Effects of steady electric fields on human retinal pigment epithelial cell orientation and migration in culture

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Abstract. Low-level, steady electric fields of 6-10 volts/cm stimulated directional orientation and translocation of cultured human retinal pigment epithelial cells. The orientative movements (galvanotropism) consisted of somatic elongation of the cells into spindle shapes, followed by pivotal alignment orthogonal to the field. The anodal edges of the cells underwent retraction of their plasmalemmal extensions, while the cathode edges and the longitudinal ends developed lamellipodia and ruffled membranes. These tropic movements were followed by a translocational movement (galvanotaxis) of the cells towards the cathode. Staining of these migrating cells for actin showed the accumulation of stress fibers at the leading (cathodal) edge, as well as at the longitudinal ends of the elongated somata. These results suggest that endogenous, biologically-generated electric fields (eg., injury currents) may play a role in the guidance and migration of retinal pigment epithelial cells after retinal injury.

Key words: cell migration - electric field - retinal pigment epithelium - galvanotaxis - galvanotropism.

Directional shape changes and movements during cell migration are controlled by several extrinsic factors, such as contact inhibition, chemotaxis, haptotaxis, and contact guidance (Trinkaus 1982). In this study, we investigate yet another possible factor that may play a role in controlling cell migration, namely electric (E) fields. The influence of physiological E-fields on the modulation of directional cell movements had previously been demonstrated in non-ocular cells (Nuccitelli 1988; Robinson 1985) and more recently in corneal cells (Soong et al. 1990a,b; Chang et al. 1991). It is now known that various cells respond to low-level, steady E-fields by undergoing a galvanotropic response (a directed, orientational shift or pivoting of cellular alignment in an E-field, concomitantly involving directional elaboration of plasmalemmal extensions), followed by a galvanotaxic response (a directed translocation of the cells in an E-field) (Nuccitelli 1988; Robinson 1985).

Biologically generated E-fields are ubiquitous in nature and may be particularly significant during wound healing and embryonic development (Nuccitelli 1988). In these situations, strong and relatively steady extracellular ionic currents are produced by the living tissue. These currents are distinct from the faster, pulsatile electrical phenomena associated with nerve and muscle action potentials (Nuccitelli 1988). During adult vertebrate tissue healing, injury currents resulting in E-fields of as high as 2 volts/cm have been measured at the cut surface of wounds by the vibrating probe technique (Robinson 1985). During morphogenesis, endogeneous E-fields are also generated by a wide variety of embryonic tissues (Nuccitelli 1988; Robinson 1985). With the progressive closure of a wound or at the conclusion of

morphogenesis, endogenously produced E-fields gradually diminish in magnitude.

Not only do tissues produce E-fields under these circumstances, but individual cells within these tissues may also be responding to the self-generated field stimuli. The threshold for galvanocelluar responses appear to be as low as 70 to 80 mV/cm (Nuccitelli 1988; Robinson 1985). It appears, therefore, that magnitudes of E-fields generated during wound healing and morphogenesis are often well above galvanotropic and galvanotaxic thresholds of the indigenous cells. Galvanocellular responses to endogenously-generated E-fields, therefore, may constitute a non-humoral, intercellular biologic feedback control system guiding cells to their final spatial location within tissue.

The contractile protein, actin, is a ubiquitous cytoskeletal protein thought to mediate cell-to-substrate adhesion, control of cell shape, elaboration of plasmalemmal extensions, and perhaps the generation of tensile forcers necessary for cell migration (Lazarides & Weber 1974; Wehland et al. 1984; Henderson & Weber 1979; Goldman et al. 1976). The intracellular distribution of actin may provide important information on the dynamics of galvanic cellular movements.

In this study, we investigated the effects of steady E-fields on the orientation, migration and re-distribution of actin in cultured human retinal epithelial (hRPE) cells.

