Journal of Cell Science 109, 1405-1414 (1996)
Printed in Great Britain © The Company of Biologists Limited 1996
JCS4102

Orientation and directed migration of cultured corneal epithelial cells in small electric fields are serum dependent

Min Zhao[1], Adriana Agius-Fernandez[2], John V. Forrester[2] and Colin D. McCaig[1,*]

[1]Department of Biomedical Sciences, Marischal College, and [2]Department of Ophthalmology, University of Aberdeen, Aberdeen AB9 1AS, Scotland, UK

*Author for correspondence (e-mail: phy059@abdn.ac.uk)

SUMMARY

Reorientation and migration of cultured bovine corneal epithelial cells (CECs) in an electric field were studied. Electric field application was designed to model the laterally directed, steady direct current electric fields which arise in an injured corneal epithelium.

Single cells cultured in media containing 10% foetal bovine serum showed significant galvanotropism, reorienting to lie perpendicular to electric field vector with a threshold field strength of less than 100 mV/mm. Cells cultured in serum-free medium showed no reorientation until 250 mV/mm. Addition of EGF, bFGF or TGF-β1 singly or in combination to serum free medium significantly restored the reorientation response at low field strengths. Both the mean translocation rate and directedness of cell migration were serum dependent. Cultured in medium with serum or serum plus added EGF, single cells showed obvious cathodal migration at 100 mV/mm. Increasing electric field strength enhanced the cathodal directedness of single cell migration. Supplementing serum free medium with growth factors restored the cathodal directed migration of single cells and highest directedness was found for the combination of EGF and TGF-β1. Corneal epithelial sheets also migrated towards the cathode in electric fields. Serum or individual growth factors stimulated CEC motility (randomly directed). Applied fields did not further augment migration rates but added a vector to stimulated migration.

Electric fields which are present in wounded cornea interact with other environmental factors and may impinge on CECs migration during wound healing. Therapies which combine the application of growth factors and eclectric fields may be useful clinically.

Key words: Corneal wound healing, Cell migration, Corneal epithelium, Electric field, Galvanotropism, Galvanotaxis, Serum, Growth factor

INTRODUCTION

Electric fields (EFs) may play an important role in wound healing. Wounded epithelia generate small direct current (dc) electric fields (EFs), whilst keratinocytes and epithelial cells, including rabbit corneal epithelial cells (CECs), reorient perpendicularly and migrate cathodally when cultured in a small dc field (Nishimura et al., 1996; reviewed by Nuccitelli, 1988, Robinson, 1985 and Vanable 1989; Soong et al., 1990a). Additionally, disrupting the naturally occurring, laterally oriented electric fields in the epithelium of experimentally wounded newt skin, prevents wound healing, whilst normal rates of re-epithelialisation are restored by passing exogenous current, which mimics the lateral electric fields (Chiang et al., 1991).

Vascular, inflammatory and neural factors are also important in wound healing. Studies with cultured CECs indicate that blood factors enhance corneal wound healing in this avascular tissue (Jumblatt and Neufeld, 1986). Based on experiments with human corneal organ cultures, Collin et al. (1995) concluded that serum factors may be more important in stimulation of epithelial cell migration than in proliferation.

EFs associated with a wound coexist with serum elements known to promote cell migration. Together these influences might interact to modulate electric field-directed cell migration. The only previous study of an EF and CECs used serum-free medium, and found a relatively high threshold for EF-directed cell migration (Soong et al., 1990a). Perhaps presenting CECs with an EF and serum elements together (as is likely to occur following a wound), might reduce the threshold field strength required for directed migration, and indicate a serum-dependency of field-directed CEC migration.

Many growth factors increase random cell motion and/or induce directional migration. EGF (epidermal growth factor), bFGF (fibroblast growth factor basic) and TGF-β1 (transforming growth factor-beta 1) all affect cell growth and movement (reviewed by Stoker and Gherardi, 1991). In vivo and in vitro investigations have shown that peptide growth factors enhance corneal wound healing, in which epithelialisation through proliferation and migration of epithelia is a key event. mRNA coding for EGF, bFGF, TGF-β1 and the protein products are found in cornea and/or its vicinity (Adamis et al., 1991; Nishida et al., 1994; van Setten et al., 1992; Wilson et al., 1992a,b, 1994). EGF receptors(EGFRs) are present on CECs (Frati et al., 1972), especially limbal stem cells (Zieske and Wasson, 1993), which

generate transient cells proliferating and migrating centripetally both to maintain a normal cornea and to assist in corneal wound healing. Receptors for bFGF exist on human corneal epithelium (Wilson et al., 1993a,b) and those for TGF-β1 are assumed to be present on epithelia, since they show specific behavioural responses to exogenously applied growth factors (Wilson et al., 1992b). These growth factors may therefore impinge on migrating epithelial cells through autocrine and/or paracrine pathways. In vitro TGF-β1 is a potent chemotactic agent for bovine and human corneal epithelium, while EGF and bFGF have both chemotactic and chemokinetic effects upon CECs (Grant et al., 1992). Although directed cell migration has been studied in the presence of either growth factors (Grant et al., 1992) or EFs alone (reviewed by Robinson, 1985; Soong et al., 1990a,b; Sulik et al., 1992), interactions between these two influences are only beginning to be studied. EFs and growth factors interact to modulate neurite growth in vitro (McCaig et al., 1995). Although apparently disparate stimuli, growth factors and EFs may exert their effects through shared mechanisms, protein kinases for example (reviewed by Schlessinger and Ullrich, 1992; Wrana et al., 1994; Chen and Weinberg 1995; Nuccitelli et al., 1993). Additionally, EGF (or serum components) and EFs redistribute and reorganise cell surface receptors and elements of the cytoskeleton (Rijken et al., 1991; Brown and Loew, 1994; Luther et al., 1983; Giugni et al., 1987; Soong et al., 1990b). Therefore there is the possibility that growth factors and EFs interact or co-operate with each other mechanistically. Clarifying the signalling systems used would further our understanding of EF-directed cell movement. Such interactions may also provide a basis for promoting corneal wound healing by enhancement of directional cell migration.

We report that cultured bovine CECs reorient to lie perpendicular to a small applied electric field and migrate cathodally. These responses are field strength-dependent and occur at field strengths similar to those associated with a wound in bovine cornea (Chiang et al., 1992). Both responses are serum-dependent and are restored specifically by the addition of optimal concentrations of individual growth factors to serum-free medium. Part of the preliminary results have been published in abstract form (Zhao et al., 1995a,b).

MATERIALS AND METHODS

Materials

Tissue culture plastic dishes, 100 mm × 20 mm (Falcon 3003), were used. Dulbecco's modified Eagle's medium (DMEM), foetal bovine serum (FBS), penicillin and streptomycin, L-glutamine, gentamicin and Fungizone were obtained from Gibco BRL (Scotland). Dispase II, Trypsin and Kanamycin were from Boehringer Mannheim (Germany). Human recombinant EGF (expressed in *Saccharomyces cerevisiae*), bFGF (from bovine pituitary glands) and TGF-β1 (from human platelets) were from Sigma. Anti-bovine bFGF monoclonal antibody (type I, monoclonal IgG1K) was from TCS biologicals Ltd. Stock solutions of EGF and bFGF were reconstituted with 5 ml of serum free DMEM. Stock solution of TGF-β1 was made by addition of 4 mM HCl containing 1 mg/ml bovine serum albumin (BSA). Samples were stored at −70°C and diluted to the final working concentration immediately before use.

