VORTEX OR WHORL FORMATION OF CULTURED HUMAN CORNEAL EPITHELIAL CELLS INDUCED BY MAGNETIC FIELDS

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SUMMARY

The terms 'vortex keratopathy' and 'hurricane keratopathy' describe two similar conditions affecting the corneal surface. In the former, a vortex or whorl pattern is seen on the corneal surface and is due to the deposition of substances such as pigment, iron or drugs in the epithelial cells. In the latter, a similar pattern is presented by migrating epithelial cells but, unlike the former, the pattern is rendered more visible by fluorescein staining. Both represent the migratory pattern of normal epithelial cells which is otherwise not visible due to the slow rate of epithelial turnover and migration. The whorl pattern has a clockwise predisposition in the majority of cases and is hypothesised to be due to the influence of ocular electromagnetic fields on the migrating epithelial cells. In this study we tested *in vitro* the effect of static magnetic fields on corneal epithelial cells. We were able to reproduce dramatic vortex or whorl patterns in response to magnetic fields, but without preferential migration towards the North or South Pole.

Terminally differentiated corneal epithelial cells are continually shed from the superficial layer of the cornea. These are replaced by the proliferation and migration of transient amplifying cells and stem cells, located in the basal layer and at the corneo-scleral junction (limbus) respectively.[1,2] Migration of cells is predominantly centripetal, from the limbus towards the centre the cornea.[3] This centripetal migration does not occur in a strictly radial manner. Clinical evidence suggests that cells originating at the limbus follow a curvilinear course, forming a vortex or whorl pattern.[4–6] We have observed that the vortex or whorl is 'clockwise' in most instances and hypothesised that its occurrence is determined by electromagnetic fields generated by the electrical potential of the eye.[7,8] In this study we tested the hypothesis by subjecting cultured human corneal epithelial cells to the influence of magnetic fields, expecting to demonstrate 'magnetotropism' and 'magenetotaxis' analogous to 'galvanotropism' and 'galvanotaxis' demonstrated by Soong and associates.[9]

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METHODS

Two to four millimetre human limbal explants, for corneal epithelial cell culture, were obtained from 24 donor rims (the ring of donor tissue left after removing the donor button) following corneal transplantation. Donor age ranged from 26 to 75 years. The endothelial layer was removed and the explants placed in the centre of each 35 mm well of a 6-well Falcon Primaria tissue culture plate. The explants were left at room temperature for 5 minutes and then covered by 150–200 μl of supplemented hormonal epithelial tissue culture medium.[10] The explants were fed twice a week with the same medium and maintained at 37 °C in an atmosphere of 5% carbon dioxide in air.

At the first sign of epithelial outgrowth, the plates were placed on permanent magnets, bar magnets of 20 gauss (G) and 250 G strength and horseshoe magnets of 25 G and 1.5 kG, embedded in polystyrene platforms. Bar magnets provided fields oriented along the plane of the bottom of the culture plates and horseshoe magnets provided fields perpendicular to the plane of migration of cultured cells. Parallel cultures, established from the same donor, were maintained in similar conditions but without magnets, and served as controls.

Cultures were observed daily until confluence was achieved. The numbers of cultures tested for the

Table I. Corneal epithelial whorl formation *in vitro*

	Bar magnet strength				Horseshoe magnet strength			
	20 G	Control	250 G	Control	25 G	Control	1.5 kG	Control
No. of cultures	9	9	5	5	5	5	5	5
No. of whorls	15	0	0	0	0	0	0	0

different types of magnets are indicated in Table I. The (corneal) epithelial nature of the cells in culture was confirmed by the polygonal morphology on phase contrast microscopy (Fig. 1) and by staining with a monoclonal antibody AE5 (ICN Biomedicals, Costa Mesa, CA) against corneal cytokeratin CK.[11]