Materials and Methods

Cell cultures

RPE cells were isolated from normal human eyes within 24 h of donor death. Briefly, the sensory retina was gently separated from the RPE monolayer. The RPE cells were then enzymatically dislodged from the underlying Bruch's membrane using trypsin (0.25%) in serum-free Dulbecco's Modified Essential Medium (DMEM) containing 0.02 mg/ml DNase I for 30 min. Following centrifugation, the RPE cells were re-suspended in DMEM containing 15% fetal bovine serum, penicillin G (100 U/ml), streptomycin sulfate (100 mg/ml), and amphotericin B (2.5 mg/ml). The cells were then seeded into fibroblast-inhibiting Falcon (Lincoln Park, NJ) Primaria™ flasks and grown to confluency. Flasks of confluent fourth to sixth passage RPE cells were used in all experiments. Cell pigmentation became minimal after multiple passages. The cultured RPE cells exhibited characteristic polygonal arrays, uniform immunohistochemical staining for elaborated basement membrane components, and established functional markers (Elner et al. 1991). Prior to E-field experiments, the cells were treated with 0.25% trypsin and seeded onto RBS™-washed (Pierce Company, Rockford, IL) 22×30 mm, # 1½ glass coverslips at a cell density of approximately 5.0×10^3 cells per coverslip. The cells were cultured on the coverslips for 48 h at 37°C and 5% CO_2. Confluence on the coverslips was avoided since this tended to obscure individual cell movements. Just prior to placement of the cell-laden coverslip into the experimental (galvanotaxis) chamber, the cells were washed in serum-free DMEM. Three different hRPE cell lines were used with the experiments, which were repeated in triplicate for each cell line.

Experimental chamber

Details of the construction of our experimental galvanotaxis chamber has been previously reported (Soong et al. 1990a,b). Chambers for observation of galvanotropism and galvanotaxis were constructed by attaching two parallel glass coverslips (0.18 mm thickness each) 5.0 mm apart onto the coverslip with the attached RPE cells, thus forming a shallow trough 0.18 mm deep and 5.0 mm wide with the cell-covered coverslip forming the floor. The trough was then covered with a glass coverslip, thus producing a thin, closed channel of $0.018 \times 0.5 \times 3.0$ cm volume. This dimensional configuration has an extremely large surface area-to-volume ratio, thus maximizing heat dissipation and resulting in negligible joule heating effects. The maximum observed temperature rise in the galvanotaxis chamber during field stimulation was 0.02°C. A waterproof seal was produced between the coverslips with sterile white petrolatum (E. Fougera Co, Melville, NY) and the channel was filled with serum-free, growth factor-free culture medium. Serum-free, growth factor-free medium was used in order to minimize electrophoretic movements of large, changed proteins. Agar bridge electrodes, filled with 2% agarose dissolved in the same medium, were used in order to prevent contamination of the cell culture medium with metallic electrode breakdown products. The chamber was placed within a custom-contrast microscope. The temperature within the incubator