Cell cultures

Fresh bovine eyes were obtained from a local abattoir immediately after killing and refrigerated until used (usually within 2-10 hours). Full-thickness sheets of corneal epithelium were obtained using modifications of a previously described method (Gipson and Grill, 1982). Blocks (about 3×3 mm^2) of limbus corneal epithelial layer (with part of stroma) next to the conjunctiva were removed under sterile conditions and transferred into a dish containing Dispase II (1.2 i.u./ml) in calcium- and magnesium-free PBS (with penicillin 250 i.u./ml, streptomycin 250 μg/ml, gentamicin 0.5 mg/ml, Fungizone 7.5 μg/ml), and incubated at 37°C for 1.5 hours. The epithelial layer was peeled off in saline under a dissection microscope and transferred to saline containing 0.25% trypsin for 5-7 minutes at 37°C with intermittent gentle shaking. Digestion was stopped by adding 10-15 ml DMEM (containing L-glutamine 0.04 mM/ml, penicillin 4 i.u./ml, streptomycin 4 μg/ml, kanamycin 50 ng/ml, Fungizone 7.5 μg/ml and 10% FBS) to deactivate the protease. The cell pellets collected after centrifugation at 1,000 rpm for 5 minutes twice in the same medium were resuspended to a cell density of $14\text{-}24\times10^6$ cells/ml and seeded in a specially made trough formed by two parallel (1 cm apart) strips of glass coverslip (No.1, length of 6.4 cm or 2.2 cm) fixed to the base of the dish with silicone grease (Don Corning, MS4). Scratch lines were made perpendicular to the long axis of the chambers with a fine sterile needle and used as reference marks for directed cell migration. The cells were incubated for 24-48 hours (37°C; 5% CO_2), allowing them to settle and adhere to the base of the dish, before a roof of No.1 coverglass was applied and sealed with silicone grease. The final dimensions of the chamber, through which current was passed, were 64 mm × 10 mm × 0.5 mm or 2.2 mm × 10 mm × 0.5 mm (Fig. 1A).

Electric field stimulation

EFs were applied within 24-48 hours after the cells were seeded and continuous illumination was avoided. Agar-salt bridges not less than 15 cm long were used to connect silver/silver chloride electrodes in beakers of Steinberg's solution, to pools of excess culture medium at either side of the chamber. This prevents diffusion of electrode products into the cultures (Fig. 1B). Field strengths were measured directly at the beginning and end of the observation period. No fluctuations in field strength were observed. Immediately prior to field application, using a push-pull technique and hand-held Pasteur pipettes, DMEM with or without 10% FBS and growth factors was exchanged into the cultures. For those experiments with antibody against bFGF, after constitution of the final solution with antibody in medium containing bFGF or 10% FBS, the medium was incubated at 37°C for 30 minutes with intermittent gentle shaking before exchanging into the culture chamber.

Quantification of the cell behaviour

Serial pictures were taken immediately before EF application and then hourly for up to 5 hours. Individual frames were stored using a Leica Image Analyser (Leica, Q500MC, Cambridge).

Perpendicular alignment

Quantification of cell orientation was as described in detail by Erickson and Nuccitelli (1984). In brief, the orientation of a cell with respect to the EF was defined as a function of $\cos2\theta$, where θ is the angle formed by the intersection of a line drawn through the long axis of each cell with a line drawn perpendicular to the field lines. This polarisation index varies from −1 to 1. A cell with its long axis parallel to the vector of EF will have a polarisation of −1, and a cell with its long axis exactly perpendicular to the EF vector will have a polarisation of 1. A randomly oriented population of cells will have an average polarisation (defined by $\Sigma n\cos2\theta/n$) of 0. The angle θ was measured using the image analyser and average polarisation for the cell population ($\Sigma n\cos2\theta/n$) was calculated. The significance of this two-dimensional orientation distribution against randomness was calculated using Rayleigh's distribution (Curray, 1956). The probability that the population is randomly oriented is given by $P=e^{-(L^2n)(10^{-4})}$, where $L=[(\Sigma n\sin2\theta/n)^2+(\Sigma n\cos2\theta/n)^2]^{\frac{1}{2}}/n(0.01)$, and n is the total number of cells. A probability level of 0.01 was used as the limit for significant polarisation.

Directed motility

Directedness and speed of movement were measured by tracing the position of cell nuclei before and after EF application from the image analyser screen onto acetate sheets. All viable cells were included in the analysis. However some cells moved very little over periods of less than 5 hours, therefore cell positions were traced at 0 hours and at 5 hours. Images were captured at intervening intervals to ensure correct identification of all cells. Total cell displacement was measured from the position in which each cell started at the beginning of the observation period to its location at the end of 5 hours with the analyser. The total displacement over 5 hours was divided by 5, to give a *mean translocation rate*. The method of Gruler and Nuccitelli (1991) was used to quantify the *directedness* of the average cellular translocation. The angle that each cell moved with respect to the imposed EF direction was also measured with the image analyser from the acetate sheets with scatter plots of cell translocation. Specifically the cosine of this angle would be equal to 1 if the cell moved directly along the field lines toward the cathode; 0 if the cell moved perpendicular to the field direction; and −1 if the cell moved directly toward the positive pole of the field. By taking the average of all the cosines, one can quantify the average *directedness* of movement. If as many cells moved toward the anode as toward the cathode, the average cosine would be 0. This average was calculated for each distribution from the equation $\Sigma i \cos\theta/n$, where θ is the angle between the field vector and the cellular translocation direction and n is the total number of cells.

Statistical analysis was made using unpaired, two-tailed Student's *t*-test, or Welch's unpaired *t*-test when s.d. was significantly different from each other. Data are expressed as mean ± s.e.m., unless stated otherwise.

RESULTS

Orientation response of CECs

Orientation at different field strengths

CECs in control cultures were randomly distributed with no predominant orientation of their long axes and thus an average polarisation very near zero (Table 1). In an applied dc EF many cells reoriented to lie perpendicular to the field vector (Fig. 2). Perpendicular realignment was voltage-dependent (Table 1), with polarisation values increasing with stronger EFs. Cells cultured in serum free medium showed slight polarisation without significant statistic difference (Table 1). Marked perpendicular reorientation of cells in serum-free medium occurred at 250 mV/mm (highest voltage used), which produces an average polarisation of 0.35-0.4 in 5 hours (a polarisation value of 1 indicates that every cell is perfectly aligned perpendicular to the field vector, while −1 indicates that every cell is perfectly aligned parallel to the field vector). A field strength of 250 mV/mm produces a voltage drop about 5 mV across an average cell that is ~20 μm wide.

Orientation in different medium

The polarisation response was dependent on the presence of serum in the medium (Table 1). Up to 200 mV/mm there was no significant perpendicular reorientation of cells in serum free medium. However for cells in medium with 10% FBS, significant perpendicular reorientation of cells occurred at 100 mV/mm, equivalent to about a 2 mV voltage drop across an average CEC. This field strength is close to that measured (42 mV/mm) following experimental corneal wounds. Cells cultured in medium with serum showed significantly higher polarisation values than those cultured in serum free medium at field strengths of 150-200 mV/mm (Table 1).

Time course of orientation response

By analysing sequential pictures of cell cultures during exposure to EFs, we studied the time course of the cell orientation response at 150 mV/mm in medium with 10% FBS. Retraction of lamellipodia facing the anode and extension towards the cathode were seen as early as 5 minutes after EF application. Polarisation values for cells from 0 hours (before EF application) up to 5 hours in EF were: 0 hours, –0.02±0.08; 1 hour, 0.10±0.07; 2 hours, 0.13±0.08; 3 hours, 0.14±0.08; 4 hours, 0.20±0.08; 5 hours, 0.35±0.08 (cell numbers analysed 61-74). Significant orientation occurred between 3 and 5 hours and at 5 hours polarisation was highly significant (P=0.0025 compared to pre-exposure value). Most cells retained this perpendicular reorientation during subsequent periods of cathodal migration (Fig. 2).