RESULTS

Fifteen whorls, 11 clockwise and 4 counterclockwise, were observed in culture wells placed on bar magnets of strength 20 G (Fig. 2). Thirteen of the 15 whorls were noted in wells that were not directly on the magnet but 25 mm adjacent to it, with a mean field strength of 15 G at the centre of the well. On two occasions, clockwise and counterclockwise whorls appeared simultaneously in the same well. Whorl formation *in vitro* was seen to be a dynamic process. The normally flat and polygonal cells became narrow and elongated (Figs. 3–5) and several such cells aligned and assumed an 'S' or reverse 'S' configuration (Figs. 3, 4). This configuration became gradually accentuated as the curves of the 'S' moved in opposing directions to form a whorl. As the whorl became tighter, cells in the centre of the whorl were closely packed together and lifted up, above the plane of the bottom of the culture well, like an inverted cone (Fig. 6). The complete process took between 10 and 12 hours. The whorls persisted and could be recognised as such for up to 24 hours, following which the spiral configuration 'unfurled' and became less distinct, until eventually only a slightly elevated ridge or wave of cells remained (Fig. 7). In three instances the whorls persisted for 6–7 days. New whorls could be seen forming, while older ones were disappearing, in the same well. Whorl formation did not occur once the culture had reached confluence. No whorls were observed with horseshoe magnets or with bar magnets of 250 G strength. Likewise, parallel control cultures from the same donors, maintained in identical conditions but without magnets, did not show any whorls.

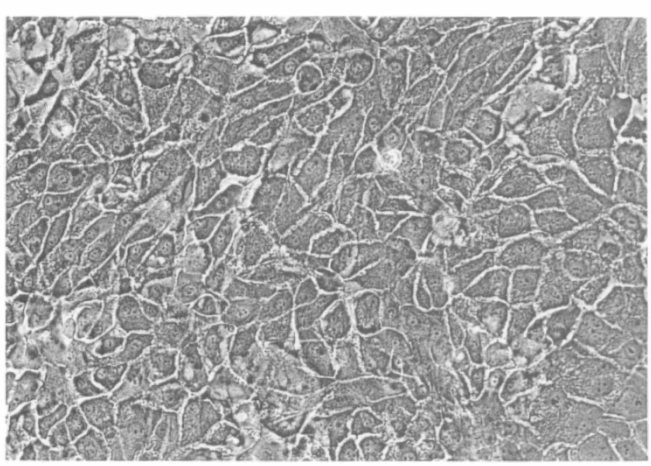

Fig. 1. *Photomicrograph of corneal epithelial cells in culture. The cells have a characteristic polygonal morphology and form a confluent monolayer on the bottom of the culture plate (phase contrast, × 400).*

DISCUSSION

During normal epithelial turnover, the path taken by epithelial cells, as they migrate from the periphery to the centre of the cornea is not visible. However, in several diverse clinical conditions, the cells are rendered visible by the intracellular deposition of substances such as pigment, iron, drug metabolites, glycogen and sphingolipid.[4,12,13] Iron, both intracellular and extracellular, is the most commonly deposited substance. It is classically seen in the Hudson-Stahli line, which is located at the migration 'null line'.[12] A vortex or whorl pattern is also often apparent on the corneal surface in such conditions, and is called vortex keratopathy. A similar pattern is seen when epithelial cell turnover is increased, as occurs in healing corneal epithelial wounds, in corneal grafts and in patients with keratoconus wearing ill-fitting rigid contact lenses.[13–15] In the latter situations, the pattern is highlighted by *in vivo* fluorescein staining of the cornea and is called 'hurricane keratopathy'. It has been suggested that rapidly migrating cells do not form tight intercellular adhesions and may be outlined by fluorescein stain either singly or in small groups.[7] The number and diversity of conditions in which a vortex or whorl pattern develops would indicate that it is not a specific disease process but represents a phenomenon that occurs during the migration of corneal epithelial cells. We studied 'hurricane keratopathy' in 30 patients and observed that the whorl had a 'clockwise' disposition in 80–90% of these patients.[16] On this basis it was postulated that the vortex or whorl pattern could represent the influence of ocular electromagnetic fields on migrating corneal epithelial cells.

The human eye, behaves like a dipole, oriented along its anteroposterior axis, with a potential difference of 6 mV, the cornea being positive to the posterior pole.[17] The electromagnetic field generated by such a current would be distributed in concentric circles, with the magnetic flux lines being clockwise

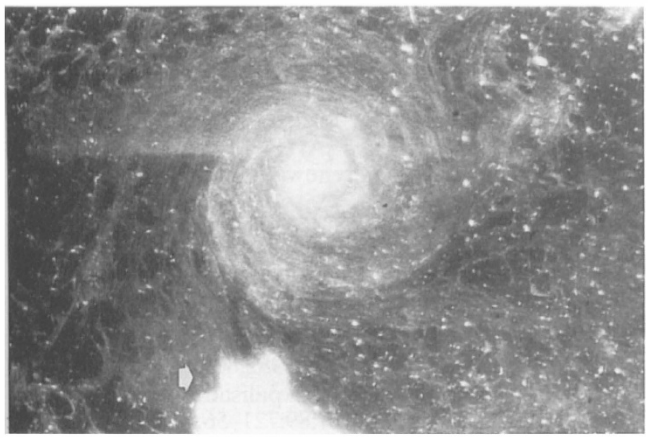

Fig. 2. *A complete clockwise whorl formed by corneal epithelial cells cultured under the influence of a bar magnet of 20 G. The bright white object seen at the bottom of the picture (arrow) is the edge of a corneal explant from which the culture was established (unstained, × 15).*

