6 ± 0.03 (69)	0.5 ± 0.03 (83)	0.7 ± 0.03 (119)[c]
Fibronectin	0.6 ± 0.03 (175)	0.6 ± 0.1 (98)	0.4 ± 0.04 (118)
Laminin	0.4 ± 0.08 (72)	0.4 ± 0.02 (85)	0.4 ± 0.04 (115)
Plastic	0.1 ± 0.01 (58)	0.1 ± 0.02 (67)	0.1 ± 0.02 (76)

[a] Cells were plated on different ECM substrates and exposed to DC electric fields as described in **Fig 1**. Mean cellular velocity was calculated as described in *Materials and Methods*.

Data are presented as μm/min ± SEM (n). n is the total number of cells analyzed in all experiments (approximately 10–15 cells/experiment).

[b] $p = 0.007$ compared with 100 mV/mm value.

[c] $p = 0.008$ compared with 100 mV/mm value.

dermis and in plasma (Hay, 1991). A significant component of the wound matrix, it is involved in cell adhesion and spreading. Our finding of decreased cell migration on a fibronectin substratum as compared with that on collagen (no applied field) has been reported previously (Stenn *et al*, 1983). The variability in enhancement of keratinocyte migration demonstrated by different matrices in the absence of an applied field can be explained in part by the structure of the matrix and the multiple adhesion mechanisms involved. The explanation for the variability in the galvanotactic response on differing matrices may be similar. Perhaps cell activation to a migratory state is a graded response depending on the relative composition of the substratum, and the degree of activation directly affects the cell's response to an electric field. Another possibility is that there is a direct effect of the field on the matrix, which promotes galvanotaxis. This explanation is less likely, considering the galvanotactic response of cells plated on tissue culture plastic. The directionality of migration (toward the cathode) on plastic was as high as that on collagen, although the velocity of migration was significantly lower. The inability of cross flow of medium to inhibit cathodal galvanotaxis also suggests a direct effect of the field on the cells, rather than on extracellular conditions.

Ours are the first reported data on the relative velocities of keratinocyte migration on different substrates **(Table I)**. It should be noted that the data reported represent the migratory velocities of previously cryopreserved keratinocytes (see *Materials and Methods*); we have observed faster rates at times (Nishimura *et al*, 1995). It is also important to note that not all cells moved. Those that did not (approximately 5%) demonstrated a round, flattened morphology quite different from the polygonal morphology with pseudopod extension characteristic of migrating cells. ECM composition significantly influenced migratory velocity. Laminin inhibited the rate of migration as compared with collagen and fibronectin. Exposure to the electric field, conversely, did not significantly affect the velocity on any of the substrates.

The relevance of these results to wound healing is suggested by the presence of endogenous electric potentials across intact human skin (Barker *et al*, 1982) and the existence of measurable electric currents that exit wounded skin (Illingworth and Barker, 1980) and that generate lateral electric fields, which point toward the center of the wound in the region beneath the epidermis (Barker *et al*, 1982). Barker *et al* (1982) measured electric fields of 100–200 mV/mm at the wound edge in the glabrous epidermis of guinea pig skin. Electric fields or currents also have been measured in wounds of newt skin (Chiang *et al*, 1991), in bovine corneal wounds (Chiang *et al*, 1992), and in human wounds (Illingworth and Barker, 1980). Chiang *et al* (1991) provided evidence for the significant role of intrinsic electric fields in the promotion of wound healing in the newt. In experiments that electrically reversed the polarity or abolished the intrinsic wound electric fields, the rate of healing was reduced by about 15%, but increasing the local field did not speed the healing process. This suggests that evolution has already optimized the cellular response mechanisms in healthy individuals, but leaves open the possibility that certain disease states might result in reduced injury currents. In those cases, increasing the local electric field near the wound might well improve the rate of wound healing. Indeed, there are several claims in the literature of enhanced wound healing by electric field application (Vanable, 1989; Gentzkow, 1993).

Mechanisms underlying the galvanotactic response of cells are largely speculative. Early evidence suggesting a requirement of Ca^{++} influx has been documented for galvanotaxis of neural crest cells (Nuccitelli and Smart, 1989) and tadpole epithelial cells (Mittal and Bereiter-Hahn, 1985). Other studies have indicated that redistribution of receptors is necessary for galvanotaxis (Brown and Loew, 1994). It has been suggested that field-induced electrophoresis and/or electroosmosis of membrane glycoproteins is responsible for this effect (Jaffe and Nuccitelli, 1977; McLaughlin and Poo, 1981).

In conclusion, we have demonstrated that the imposition of DC electric fields at strengths equivalent to those measured at wound edges *in vivo* results in directed migration of human keratinocytes, regardless of the composition of the underlying substrate. The magnitude of the response is variable and dependent on the substratum composition. The mechanism of the response is unknown, but probably involves a direct effect on the cell membrane in combination with complex cell-matrix interactions. These results have potential implications in the area of both acute and chronic wound healing.

D. M. Sheridan was supported by a UC Davis Medical Scholar grant from the School of Medicine. This work was supported by National Institutes of Health Grant ES07133.