Growth factor addition to serum free medium restores perpendicular reorientation

Since the perpendicular alignment of cultured CECs was serum-dependent at low field strengths (100-200 mV/mm) (Table 1), we supplemented serum free medium with growth factors to investigate whether reorientation could be restored at 150 mV/mm. Addition of EGF, bFGF or TGF-β1 to serum free medium significantly restored perpendicular reorientation of CECs in EFs (Table 1). bFGF had the strongest effect. Significantly higher polarisation than that in medium with 10% FBS was found in serum free medium with 100 or 200 ng/ml of bFGF added (P<0.05; Table 1). Higher polarisation values than found in serum, also arose for cells in medium with EGF or TGF-β1 at certain concentrations. The reorientation response was restored in a concentration-dependent manner by each growth factor alone. We varied the concentration of GFs (Table 1) and

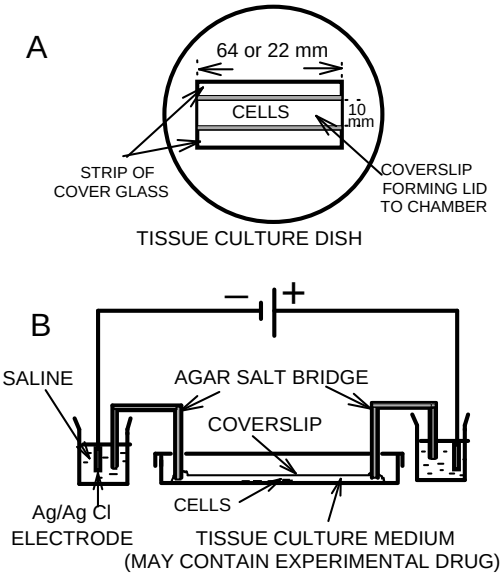

Fig. 1. Experimental design of culture chamber and field application. (A) Chamber constructed within a tissue culture plastic dish, viewed from above. (B) Side-on view includes dc power supply and Ag/AgCl electrodes isolated from the culture chamber using agar-gelled salt bridges.

Table 1. Average polarisation of corneal epithelial cells after 5 hours in indicated medium and field strength (mean ± s.e.m.)

Medium (DMEM +)	Field strength (mV/mm)	Polarisation (cos2θ) (mean ± s.e.m.)	Probability that distribution is random
Serum-free	0	0.03±0.11(49)	0.88
	150	0.13±0.08(77)	0.15
	200	0.17±0.09(57)	0.15
	250	0.35±0.10(51)*	0.0014
10% FCS	0	–0.037±0.07(92)	0.14
	100	0.207±0.08(69)*	0.05
	150	0.365±0.09(59)†,‡	0.0004
	200	0.461±0.08(67)†,§	3.71E-07
	250	0.482±0.09(47)†	1.39E-05
10% FCS	0	–0.01±0.09(54)	0.135
+ EGF (25 ng/ml)	100	0.33±0.10(59)†	0.00051
	150	0.35±0.08(61)†,‡	0.000237
	200	0.47±0.08(64)†,§	2.32E-06
	250	0.40±0.07(61)†	4.97E-05
bFGF (ng/ml)			
25	150	0.38±0.08(72)§	7.6E-06
50	150	0.35±0.09(60)§	7.0E-04
100	150	0.63±0.07(55)¶	4.64E-10
200	150	0.64±0.07(59)¶	9.4E-12
EGF (ng/ml)			
1.25	150	0.21±0.10(46)	0.111
6.25	150	0.27±0.10(56)	0.012
12.5	150	0.59±0.06(77)¶	4.77E-12
25	150	0.52±0.05(129)¶	4.44E-16
50	150	0.60±0.09(51)¶	9.79E-09
100	150	0.36±0.08(61)	0.000439
TGF (pg/ml)			
0.5	150	0.21±0.08(71)	0.01248
1.0	150	0.34±0.08(69)	0.000228
1.5	150	0.40±0.09(54)§	0.000171
2.0	150	0.58±0.06(59)¶	1.72E-10
bFGF (100 ng/ml) + TGF (1 pg/ml)	150	0.40±0.07(74)§	2.69E-06
EGF (25 ng/ml) + bFGF (100 ng/ml)	150	0.53±0.06(100)¶	7.37E-13
EGF (25 ng/ml) + TGF (1 pg/ml)	150	0.39±0.10(34)‡	0.001987
EGF (25 ng/ml) + bFGF (100 ng/ml) + TGF (1 pg/ml)	150	0.40±0.09(65)§	1.35E-05

Compared with that in same medium without applied EF: *P<0.05; †P<0.01.
Compared with that in serum-free medium at same field strength: ‡marginally significant (P very close to 0.05); §P<0.05; ¶P<0.01.

established the concentration dependency of the reorientation response. Significant cell polarisation occurred in medium with 6.25 ng/ml of EGF. Highest polarisation of cells was found with an EGF concentration in the range of 12.5-50 ng/ml. The threshold for a response lay between 1 and 6.25 ng/ml EGF.

TGF-β1 in serum free medium showed a similar concentration-dependent effect on the polarisation. Significant polarisation arose between 0.5 and 1.5 pg/ml and polarisation increased with growth factor concentration. As BSA was used in preparing a stock solution of TGF-β1 (see Materials and Methods), serial concentrations of albumin corresponding to those present in the added TGF-β1 experiments were also tested. Four albumin concentrations (corresponding to each of the four points of TGF-β1, Table 1) yielded polarisation of the following values, respectively: –0.03±0.08 (n=74), 0.17±0.09 (n=51), 0.15±0.09 (n=55) and 0.11±0.09 (n=48). Thus the concentration dependency of the restoration of field-induced reorientation by TGF-β1 cannot be attributed to BSA and must be a specific effect of the growth factor.

To test whether the orientation response restored in medium with bFGF was growth factor specific, we used a monoclonal antibody against bFGF. Neutralisation of bFGF did not affect the reorientation response of cells in medium containing 10% FBS (polarisation 0.37±0.09 (n=46) versus 0.28±0.02 (n=31) in neutralised medium, P=0.11). However, in serum-free medium with added bFGF, there was a significant decrease in polarisation value after neutralisation of bFGF with antibody (0.33±0.08 (n=80), versus 0.63±0.07 (n=55) in non-neutralised medium, P=0.014).

Combinations of growth factors also restored perpendicular orientation (Table 1), but were no better than single growth factors.

Migration of single cells

Single CECs in EFs become perpendicular to the field and migrate cathodally (Fig. 2). The cells, ~20 μm in diameter, extended membrane preferentially at the leading edge or at both ends of the long axis. Fig. 3 shows scatter plots of the cellular distribution after 5 hours in EFs of different strength and in different medium. We quantified cell migration using two components: *directedness* of cell migration and *mean translocation rate* of migration (see Materials and Methods).