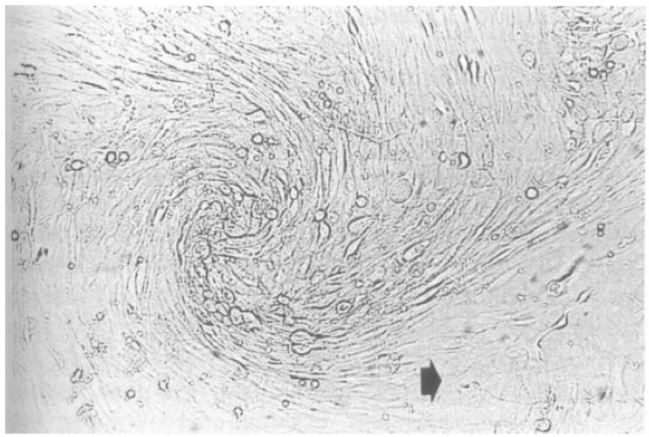

Fig. 3. *Early clockwise whorl formation showing the reverse 'S' configuration. The polygonal outlines of the normal flat monolayer of corneal epithelial cells are visible at the periphery of the whorl (bottom right-hand corner of the picture, indicated by broad arrow). Cells participating in the whorl become narrow and elongated (unstained, × 100).*

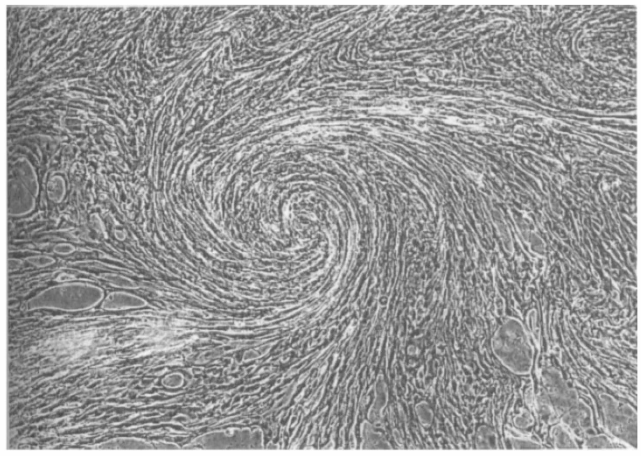

Fig. 4. *Early anticlockwise whorl formation showing the 'S' configuration. Occasional islands of polygonal cells are seen at the periphery of the whorl. The transition from polygonal to elongated, spindle-shaped cells is clearly visible at the periphery (phase contrast, × 100).*

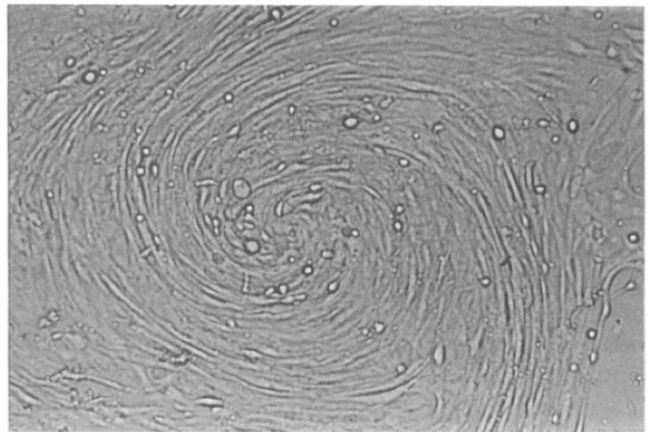

Fig. 5. *Completed anticlockwise whorl of cultured epithelial cells. The whorl is composed of elongated, spindle-shaped corneal epithelial cells. A patch of polygonal cells is visible at the top left-hand corner of the illustration (unstained, × 100).*