REFERENCES

Assimacopoulos D: Low intensity negative electric current in the treatment of ulcers of the leg due to chronic venous insufficiency. *Am J Surg* 115:683–687, 1969

Barker AT, Jaffe LF, Vanable JW Jr: The glabrous epidermis of cavies contains a powerful battery. *Am J Physiol* 242:R358–366, 1982

Brown MJ, Loew LM: Electric field-directed fibroblast locomotion involves cell surface molecular reorganization and is calcium independent. *J Cell Biol* 127:117–128, 1994

Carley PJ, Wainapel SF: Electrotherapy for acceleration of wound healing: low intensity direct current. *Arch Phys Med Rehabil* 66:795–801, 1985

Chiang M, Cragoe EJ Jr, Vanable JW Jr: Intrinsic electric fields promote epithelialization of wounds in the newt, *Notophthalmus viridescens*. *Dev Biol* 146:377–385, 1991

Chiang M, Robinson KR, Vanable JW: Electrical fields in the vicinity of epithelial wounds in the isolated bovine eye. *Exp Eye Res* 54:999–1003, 1992

Chu CS, McManus DT, Mason ADJ, Okerberg CV, Pruitt BAJ: Multiple graft harvestings from deep partial-thickness scald wounds healed under the influence of weak direct current. *J Trauma* 30:1044–1049, 1990

Erickson CA, Nuccitelli R: Embryonic fibroblast motility and orientation can be influenced by physiological electric fields. *J Cell Biol* 98:296–307, 1984

Gentzkow GD: Electrical stimulation to heal dermal wounds. *J Dermatol Surg Oncol* 19:753–758, 1993

Grinnell F: The activated keratinocyte: up regulation of cell adhesion and migration during wound healing. *J Trauma* 30:144–149, 1990

Hay E (ed.). *Cell Biology of the Extracellular Matrix*. 2nd ed. Plenum Press, New York, 1991

Illingworth CM, Barker AT: Measurement of electrical currents emerging during the regeneration of amputated fingertips in children. *Clin Phys Physiol Meas* 1:87–89, 1980

Isseroff RR, Ziboh VA, Chapkin RS, Martinez DT: Conversion of linoleic acid by cultured murine and human keratinocytes. *J Lipid Res* 28:1342–1349, 1987

Jaffe LF, Nuccitelli R: Electrical controls of development. *Annu Rev Biophys Bioeng* 6:445–475, 1977

McLaughlin S, Poo M-M: The role of electro-osmosis in the electric field-induced movement of charged macromolecules on the surfaces of cells. *Biophys J* 34:85–93, 1981

Mittal AK, Bereiter-Hahn J: Ionic control of locomotion and shape of epithelial cells: I Role of calcium influx. *Cell Motility* 5:123–136, 1985

Nishimura KY, Isseroff RR, Nuccitelli R: Human keratinocytes migrate to the negative pole in DC electric fields comparable to those measured near mammalian wounds. *J Cell Sci* (in press)

Nuccitelli R: Physiological electric fields can influence cell motility, growth, and polarity. *Adv Cell Biol* 2:213–233, 1988

Nuccitelli R, Erickson CA: Embryonic cell motility can be guided by physiological electric fields. *Exp Cell Res* 147:195–201, 1983

Nuccitelli R, Smart T: Extracellular calcium levels strongly influence neural crest cell galvanotaxis. *Biol Bull* 176:130–135, 1989

O'Keefe EJ, Payne REJ, Russell N, Woodley DT: Spreading and enhanced motility of human keratinocytes on fibronectin. *J Invest Dermatol* 85:125–130, 1985

Rheinwald JG, Green H: Serial cultivation of strains of human keratinocytes: the formation of keratinizing colonies from single cells. *Cell* 6:331–342, 1975

Sarret Y, Woodley DT, Grigsby K, Wynn K, O'Keefe EJ: Human keratinocyte locomotion: the effect of selected cytokines. *J Invest Dermatol* 98:12–6, 1992

Shipley GD, Pittelkow MR: Control of growth and differentiation in vitro of human keratinocytes cultured in serum-free medium. *Arch Dermatol* 123:1541a–1544a, 1987

Stenn KS, Madri JH, Tinghitella T, Terranova VP: Multiple mechanisms of dissociated epidermal cell spreading. *J Cell Biol* 96:63–67, 1983

Vanable JW Jr: Integumentary potentials and wound healing. In: Borgens RB, Robinson KR, Vanable JW Jr, McGinnis ME (eds.). *Electric Fields in Vertebrate Repair*. Alan R. Liss, New York, 1989, pp 171–224

Woodley DT, Bachman PM, O'Keefe EJ: Laminin inhibits human keratinocyte migration. *J Cell Physiol* 136:140–146, 1988

Woodley DT, Chen JD, Kim JP, Sarret Y, Iwasaki T, Kim YH, O'Keefe EJ: Re-epithelialization, human keratinocyte locomotion. *Dermatol Clin* 11:641–646, 1993

Woodley DT, O'Keefe EJ, Prunieras M: Cutaneous wound healing: a model for cell-matrix interactions. *J Am Acad Dermatol* 12:420–433, 1985