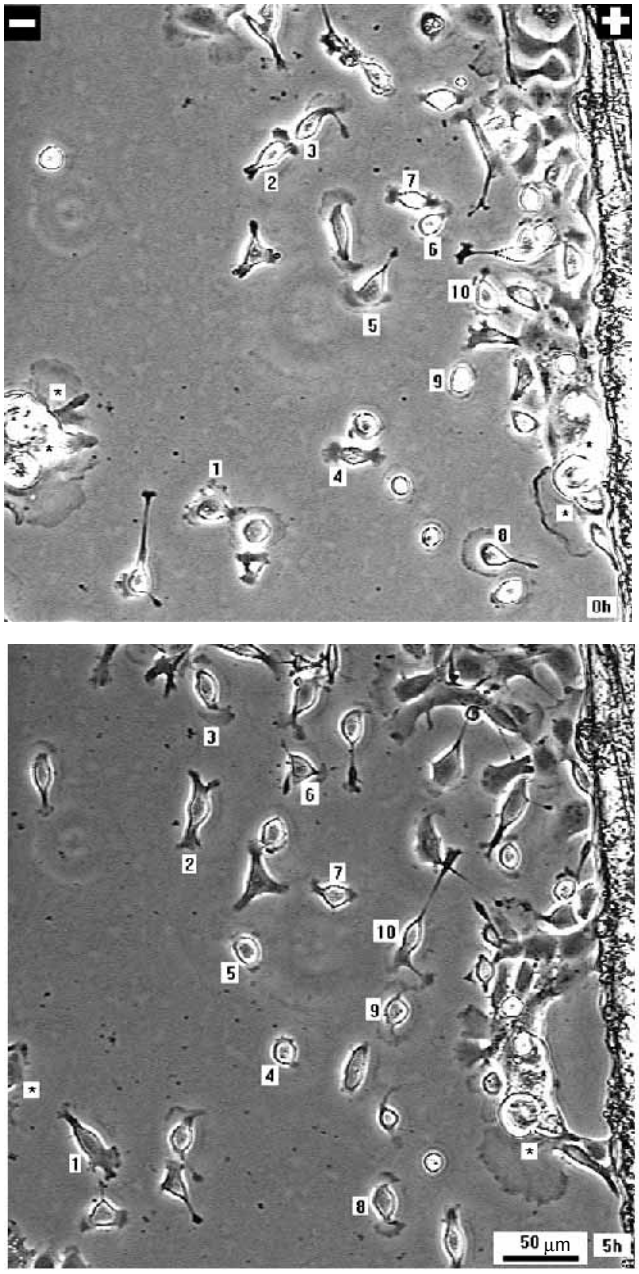

Fig. 2. Bovine corneal epithelial cells migrated cathodally and aligned perpendicular to the EF vector. The reference scratch is to the right of the picture. Same cells are indicated by same numbers before and after 5 hours in EF. Cell sheets (*) also migrated towards the cathode. EF = 150 mV/mm, polarity as shown. Cultured in serum free medium with 25 ng/ml EGF, 100 ng/ml bFGF and 1 pg/ml TGF-β1.

Directedness of the cell migration

At 150 mV/mm in medium with 10%FBS, about 10% of single cells had moved toward the cathode within 30 minutes, with 75% of cells migrating cathodally after 1 hour. The time it took for half of the cells to begin migrating directionally in EF at the field strength of 150 mV/mm is about 45 minutes. Cathode-directed migration of single CECs was striking after 5 hours in EFs (Fig. 3). The directedness of cell migration varied directly with EF strength. However, even at the lowest field strength tested (100 mV/mm), significantly more migration was directed cathodally than anodally over 5 hours (Figs 3, 4). Thus, the threshold for directed migration is less than 100 mV/mm. For cells in serum free medium exposed to EFs up to 200 mV/mm, the average cosine of this distribution of migration directions ($\Sigma i\cos\theta/n$) was not significantly different from that in the absence of a field (Fig. 4). Thus the directedness of migration in EFs below 250 mV/mm was serum dependent (Figs 3, 4). When the current polarity was reversed the direction of cell migration was reversed (toward the new cathode side) (Fig. 5).

Effects of growth factors on the directedness of cell migration

Since the directedness of cell migration at low field strength was serum-dependent, we asked whether adding individual growth factors to serum free medium could restore directedness of CEC migration. Specific growth factors significantly restored cathodal directedness in a concentration-dependent manner, although this differed from the concentration-dependent profile seen for cell reorientation (Figs 3, 6; Table 1). Each single growth factor restored cathodal directedness to about the same as that in medium with 10% FBS (Fig. 6) (~0.6-0.8, a value of $\Sigma i\cos\theta/n$ closer to 1 means more cells move toward the cathode, closer to −1 means more cells move to the opposite direction). BSA control experiments showed that the directedness induced with TGF-β1 was not caused by the presence of albumin in the stock solution of TGF-β1 (Fig. 6).

Some combinations of growth factors did not increase further the directedness of cell migration over single growth factors with $\Sigma i\cos\theta/n$ values of: 0.29±0.09 for EGF (25 ng/ml) + bFGF (100 ng/ml); 0.48±0.08 for bFGF (100 ng/ml) + TGF-β1(1 pg/ml) (compared with Fig. 6). However, using a combination of EGF (25 ng/ml) + TGF-β1 (1 pg/ml) or the three growth factors together (EGF (25 ng/ml) + bFGF (100 ng/ml) + TGF-β1 (1 pg/ml)), restored the directedness of cathodal migration completely with $\Sigma i\cos\theta/n$ values of 0.81±0.04 and 0.73±0.05, respectively (compare with Fig. 6).

Mean translocation rate of cultured CECs

Mean translocation rate of CECs varied significantly in different media. In serum free medium (no electric field), single CECs moved slowly and randomly with a track velocity of 4.8±0.8 μm/hour (n=43) (Table 2). In the presence of 10% FBS, or 10% FBS with added EGF (25 ng/ml) (still no applied field) translocation rates increased 3- to 4-fold and were between 13 and 16 μm/hour (Table 2). Thus serum markedly augmented cell motility. Electric field application (100-250 mV/mm) did not increase translocation rates either in serum free medium, medium with 10% FBS, or in medium with 10% FBS and added EGF (25 ng/ml) (Table 2).

Specific concentrations of single growth factors (added to serum free medium) increased the mean translocation rate of CECs exposed to 150 mV/mm, but to a lesser extent than when whole serum was added. Significant increases (P<0.01) in mean translocation rate were found for bFGF (100 ng/ml): 7.6±0.7 (n=51), EGF (25 ng/ml): 7.6±0.4 (n=127) and TGF-β1 (1 pg/ml): 6.2±0.5 (n=75), compared with a translocation rate in serum free medium alone of 4.2±0.3 (n=51) and a translocation rate at 150 mV/mm in 10% FBS of 14 μm/hour (Table 2). Combinations of growth factors (concentrations as above) also sig-

nificantly increased the mean translocation rate of cell migration as follows, with EGF + TGF-β1 giving the greatest increase (all in μm/hour): EGF + bFGF, 7.7±0.5 (n=63); EGF + TGF-β1, 10.7±0.9 (n=61); bFGF + TGF-β1, 5.9±0.4 (n=66); EGF + bFGF + TGF-β1, 7.5±0.6 (n=82) (P<0.01).

Electric field directed migration of cell sheets

Marked migration of sheets of CECs (~3 to 50 cells together) towards the cathode was observed in EFs (Fig. 2). As for single CECs this was voltage and serum dependent. In serum free medium, the translocation of sheets of CECs cathodally was also restored by addition of specific growth factors. Since it is very difficult to quantify the directional migration of cell sheets in the way we have for single cells, a different quantification method was used and detailed results on directed migration of CEC sheets are presented elsewhere (Zhao et al., 1996).

DISCUSSION

EFs, directed cell migration and wound healing

Endogenous and applied EFs direct cell growth, with implications for development (Hotary and Robinson, 1990, 1992, 1994) and wound healing (Nishimura et al., 1996; Chiang et al., 1991). A potential difference exists across the entire cornea (Maurice, 1967; Klyce, 1972; Chiang et al., 1992), with most of the potential drop across the epithelium. Lesion-induced disruption to this transepithelial potential difference (TEP), instantaneously results in a steep, laterally oriented voltage gradient close to the newly created current sink. In bovine cornea, EFs of 42 mV/mm close to a wound have been measured directly and may be underestimates (Chiang et al., 1992). In newts, wound currents are implicated in re-epithelialisation. Disruption of the naturally occurring wound current of the skin inhibits wound healing, whilst normal rates of epithelialisation are restored by passing exogenous currents to mimic the inhibited wound current (Chiang et al., 1991). A wounded cornea, covered by a tear film to keep the outer surface moist, resembles amphibian skin, in which EFs play important roles in wound healing (reviewed by Vanable, 1989; Chiang et al., 1991). (However there are important differences; see Physiological/clinical significance, below).