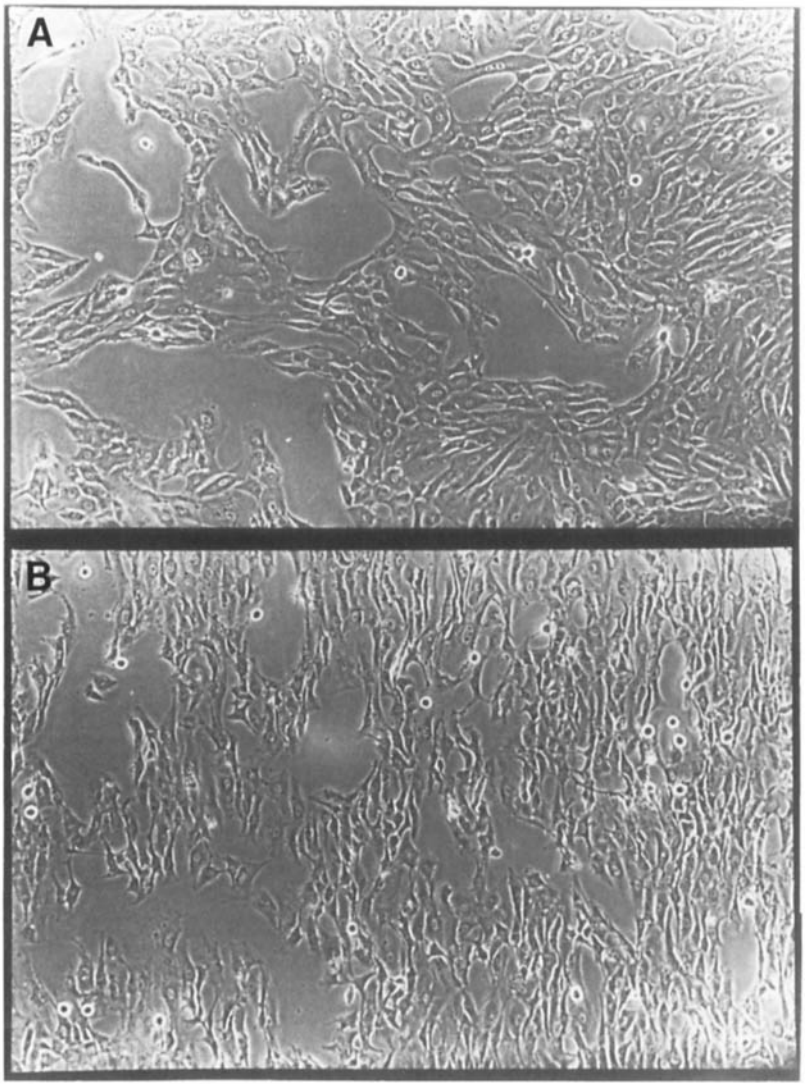

Fig. 1.
Low-power (× 110) inverted phase micrographs of cultured hRPE cells (a) before applying field, and (b) after 4.5 h of exposure to E-field (6 volts/cm), showing galvanotropic alignment of elongated cells perpendicular to applied E-field. Anode is to right and cathode is to left.

was maintained at 37°C with a thermistor-controlled heater system. The temperature in the immediate vicinity of the microscope objective lens was continuously monitored with a separate temperature-sensitive probe. The atmosphere within the chamber was maintained at 5% CO_2. E-field stimuli of 4-10 volts/cm were used in this study.

Detection of cell movement

Cells were photographed at 30 min time intervals with a Nikon 35 mm camera onto ASA 400 film (Kodak Tri-X). A Cartesian grid with 1-mm spacing on a plastic transparency was later superimposed onto enlarged photographic prints of the cells. From the known enlargement and magnification factors, cellular dimensions were calculated. For each time-lapse photographic frame, hRPE cells were analyzed after being each assigned Cartesian coordinates designating the top and bottom ends of the cell, the right and left cell borders, the nuclear center, and the approximate 'center of gravity' of the cell. In addition, the axial angle

relative to the horizontal was determined. The velocities of individual cells that manifested translocation were recorded. These values were averaged and standard errors were calculated. These data were then used to ascertain the magnitude of galvanotropic and galvanotaxic response.

Fluorecent staining for actin

Both E-field treated and control hRPEs (no E-field application) on coverslips were rinsed in phosphate-buffered saline (PBS), pH 7.0, fixed in 3% formaldehyde solution for 10 min at room temperature, treated with 0.05% triton X-100 for 60 sec, and rinsed again in PBS. Both the E-field exposed cells and the controls were labelled for actin with 0.3 μmol/l rhodamine phalloidin for 30 min at room temperature. The coverslips with the adherent cells were covered with a glycerol-based mounting medium and inverted cell-side-down onto glass slides. The edges of the coverslips were sealed with fingernail polish. The cells were examined with an epifluorescence light micropscope (Olympus AH2) and photographed with ASA 400 (Kodak Tri-X) film push-processed to ASA 1000.

Results

At a field strength of 4 volts/cm, no significant changes in cell shape, orientation, or displacement were observed after 2 h when compared with controls that were subjected to no E-fields. After 2.5 h, however, a few cells extended minimal ruffled membranes in the cathodal direction, but there was no measurable cellular translocation. Controls showed only random cellular movements with little or no net directional cell translocation, shape alterations, or orientational changes.

At 6 volts/cm, hPRE cells displayed easily discernible galvanotropism and galvanotaxis. At this stimulus level, all cells manifested galvanotropic movements as early as 30 min after commencement of the E-field. Galvanotropic movements were manifested by elongation of the cells in the direction perpendicular to the field. Cells which were already elongate in shape prior to field application appeared to undergo elongation or rotation (pivoting) to achieve orientation 90° to the E-field. These orientational movements continued for another 2 h, until all the cells were aligned along the same axis (Fig. 1). During the galvanotopic response, lamellipodia, ruffled membranes, and pseudopodia were produced in large numbers on the cathodal side, while similar plasmalemmal protrusions on the anodal side underwent retraction.

The onset of galvanotropic movements was followed by the onset of cellular translocation in at least 50% of the cells (latency of approximately 30 min) toward the cathode at a rate of approximately 16 ± 6 mm/h. Most cells undergoing this process were generally crescent shaped, with the convex side facing the cathode.