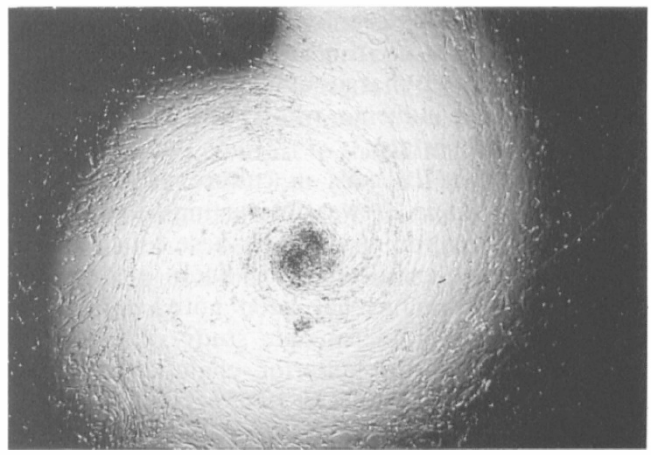

Fig. 6. *Completed clockwise whorl of cultured corneal epithelial cells. The centre of the whorl is made up of closely packed cells which were also above the plane of the culture plate, giving the whorl an inverted cone appearance (unstained, × 25).*

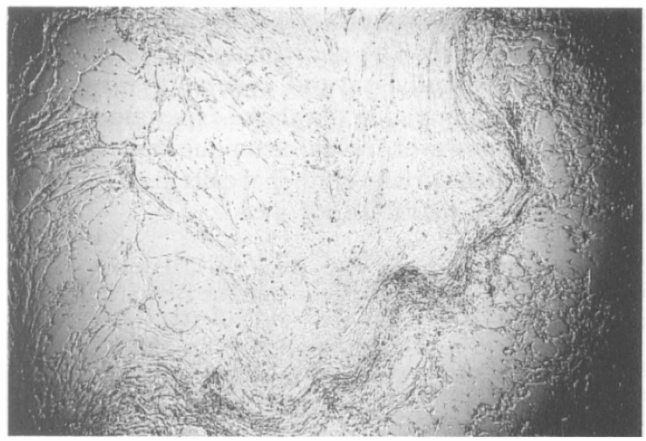

Fig. 7. *The appearance of the whorl in Fig. 6 18 hours after completion. The whorl has 'unfurled' and only a ridge of epithelial cells remains as a wavy line (unstained, × 25).*

and the flux density gradually increasing from the apex of the cornea to the periphery.[7] Although this provides a theoretical basis for an explanation of the vortex and whorl patterns, there is no direct evidence that corneal epithelial cells respond to magnetic fields.

Several cells and organisms that contain ferromagnetic substances such as magnetite (iron oxide) and greigite (iron sulphide) are known to respond to magnetic fields.[18–22] In magnetotactic bacteria, honey bees, homing pigeons and dolphins' heads, deposits of magnetite are believed to be associated with receptors for geomagnetic fields.[18–22] Although such substances have not been detected in the corneal epithelium, the normal corneal epithelial cells are known to have a high iron content.[23] We were able to estimate a total iron content of 540 ng in the lysate of 1.5×10^5 cultured corneal epithelial cells which had previously demonstrated whorl formation in magnetic fields (unpublished observation). The unique electromagnetic environment of the eye together with the presence of iron and other paramagnetic substances, or ionic charges of epithelial cells, may be responsible for inducing whorl formation on the human corneal surface.

Corneal epithelial cells in culture are normally polygonal in shape. It was interesting to note that during the formation of a whorl, these cells became elongated and spindle-shaped. Such a dramatic change in morphology has been noted *in vivo* as well. An electron microscopic study of epithelial downgrowth into the anterior chamber revealed elongated epithelial cells (W. Lee, personal communication). A similar morphology of both corneal epithelial cells and keratocytes was noted by Soong *et al.*[9] in response to electric fields. It is likely that during migration the normally polygonal-shaped cells assume an elongated morphology.

The hypothesis that we originally set out to test is not totally substantiated by the above experiment. The response of corneal epithelial cells to magnetic fields *in vitro* does not prove that the same occurs on the ocular surface. The electromagnetic field of the eye is, theoretically, several orders of magnitude smaller than that used in the above experiments. This study does, however, reveal a unique behaviour of cultured human corneal epithelial cells in response to static magnetic fields.

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Key words: Hurricane keratopathy, Vortex keratopathy, Magnets, Corneal epithelium.

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