Soong et al. (1990a) have shown that rabbit CECs change shape to lie perpendicular to an applied field and migrate

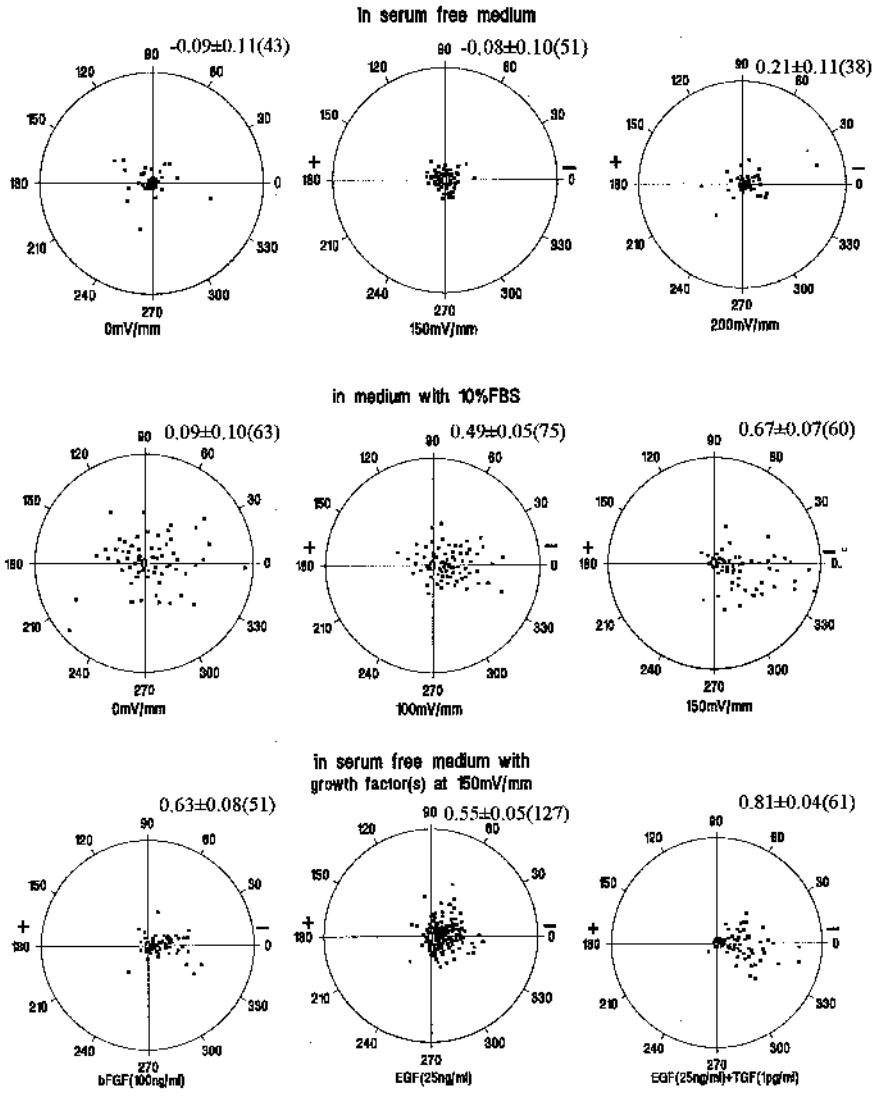

Fig. 3. Translocation response of bovine corneal epithelial cells during a 5 hour period in the indicated field strength and culture medium. After tracing from a computer screen the position of each cell before and after 5 hours in the field, the initial point was placed at the origin and the final location plotted as a single point on the circular graph. The radius of each circle represents 200 μm of translocation distance, and the average cell length is ~20 μm. The average cosine of the distribution ± s.e.m., and the number of cells plotted, are indicated to the upper right of each distribution.

Table 2. Mean translocation rate over 5 hours and directedness of corneal epithelial cell migration (mean ± s.e.m.)

Group	Field strength (mV/mm)	Track velocity (μm/hour)	Average cosine
Serum-free	0	4.84±0.76(43)	–0.06±0.73
	150	4.24±0.29(51)	–0.08±0.10
	200	5.04±0.74(38)	0.21±0.10
	250	2.77±0.33 (28)*	0.28±0.12†
10% FCS	0	12.77±1.08(63)‡	0.08±0.09
	150	13.83±1.47(60)‡	0.67±0.06‡,§
	200	10.68±1.07(71)‡	0.63±0.05‡,§
	250	7.84±0.99(32)‡,§	0.68±0.08‡,§
10% FCS	0	16.26±1.89(40)‡	–0.02±0.11
+ EGF (25 ng/ml)	100	9.89±0.65(75)§	0.49±0.07§
	150	15.25±1.55(42)‡	0.75±0.06‡,§
	200	12.75±1.85(28)‡	0.57±0.10‡
	250	11.42±1.49(35)‡	0.50±0.06§
Serum-free DMEM			
+ EGF (25 ng/ml)	150	7.56±0.70(127)‡	0.63±0.08‡
+ bFGF (100 ng/ml)	150	7.57±0.37(51)‡	0.55±0.05‡
+ TGF-β1 (1 pg/ml)	150	6.24±0.50(75)‡	0.50±0.07‡

Compared with that in same medium without applied EF: *P<0.05; §P<0.01.

Compared with that in serum-free medium at same field strength: †P<0.05; ‡P<0.01.

towards the cathode. However, the threshold for these events (400 mV/mm) was almost tenfold higher than the fields measured at wounds in bovine cornea (Chiang et al., 1992). We have extended these observations using bovine CECs and a much more quantitative approach and confirm that cells elongate perpendicular to a small applied EF and migrate cathodally. Additionally for bovine cells: (1) field-directed responses required serum in the culture medium; (2) adding single growth factors to serum-free medium restored field-induced responses; (3) large sheets of cells also changed shape and migrated cathodally; (4) bovine CECs responded to much smaller EFs than rabbit CECs; and (5) serum (no EF) stimulated random movement of bovine CECs.

Corneal epithelial cells change shape in an EF

The endogenous EF at a wound is only one element which could contribute to corneal wound healing. Serum and plasma, for example, induced significantly greater epithelial growth into a wound than occurred in serum free medium (Jumblatt and Neufeld, 1986; Collin et al., 1995). Perhaps interactions occur between EFs in wounded cornea and serum/growth factor elements, which gain access to the epithelium from stroma or from the tear film.

Many cell types respond to a small applied EF by assuming a perpendicular orientation. The mechanism is unclear but may involve an asymmetric redistribution of charged cell surface receptors, asymmetric activation of second messenger signalling pathways and asymmetric addition of new membrane and cytoskeletal polymerisation (McCaig and Dover, 1991). For CECs, the threshold for perpendicular reorientation was markedly reduced by serum, and by individual growth factors (EGF, bFGF and TGF-β1; Table 1).

In an EF, the EGF receptor may accumulate cathodally on CECs as it does on fibroblasts (Giugni et al., 1987). Whether receptors for bFGF and TGF-β1 also accumulate cathodally on CECs is not known. However a receptor-tyrosine kinase link to increased microfilament polymerisation in cathodally reoriented leading lamellae might be expected, since serum induces a relocalization of β-actin mRNA to the leading lamellae of serum-starved chick fibroblasts (Latham et al., 1994). Additionally, electric field-induced receptor clustering (acetylcholine receptors on frog myoblasts) is prevented by receptor tyrosine kinase inhibitors (Peng et al., 1993). Thus a potential mechanism underlying the perpendicular realignment of CECs is as follows. EGF, or bFGF or TGF-β1 (all are assumed present in serum) activate their cathodally clustered receptors, with subsequent kinase activation. Cathodally localised EGFR activation may result in localised membrane ruffling and local reorganisation of stress fibres and focal adhesions, through activation of members of the rac and rho subfamilies of GTP-binding proteins (Ridley and Hall, 1992; Ridley et al., 1992). Cathodally localised adhesion sites may create a vector of tension which re-directs actin polymerisation perpendicular to the EF. However, cytoskeleton rearrangement due to a more direct effect of the EF, which may not require ligand binding, cannot be excluded, since cells in serum-free medium also showed reorientation, although only at considerably higher field strengths (250 mV/mm, Fig. 2) (Soong et al., 1990a,b; Sulik et al., 1992).

Combined treatments with EGF, bFGF and TGF-β1 were no better than addition of a single growth factor in restoring EF-induced perpendicular reorientation (Table 1). The downstream signalling pathways activated by individual growth factors may be common, and may become ‘saturated’ by single ligand activation, thus preventing additive effects. The finding that this response does not depend on a single growth factor, but is activated by any of three separate growth factors, may indicate a central importance of cellular reorientation in re-epithelialisation.