At 8 volts/cm, some cells underwent galvanotaxis toward the cathode at rates of as high as 47 mm/h (maximum observed velocity) (Fig. 2), again with a latency of approximately 30 min. Cells that were relatively round prior to E-field exposure responded by producing many plasmalemmal extensions in the cathodal direction, while simultaneously elongating orthogonally to the E-field (Fig. 2). At 10 volts/cm, the average galvanotaxic rate dropped to approximately 12 ± 7 mm/h, but once again the latency remained approximately 30 min. Thus, the galvanotaxic rate appeared to peak at a stimulus strength of 8 volts/cm.

Staining for actin in aligned cells revealed two prominent stress fiber patterns: (1) stress fibers oriented along the longest cellular axis, orthogonal to the E-field, and (2) stress fibers extending towards the cathodal edge of the cell and frequently into the cell membrane extensions on that side (i.e., parallel to the E-field). Controls showed random distributions of actin. In some cells undergoing galvanotaxic translocation, a dense band of actin was observed on the cathodal side, at the leading edge of migration (Fig. 3). Often, from this dense band of actin, stress fibers extended into lamellipodia and filopodia in the direction of migration.

Discussion

Galvanically-induced movements of cells were first observed 80 years ago (Nuccitelli 1988); since then, such movements have been observed in a wide variety of cells, including slime mold (Anderson 1951), amoeba (Verwon 1986) neural crest cells (Stump & Robinson 1983; Nuccitelli & Erickson 1983), regenerating nerves (Jaffe & Stern 1979; patel & Poo 1982), fibroblasts (Yang et al. 1984; Erickson & Nuccitelli 1984), leukocytes (Dineur

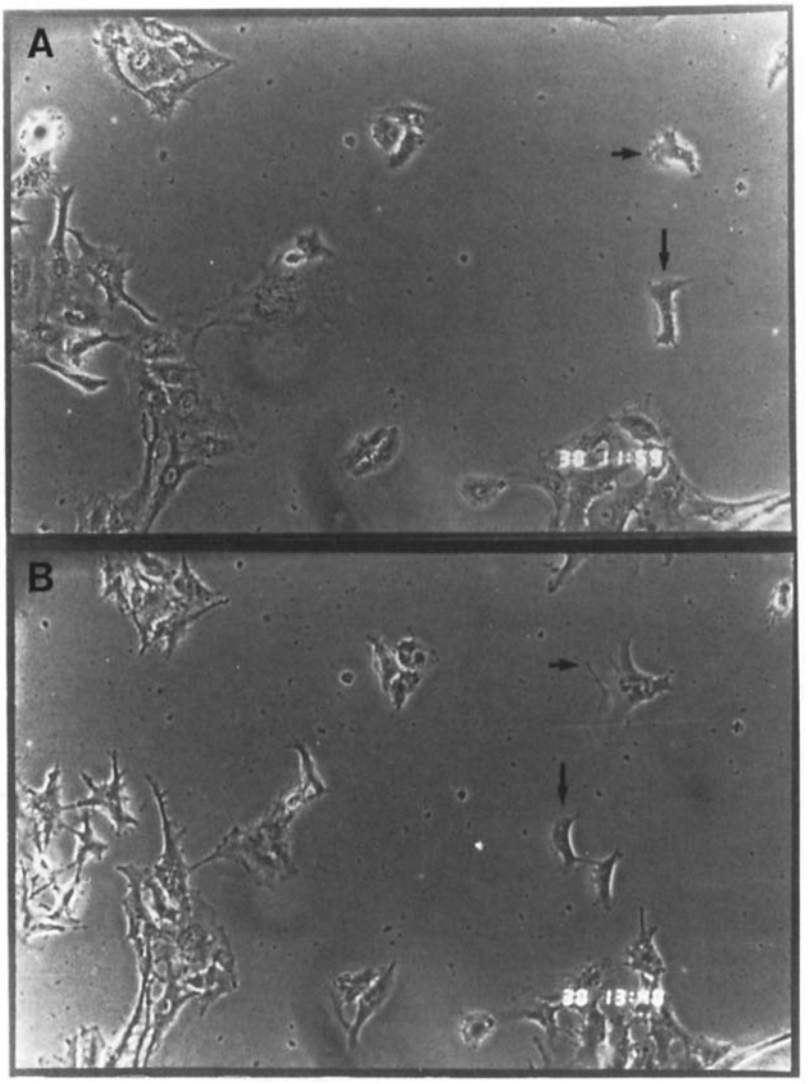

Fig. 2.