EFs, serum components and growth factors interact in modulating CEC migration

Cathodal directedness of CEC migration was absent or weak in serum-free medium until above 200 mV/mm, but was robust at 100 mV/mm in both serum-containing medium and medium with EGF supplementing the serum (Figs 3 and 4). Thus serum markedly reduced the threshold for EF-directed migration to

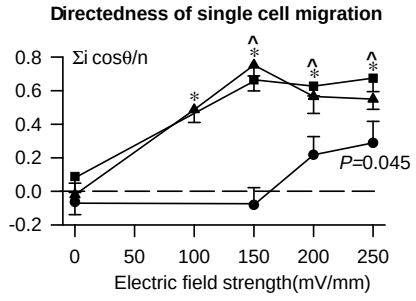

Fig. 4. Voltage dependency of the directedness (mean ± s.e.m) of cell migration in DMEM with (●): no serum; (■): 10% FBS; (△): 10% FBS and 25 ng/ml EGF. *P<0.01 when compared to that with no EF control; ^P<0.01 when compared to that in serum free medium at same voltage.

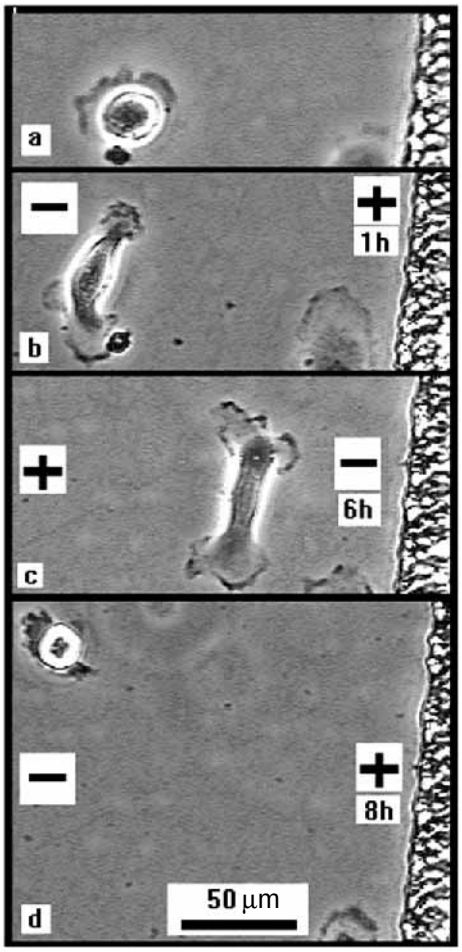

Fig. 5. Effect of reversing polarity of EF. The reference scratch is to the right and EF polarity as shown. (a) Before EF exposure; (b) 1 hour in EF with the cathode to the left, then the polarity was reversed; (c) 6 hours in EF with reversed polarity and the cell moved to the scratch (now the cathode side), then the polarity was reversed again; (d) 8 hours in re-reversed EF, the cell moved away from the scratch toward the new cathode side, although by that stage its shape had changed. Cultured in DMEM with 10% FBS, at 150 mV/mm.

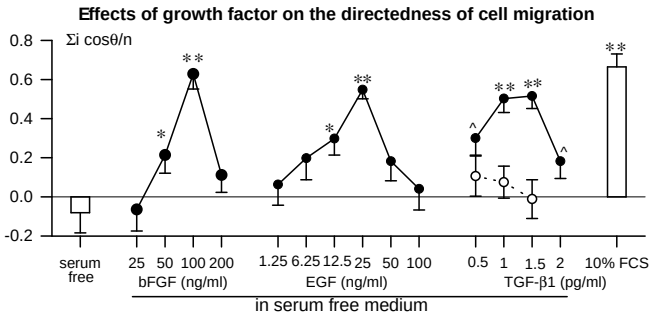

Fig. 6. Restoration of directedness (mean ± s.e.m.) of cell migration towards the cathode showed a concentration dependence on the growth factor present in serum free medium. ^Marginally significant (*P* very near 0.05); **P*<0.05, ***P*<0.01 when compared to serum free control. ○, directedness of cell migration cultured in serum free medium with bovine serum albumin as control for the TGF-β1 experiments. Each point of the control experiments contained an albumin concentration corresponding to that in the medium with TGF-β1. Number of cells measured for each point: 45-127.

below 100 mV/mm, i.e. close to the field strength at a corneal wound. Field-directed migration of CECs was enhanced therefore by more closely mimicking the in vivo situation with added environmental factors.

EF-induced gradients of growth factors might arise in culture and promote chemotactic migration of CECs. We have not ruled this out directly, but consider it unlikely. Negatively charged growth factor proteins would be expected to accumulate anodally, as has been demonstrated directly for BSA (Robinson and Messerli, 1995). However, bovine CECs migrated cathodally. Unless any gradients of growth factors were repulsive, cathodally directed migration is likely to be a direct effect of the EF. Nonetheless, the endogenous EF of wounded corneal epithelium may be sufficient to establish gradients of growth factors (Robinson and Messerli, 1995), which in vivo may interact with the directing effect of the EF.

In serum-free medium, individual growth factors restored field-induced directedness of CEC migration.The extent of EF-induced directedness of migration of CECs varied with growth factor concentration, and was a specific response to the individual growth factors (Fig. 6). Interestingly, the dose-dependence for growth factor stimulation of EF-induced directional migration was similar to that for growth factor induction of chemotaxis: peak migration rates occurring at 25 ng/ml for EGF, 100 ng/ml for bFGF and 1-1.5 pg/ml for TGF-β1 (Fig. 6) (Grant et al., 1992). At higher concentrations of EGF and TGF-β1, directedness of CEC migration decreased or virtually disappeared (Fig. 6). This may reflect a down regulation of growth factor receptors. Down regulation of EGFRs for instance (Kitazawa et al., 1990; Matthay et al., 1993; Coleman and Daniel, 1990; Abbott and Pratt, 1988), results from receptor internalisation and degradation (Honegger et al., 1990). As was the case for chemotaxis (Grant et al., 1992), TGF-β1 effectively stimulated migration at an extremely low concentration (producing optimal cathodal migration at 1/25,000 of the concentration of EGF and 1/100,000 of that of bFGF), although its molecular mass (25 kDa) is similar to that of EGF (6 kDa) and bFGF(16.4 kDa).

Combinations of growth factors had additive effects on directional migration of CECs. EGF + TGF and EGF + bFGF + TGF showed a higher directedness of CEC migration than the individual growth factors (*P*=0.07). TGF-β1 modulates the effect of EGF on corneal cells (Mishima et al., 1992; Hongo et al., 1992). Exposure of corneal keratocytes to TGF-β1 increased the number of high affinity receptors for EGF as much as tenfold, although there was no effect on CECs (Hongo et al., 1992). It is not clear whether a similar modulation may be occurring here. Additionally, TGF-β1 binding to its receptors activates different kinases (serine/threonine kinases) from those activated by EGF and bFGF (tyrosine kinases) and may be capable of recruiting different, parallel signalling pathways, involved in directed cytoskeleton reorganisation.

The following mechanisms may underlie cathodal-directed migration of CECs (and cell sheets). Again the key involves field-induced receptor redistribution and accumulation on cathodal-facing membranes, since if this is prevented in neuronal growth cones, or fibroblasts, cathodal-directed

motility ceases (Patel and Poo, 1982; Brown and Loew, 1994). GF receptors may accumulate cathodally on CECs as they do on fibroblasts (Giugni et al., 1987: Brown and Loew, 1994). Thereafter, two parallel but not mutually exclusive mechanisms may be involved. Ligand-induced autophosphorylation of tyrosine kinase domains may activate phospholipase C, which associates preferentially with the EGFR and promotes cathodally localised, ligand-directed actin polymerisation. Alternatively or additionally, cathodally localised EGFR activation may result in localised membrane ruffling and local reorganisation of stress fibres and focal adhesions, through activation of members of the rac and rho subfamilies of GTP-binding proteins (Ridley and Hall, 1992; Ridley et al., 1992). These events, coupled with an asymmetric, ligand-induced localisation of actin to the cathodal-facing leading lammellae, may underlie cathodal-directed migration of CECs. A further element is required to allow cathodal-directed migration, namely field-directed release of substrate adhesions. In migrating neutrophils this arises by calcium- and calcineurin-dependent endocytosis of integrin receptors at the trailing edge, with subsequent recycling and reinsertion of receptors at the leading edge (Lawson and Maxfield, 1995).

Possible mechanisms underlying the interaction of growth factors with EFs

CECs migrated slowly in the absence of serum (mean translocation rate of 4-5 μm/hour). With serum in the medium, or with serum plus added EGF, the mean translocation rate increased 3- to 4-fold, to 12-16 μm/hour, albeit movement was randomly-directed (Table 2). Single growth factors also enhanced translocation rates. Serum or certain growth factors can promote motility (Latham et al., 1994; Hill et al., 1994), as we show here for CECs. Additionally, an applied electric field adds a vector to cell movement, without itself stimulating migration rates. This vector may arise from induced asymmetry of growth factor or integrin receptors. An applied electric field induces gradients of receptors for both growth factors and adhesion molecules on fibroblasts (Giugni et al., 1987; Brown and Loew, 1994). The contribution made by each receptor type in migrating CECs is unknown; one may provide a vector for field-directed growth, whilst another could be involved in serum/growth factor-induced increase in general motility. Given the additive effects of individual growth factors on cathodal migration, it may be that many growth factor receptors accumulate cathodally with each making a parallel contribution through separate second messenger pathways, such as tyrosine and/or serine/threonine kinase activation and downstream phosphorylation (Chen et al., 1994; Schlessinger and Ullrich, 1992; Wrana et al., 1994; Chen and Weinberg 1995) to locally activated cell motility. Common signalling pathways may be shared by growth factors and an EF, since EFs influence cell growth and movements through tyrosine kinase activation and downstream phosphorylation events (Peng et al., 1993; Nuccitelli et al., 1993).

Physiological/clinical significance

Single CECs and, significantly, sheets of CECs migrate cathodally in an applied EF, and in the presence of serum and added growth factors, the threshold for migration is less than 100 mV/mm, i.e. close to the EF values measured directly at wounds in bovine corneal epithelium (42 mV/mm was a low estimate) (Chiang et al., 1992). Other growth factors and extracellular matrix elements also may interact with the EF. Complex interactions (competing and synergistic) may exist during corneal epithelial wound healing and these will determine the prospects for using EFs clinically. A complicating factor is that the polarities of the endogenous field at a wounded corneal epithelium and of the applied field which causes cathodal migration, are opposite. In a wounded cornea, current is driven from the wound through the tear film to establish a lateral field of 42±3 mV/mm, with the wound site positive. CECs migrate to the negative pole in vitro, although stromal fibroblasts migrate anodally (Soong et al., 1990a). Does the endogenous EF in a wounded corneal epithelium actively prevent re-epithelisation? If so, would the effect of the endogenous field need to be more than nullified by an applied field in order to promote cell ingrowth to the wound? Additionally, it may be possible to use applied fields of appropriate polarity, not simply to promote migration in a desired direction, but also to prevent CECs from migrating in a deleterious direction, as can occur excessively in corneal scar tissue with resultant compromised vision.

This work was supported by the Wellcome Trust.

REFERENCES

Abbott, B. D. and Pratt, R. M. (1988). EGF receptor expression in the developing tooth is altered by exogenous retinoic acid and EGF. *Dev. Biol.* **128**, 300-304.

Adamis, A. P., Meklir, B. and Joyce, N. C. (1991). In situ injury-induced release of basic-fibroblast growth factor from corneal epithelial cells. *Am. J. Pathol*. **139**, 961-967.

Brown, M. J. and Loew, L. M. (1994). Electric field-directed fibroblast locomotion involves cell surface molecular reorganization and is calcium independent. *J. Cell Biol*. **127**, 117-128.

Chen, F. and Weinberg, R. A. (1995). Biochemical evidence for the autophosphorylation and transphosphorylation of transforming growth factor beta receptor kinases. *Proc. Nat. Acad. Sci. USA* **92**, 1565-1569.

Chen, P., Gupta, K. and Wells, A. (1994). Cell movement elicited by epidermal growth factor receptor requires kinase and autophosphorylation but is separable from mitogenesis. *J. Cell. Biol.* **124**, 547-555.

Chiang, M., Cragoe, E. J. Jr and Vanable, J. W. Jr (1991). Intrinsic electric fields promote epithelisation of wounds in the newt, *Notophthalmus viridescens*. *Dev. Biol.* **146**, 377-385.

Chiang, M., Robinson, K. R. and Vanable, J. W. Jr (1992). Electrical fields in the vicinity of epithelial wounds in the isolated bovine eye. *Exp. Eye Res.* **54**, 999-1003.

Coleman, S. and Daniel, C. W. (1990). Inhibition of mouse mammary ductal morphogenesis and down-regulation of the EGF receptor by epidermal growth factor. *Dev. Biol.* **137**, 425-433.

Collin, H. B., Anderson, J. A., Richard, N. R. and Binder, P. S. (1995). In vitro model for corneal wound healing; organ-cultured human corneas. *Curr. Eye Res*. **14**, 331-339.

Curray, J. R. (1956). The analysis of two-dimensional orientation data. *J. Geol*. **64**, 117-130.

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

Frati, L., Daniele, S., Delogu, A. and Covelli, I. (1972). Selective binding of the epidermal growth factor and its specific effects on the epithelial cells of the cornea. *Exp. Eye Res.* **14**, 135-141.

Gipson, I. K. and Grill, S. M. (1982). A technique for obtaining sheet of intact corneal epithelium. *Invest. Ophthalmol. Vis. Sci*. **23**, 269-273.

Giugni, T. D., Brastan, D. L. and Haigler, H. T. (1987). Electric field-induced redistribution and post-field relaxation of epidermal growth factor receptors on A431 cells. *J. Cell Biol*. **104**, 1291-1297.

Grant, M. B., Khaw, P. T., Schultz, G. S., Adams, J. L. and Shimizu, R. W. (1992). Effects of epidermal growth factor, fibroblast growth factor, and

transforming growth factor-β on corneal cell chemotaxis. *Invest. Ophthalmol. Vis. Sci.* **33**, 3292-3302.

Gruler, H. and Nuccitelli, R. (1991). Neural crest cell galvanotaxis: new data and a novel approach to the analysis of both galvanotaxis and chemotaxis. *Cell Motil. Cytoskel.* **19**, 121-133.

Hill, M. A., Schedlich, L. and Gunning, P. (1994). Serum induced signal transduction determines the peripheral location of β-actin mRNA within the cell. *J. Cell Biol.* **126**, 1221-1230.

Honegger, A. M., Schmidt, A., Ullrich, A. and Schlessinger, J. (1990). Separate endocytic pathways of kinase-defective and -active EGF receptor mutants expressed in same cells. *J. Cell Biol.* **110**, 1541-1548.

Hongo, M., Itoi, M., Yamaguchi, N. and Imanishi, J. (1992). Distribution of epidermal growth factor (EGF) receptors in rabbit corneal epithelial cells, keratinocytes and endothelial cells, and the changes induced by transforming growth factor-beta 1. *Exp. Eye Res.* **54**, 9-16.

Hotary, K. B. and Robinson, K. R. (1990). Endogenous electrical currents and the resultant voltage gradients in the chick embryo. *Dev. Biol.* **140**, 149-160.

Hotary, K. B. and Robinson, K. R. (1992). Evidence of a role for endogenous electrical fields in chick embryo development. *Development* **114**, 985-996.