Higher-magnification (×220) inverted phase micrographs of hRPEs (a) before applying field, and (b) after ⅔ h of E-field (8 volts/cm), showing galvanotropic changes and glavanotaxis. Note ruffled membranes on cathodal side of cells and translocation of cells in cathodal direction (vertical arrows indicate corresponding cells before and after translocation). Horizontal arrows show new lamellipoidal extensions towards cathode after E-field application. Anode is to right and cathode is to left.

1891; Orida & Feldman 1982), epidermal cells (Robinson 1985; Cooper & Schliwa 1986; Luther et al. 1983), myoblasts (Hinkle et al. 1981), and mammalian osteroblasts and osteoclasts (Ferrier et al. 1986). Our laboratory has also observed galvanotropic and galvanotaxic movements in corneal epithelial, stromal, and endothelial cells (Soong et al. 1990a,b; Chang et al. 1990).

With the development of the vibrating probe technique by Jaffe in 1974 (Jaffe & Nuccitelli 1974), it became feasible to measure and map the current densities, and thus the associated E-fields surrounding living cells, at minute spatial displacements of as little as 10 μm apart. Subsequent investigations using this method have shown the existence of 'physiological', self-generated E-fields surrounding tissue wounds and developing embryos (Nuccitelli 1988). Epithelial monolayers that cover

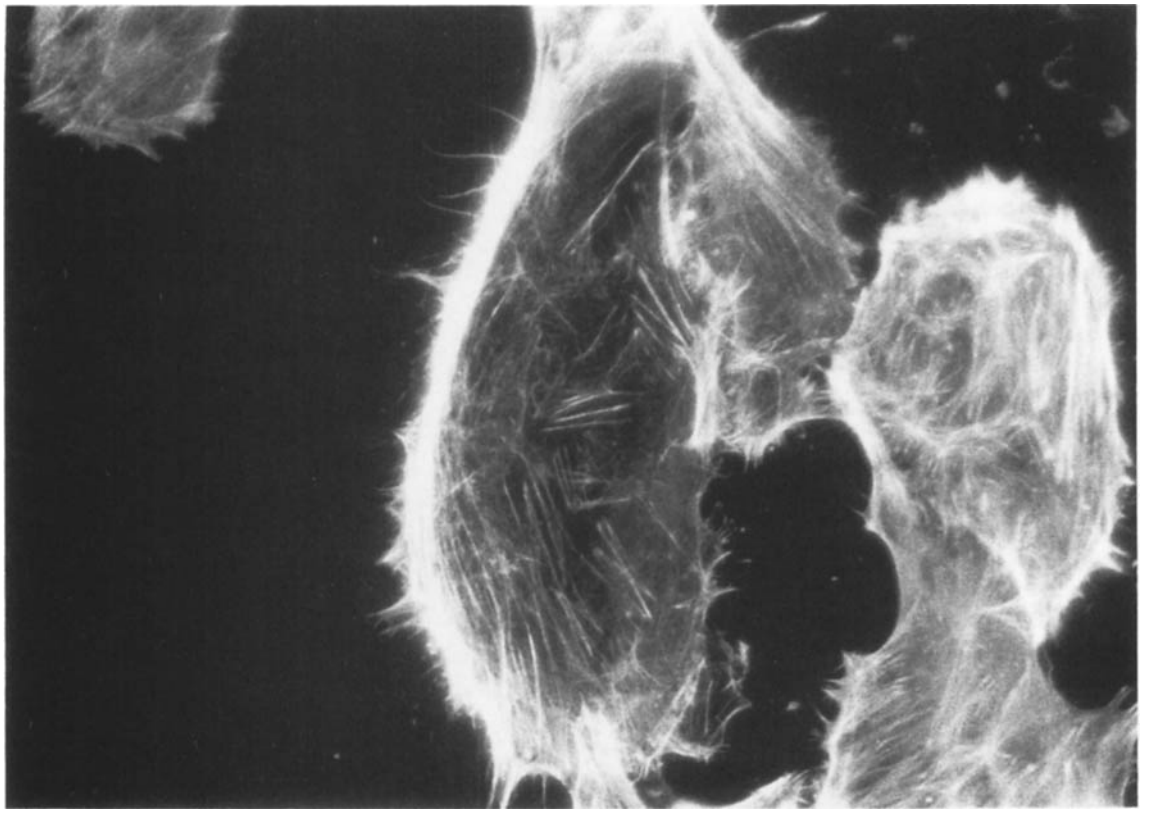

Fig. 3.
Actin distribution in cultured hPRE cells after exposure to E-field (8 volts/cm) for 2.5 h. Note heavy band of actin accumulation located along leading edge (cathodal side), stress fibers oriented perpendicular to field (along long axes of cells), and stress fibers extended in direction of migration (parallel to field). Anode is to right and cathode is to left. (Magnification = ×850).

adult tissue and developing embryos produce a potential difference of as large as 100 mV between the basal and apical surfaces (Jaffe & Stern 1979). It follows that multicellular epithelial layers may generate even larger potential differences than monolayers. These cellular layers can therefore move substantial secondary ionic injury currents out of discontinuities that occur after wounding (Nuccitelli 1988; Robinson & Stump 1984; McCaig & Robinson 1982).

Our previous investigations have shown that corneal epithelial cells move toward the cathode, whereas corneal stromal fibroblasts and corneal endothelial cells migrate toward the anode (Soong 1990a,b; Chang et al. 1991). Corneal epithelial cells (being of ectodermal origin) and RPE cells (being of neuroectodermal origin), both of which exhibit epithelial phenotypes, respond to applied E-fields by migrating toward the cathode. In contrast, corneal stromal fibroblasts and endothelial cells, both of neural crest origin, respond by migrating in the opposite direction. It is, therefore, possible that embryonic origin may influence the directional movements of cells.

In hRPE cells exposed to steady E-fields, the peculiar actin distribution appears to correspond to the galvanically-induced cell shape changes and movements. It is not clear whether the actin cytoskeleton responds directly to the E-field or if the cytoskeletal redistribution occurs as an indirect result of cell surface charges altered by the imposed E-field. Luther and co-workers (1983) reported field-induced cytoskeletal changes in *Xenopus* epithelial cells. In contrast to hRPE cells, these nonmammalian epithelial cells did not manifest a subpopulation of stress fibers that were oriented in the

direction of galvanotaxis, but contained only a band of concentrated actin along the leading edge during galvanotaxis. Recent reports indicate that E-field induced cellular shape changes, galvanotropism, galvanotaxis, and cytoskeletal re-organization may be Ca^{2+} and calmodulin dependent (Nuccitelli 1988; Chang et al. 1990).

RPE migration occurs during retinal wound healing and possibly during embryonic retinal development. In some diseases, such as proliferative vitreoretinopathy, RPE migration may be deleterious leading to vitreal and periretinal fibrous bands which cause recurrent retinal detachment. It is reasonable to hypothesize that injuries to the retina, involving the underlying RPE, may generate injury currents produced by the imbalance in the overall mass distribution of cells. E-fields may constitute one of the components (in addition to contact inhibition, chemotaxis, haptotaxis, and contact guidance) in controlling RPE migration in the eye. Since the electrical potential across an intact RPE monolayer is in the order of 10 mV (retinal side positive) and the RPE cells are approximately 10 μm thick, a small hole through the RPE monolayer would therefore experience a field of ~10 volts/cm, or well within the stimulation magnitudes utilized in our study. It is still unknown if externally-applied E-fields may be utilized to enhance the proliferation and spreading of transplanted RPE cells, on the one hand, or to inhibit proliferative vitreoretinopathy, on the other hand. Further studies are needed to answer these questions.

References

Anderson J D (1951): Galvanotaxis of slime mold. J Gen Physiol 35: 1-16.

Chang P C T, Sulik G L, Soong H K, Parkinson W C, Qu X & Meyer R F (1991): Galvanotropic and galvanotaxic responses of corneal endothelial cells are inhibited by cytochalasin D and W-7. Invest Ophthalmol Vis Sci (suppl) 32: 1219.

Cooper M S & Schliwa M (1986): Motility of cultured fish epidermal cells in the presence and absence of direct current electric fields. J Cell Biol 102: 1384-1399.

Dineur E (1891): Note sur la sensibilite des leucocytes a l'electricite. Bull Seances Soc Belge Microscopie (Bruxelles) 18: 113-118.

Elner S G, Strieter R M, Elner V M, Rollins B J, Del Monte M A & Kunkel S L (1991): Monocyte chemotactic protein gene expression by cytokine-treated human retinal pigment epithelial cells. Lab Invest 64: 819-825.