Hotary, K. B. and Robinson, K. R. (1994). Endogenous electrical currents and voltage gradients in Xenopus embryos and the consequences of their disruption. *Dev. Biol.* **166**, 789-800.

Jumblatt, M. M. and Neufeld, A. H. (1986). A tissue culture assay of corneal epithelial wound closure. *Invest. Ophthalmol. Vis. Sci.* **27**, 8-13.

Klyce, S. D. (1972). Electrical profiles in the corneal epithelium. *J. Physiol.* **226**, 407-429.

Kitazawa, T., Kinoshita, S., Fujita, K., Araki, K., Watanabe, H., Ohashi, Y. and Manabe, R. (1990). The mechanisms of accelerated corneal epithelial healing by human epidermal growth factor. *Invest. Opthalmol. Vis. Sci.* **9**, 1773-1778.

Latham, V. M. Jr, Kislauskis, E. H., Singer, R. H. and Ross, A. F. (1994). β-actin mRNA localization is regulated by signal transduction mechanisms. *J. Cell Biol.* **126**, 1211-1219.

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

Lawson, M. A. and Maxfield, F. R. (1995). Ca^{2+}- and calcineurin-dependent recycling of an integrin to the front of migrating neutrophils. *Nature* **377**, 75-79.

McCaig, C. D. and Dover, P. J. (1991). Factors influencing perpendicular elongation of embryonic frog muscle cells in a small applied electric field. *J. Cell Sci.* **98**, 497-506.

McCaig, C. D., Zietlow, R., Stewart, R. and Sangster, L. (1995). The neutrophin NT3 enhances electric field-stimulated nerve growth and guidance. *Soc. Neurosci.* **21**, 322.3 (abstract).

Maurice, D. M. (1967). Epithelial potential of the cornea. *Exp. Eye Res.* **6**, 138-140.

Matthay, M. A., Thiery, J. P., Lafont, F., Stampfer, M. F. and Boyer, B. (1993). Transient effect of epidermal growth factor on the motility of an immortalized mammary epithelial cell line. *J. Cell Sci.* **106**, 869-878.

Mishima, H., Nakamura, M., Murakami, J., Nishida, T. and Otori T. (1992). Transforming growth factor-beta modulates effects of epidermal growth factor on corneal epithelial cells. *Curr. Eye Res.* **11**, 691-696.

Nishida, K., Kinoshita, S., Yokoi, N., Kaneda, M., Hashimoto, K. and Yamamoto, S. (1994). Immunohistochemical localization of transforming growth factor-beta 1, -beta 2, and -beta 3 latency-associated peptide in human cornea. *Invest. Ophthal. Vis. Sci.* **35**, 3289-3294.

Nishimura, K. Y., Isseroff, R. R. and Nuccitelli, R. (1996). Human keratinocytes migrate to the negative pole in direct current electric fields comparable to those measured in mammalian wounds. *J. Cell Sci.* **109**, 199-207.

Nuccitelli, R. (1988). Physiological electric fields can influence cell motility, growth and polarity. *Advan. Cell Biol.* **2**, 213-234.

Nuccitelli, R., Smart, T. and Ferguson, J. (1993). Protein kinases are required for embryonic neural crest cell galvanotaxis. *Cell Motil. Cytoskel.* **24**, 54-66.

Patel, N. and Poo, M. M. (1982). Orientation of neurite growth by extracellular electric fields. *J. Neurosci.* **2**, 483-496.

Peng, H. B., Baker, L. P. and Dai, Z. (1993). A role of tyrosine phosphorylation in the formation of acetylcholine receptor clusters induced by electric fields in cultured Xenopus muscle cells. *J. Cell Biol.* **120**, 197-204.

Ridley, A. and Hall, A. (1992). The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell* **70**, 389-399.

Ridley, A., Paterson, H., Johnston, C., Diekmann, D. and Hall, A. (1992). The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell* **70**, 401-410.

Rijken, P. J., Hage, W. J., wan Bergen en Henegouwen, P. M., Verkleij, A. J. and Boonstra, J. (1991). Epidermal growth factor induces rapid reorganization of the actin microfilament system in human A432 cells. *J. Cell Sci.* **100**, 491-499.

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

Robinson, K. R. and Messerli, M. R. (1995). Electric embryos: The embryonic epithelium as a generator of developmental information. In *Axon Growth and Axon Guidance* (ed. C. D. McCaig). Portland Press (in press).

Schlessinger, J. and Ullrich, A. (1992). Growth factor signalling by receptor tyrosine kinases. *Neuron* **9**, 383-391.

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

Stoker, M. and Gherardi, E. (1991). Regulation of cell movement: the motogenic cytokines. *Biochim. Biophys. Acta* **1072**, 81-102.

Sulik, G. L., Soong, H. K., Chang, P. C. T., Parkinson, W. C., Elner, S. G. and Elner, V. M. (1992). Effects of steady electric fields on human retinal pigment epithelial cell orientation and migration in culture. *Acta Ophthalmol.* **70**, 115-122.

Vanable, J. W. Jr (1989). Integumentary potentials and wound healing. In *Electric Fields in Vertebrate Repair* (ed. R. Borgens, K. Robinson, J. Vanable and M. McGinnis), pp. 171-224. Alan R. Liss, New York.

van Setten, G. B., Tervo, T., Tervo, K. and Tarkkanen, A. (1992). Epidermal growth factor (EGF) in ocular fluids: presence, origin and therapeutical considerations. *Acta Ophthalmol.* (suppl.) **202**, 54-59.

Wilson, S. E., He, Y. G. and Lloyd, S. A. (1992a). EGF, EGF receptor, basic FGF, TGF beta-1, and IL-1 alpha mRNA in human corneal epithelial cells and stromal fibroblasts. *Invest. Ophthal. Vis. Sci.* **33**, 1756-1765.

Wilson, S. E., Lloyd, S. A. and He, Y. G. (1992b). EGF, basic FGF, and TGF-β1 messenger RNA production in rabbit corneal epithelial cells. *Invest. Opthal. Vis. Sci.* **33**, 1987-1995.

Wilson, S. E., Lloyd, S. A. and He, Y. G. (1993a). Fibroblast growth factor-1 receptor messenger RNA expression in corneal cells. *Cornea* **12**, 249-254.

Wilson, S. E., Walker, J. W., Chwang, E. L. and He, Y. G. (1993b). Hepatocyte growth factor, keratinocyte growth factor, fibroblast growth factor receptor-2 and the cells of the cornea. *Invest. Opthal. Vis. Sci.* **33**, 1987-1995.

Wilson, S. E., Schultz, G. S., Chegini, N., Weng, J. and He, Y. G. (1994). Epidermal growth factor, transforming growth factor alpha, transforming growth factor beta, acidic fibroblast growth factor, basic fibroblast growth factor, and interleukin-1 proteins in the cornea. *Exp. Eye Res.* **59**, 63-71.

Wrana, J. L., Attisano, L., Wieser, R., Ventura, F. and Massague, J. (1994). Mechanism of activation of the TGF-β receptor. *Nature* **370**, 341-347.

Zhao, M., Aguis-Fernandez, A., Forrester, V. D. and McCaig, C. D. (1995a). Serum dramatically enhances orientation and migration of cultured bovien corneal epithelial cells in an applied electric field. *Vision Res.* **35**, S177.

Zhao, M., Aguis-Fernandes, A., Forrester, V. D. and McCaig, C. D. (1995b). Cultured bovine corneal epithelial cells in a small applied electric field: effects of EGF, bFGF and TGF. *Vision Res.* **35**, S112.

Zhao, M., Aguis-Fernandes, A., Forrester, V. D. and McCaig, C. D. (1995c). Electric field-induced directional migration of cultured corneal epithelial sheets is serum and growth factor dependent. *Invest. Ophthal. Vis. Sci.* (in press).

Zieske, J. D. and Wasson, M. (1993). Regional variation in distribution of EGF receptor in developing and adult corneal epithelium. *J. Cell Sci.* **106**, 145-152.

(Received 2 October 1995 - Accepted 5 March 1996)