Erickson C A & Nuccitelli R (1984): Embryonic fibroblast motility and orientation can be influenced by physiological electric fields. J Cell Biol 98: 296-307.

Ferrier J, Ross S M, Kanehisa J & Aubin J E (1986): Osteoclasts and osteoblasts migrate in opposite directions in response to a constant electric field. J Cell Physiol 129: 283-288.

Goldman R D, Schloss J A & Starger J M (1976): Organizational changes of actin-like microfilaments during animal cell movement. In: Goldman R D, Pollard T & Rosenbaum J (eds). Cell Motility, p 217. Cold Spring Harb Press, Cold Spring Harbor (ME).

Henderson D & Weber K (1979): Three-dimensional organization of microfilaments and microtubules in the cytoskeleton. Exp Cell Res 124: 301-309.

Hinkle L, McCaig C D & Robinson K R (1981): The direction of growth of differentiating neurones and myoblasts from frog embryos in an applied electric field. J Physiol (London) 314: 121-135.

Jaffe L D & Nuccitelli R (1974): An ultrasensitive vibrating electrode for measuring steady extracellular currents. J Cell Biol 63: 614-628.

Jaffe L F & Stern C D (1979a): Neurites grow faster towards the cathode than the anode in a steady field. J Exp Zool 209: 115-128.

Jaffe L F & Stern C D (1979b): Strong electrical currents leave the primitive streak of chick embryos. Science 206: 569-571.

Lazarides E & Weber K (1974): Actin antibody: The specific visualization of actin filaments in non-muscle cells. Proc Natl Acad Sci USA 71: 2268-2272.

Luther P W, Peng H B & Lin J J (1983): Changes in cell shape and actin distribution induced by constant electric fields. Nature (London) 303: 61-64.

McCaig C D & Robinson K R (1982): The ontogeny of the transepithelial potential difference in from embryos. Dev Biol 90: 335-339.

Nuccitelli R (1938): Physiological electric fields can influence cell motility, growth, and polarity. In: Miller K (ed). Advances in Cell Biology. Vol 2, p 213. JAI Press, Greenwich.

Nuccitelli R & Erickson C A (1983): Embryonic cell motility can be guided by physiological electric fields. Exp Cell Res 147: 195-201.

Orida N & Feldman J D (1982): Directional protrusive pseudopodial activity and motility in macrophages induced by extracellular electric fields. Cell Motil Cytoskeleton 2: 243-255.

Patel N & Poo M-m (1982): Orientation of neurite growth by extracellular electric fields. J Neurosci 2: 483-496.

Robinson K R (1985): The responses of cells to electric fields: A review. J Cell Biol 101: 2023-2027.

Robinson K R & Stump R F (1984): Self generated electrical currents through Xenopus neurulae. J Physiol 352: 339-352.

Soong H K, Parkinson W C, Bafna S, Sulik G L & Huang S (1990a): Movements of cultured corneal epithelial cells

and stromal fibroblasts in electric fields. Invest Ophthalmol Vis Sci 31: 2278-2282.

Soong H K, Parkinson W C, Sulik G L & Bafna S (1990b): Effects of electric fields on cytoskeleton of corneal stromal fibroblasts. Curr Eye Res 9: 893-901.

Stump R F & Robinson K R (1983): Xenopus neural crest cell migration in an applied electric field. J Cell Biol 97: 1226-1233.

Trinkaus J P (1982): Some thoughts on directional cell movement during morphogenesis. In: Bellairs R, Curtis A & Dunn G (eds). Cell Behaviour, p 471. Cambridge University Press, Cambridge.

Verwon M (1896): Untersuchungen über die polare Erregung der lebendigen Substanz durch den konstanten Strom. III. Mitteliung. Pflugers Arch Eur J Physiol 62: 415-450.

Wehland J, Osborn M & Weber K (1984): Cell to substate contacts in living cells, a direct correlation between interference reflexion and indirect immunofluorescent microscopy using antibodies against actin and neurulae. J Physiol 352: 339-352.

Yang W P, Onuma E K & Hui S W (1984): Response of C3H/10T1/2 fibroblasts to an external steady electric field stimulation: Reorientation, shape change, Con A receptor and intramembranous particle distibution and cytoskeleton reorganization. Exp Cell Res 155: 91-104.

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