Topographical control of cell behaviour: II. multiple grooved substrata

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Summary

Electronics miniaturisation techniques have been used to fabricate substrata to study contact guidance of cells. Topographical guidance of three cell types (BHK, MDCK and chick embryo cerebral neurones) was examined on grooved substrata of varying dimensions (4–24 μm repeat, 0.2–1.9 μm depth). Alignment to within $10^\circ$ of groove direction was used as our criterion for guidance. It was found that repeat spacing had a small effect (alignment is inversely proportional to spacing) but that groove depth proved to be much more important in determining cell alignment, which increased with depth. Measurements of cell alignment and examination by scanning electron microscopy showed that BHK cells and MDCK cells interacted differently with grooved substrata, and also that the response of MDCK cells depended on whether or not the cells were isolated or part of an epithelial cell island. Guidance by a multiple topographical cue is greater than could be predicted from cells' reactions to a single cue (Clark \textit{et al.} \textit{Development} 99: 439–448, 1987). Substratum topography is considered to be an important cue in many developmental processes. Cellular properties such as cytoskeletal organisation, cell adhesion and the interaction with other cells are discussed as being factors determining a cells susceptibility to topography.

Key words: contact guidance, BHK cells, MDCK cells, neurones, epithelial cells, photolithography, grooved substrata.

Introduction

Topographical guidance of animal tissue cells is believed to be an important factor in the determination of cell shape and movement in a number of developmental situations (Bard and Higginson, 1977; De Haan, 1963; Lofberg \textit{et al.} 1980; Newgreen, 1989; Wood and Thorogood, 1984, 1987; Wylie \textit{et al.} 1979). It has been known, since the earliest days of cell culture, that the shape of their environment strongly affects the behaviour of cells (Harrison, 1911; see Dunn, 1982).

Recently, there have been a number of studies of topographical guidance of animal cells on substrata patterned using the microfabrication techniques developed for the microelectronics industry (Brunette, 1986a,b; Dunn and Brown, 1986; Clark \textit{et al.} 1987; Dow \textit{et al.} 1987; Wood, 1988). These techniques have proved to be extremely useful because they allow the precise fabrication of a potentially limitless number of patterned structures in a variety of materials.

As it is hardly practical to eliminate other possible guidance factors \textit{in vivo}, it is necessary to examine the types and sizes of structures that affect cell behaviour \textit{in vitro} to determine a mechanism for guidance, and to show whether or not topographical environments found in nature could act as cues. This approach has recently been used for the mesenchymal cells of the developing teleost fin bud. These cells are believed to the contact guided by collagen actinotrichia forming a double layer of ridge substratum through which they migrate into cell-free space (Wood and Thorogood, 1984, 1987), and were found to be contact guided in a similar manner by artificially grooved substrata (Wood, 1988).

Following on from our work on the reaction of cells to single steps in a planar substratum (Clark \textit{et al.} 1987), we have examined how various parallel grooved substrata affect the alignment of different cell types. Grooved substrata of different pitches and depths, patterned photolithographically, were used to assess the extent to which multiple topographical cues alter the alignment of baby hamster kidney (BHK) cells, Madin Darby canine kidney (MDCK) cells and cultured chick embryo cerebral neurons.

Materials and methods

Cell culture

BHK and chick embryo cerebral neurones were cultured as described previously (Clark \textit{et al.} 1987). BHK cells were cultured in Hepes-buffered Glasgow-modified MEM (Gibco, UK) supplemented with 10% calf serum, 10% tryptose phosphate broth, glutamine and antibiotics. After dissection of cerebral hemispheres from 8-day chick embryos, this tissue was incubated in trypsin–EDTA. The cells were dissociated by trituration after the addition of serum-containing medium and were cultured in bicarbonate-buffered Glasgow-modified...
MEM and 15% horse serum and 5% chick embryo extract and glutamine and antibiotics in a 95% air/5% CO₂ atmosphere.

Stocks of MDCK cells are maintained in our laboratory. Cell suspensions were prepared, after rinsing in Ca²⁺/Mg²⁺-free Hank's basic salt solution followed by EDTA, using the same cell suspension technique as employed with the BHK cells. The cells were maintained in bicarbonate-buffered Glasgow MEM with 10% calf serum, 10% tryptose phosphate broth, glutamine and antibiotics in a 95% air/5% CO₂ atmosphere.

Substratum patterning
Grooved structures were made in Perspex using the photolithographic and dry etching techniques previously described (Clark et al. 1987). Briefly, 25 mm square pieces of 2 mm thick Perspex were coated by vacuum evaporation of aluminium. The aluminium surface was spin coated with photoresist (Shipley 1450J) which was exposed to UV light through chrome masks of the desired grating pattern. After development in 1:1 AZ developer/water, which removes the exposed areas of resist, the exposed aluminium was etched in aluminium etch, and the remaining resist removed by overall exposure to UV light and development (as the customary solvent wash attacks the Perspex). The aluminium pattern was then used as an ion resistant mask for reactive ion etching in an oxygen plasma, after which it was removed by the same etch to leave a grooved pattern in the Perspex.

Grooved substrata were formed on 4, 6, 12 and 24 μm pitches with equal groove and ridge widths. For each pitch, samples were etched to different depths (0.20, 0.56, 1.10 and 1.9 μm) (measured using Talystep profilometer at a step edge) and so in all, sixteen different grooved substrata were obtained. The samples were all briefly exposed to the oxygen plasma to ensure a similar chemistry on all surfaces.

Scanning electron microscopy
Cells on Perspex substrata were processed for scanning electron microscopy as previously described (Clark et al. 1987); briefly, after double fixation, the samples were freeze dried, sputter coated with gold-palladium and examined in a Philips SEM 500.

Determination of cell alignment
BHK and MDCK cells were seeded onto the substrata at low density (approximately 7000 cells cm⁻²). 24 h after seeding, the cells were examined and photographed under phase contrast, and the angle of the long axis (i.e. the direction of the maximum caliper diameter) of each cell relative to the grooves was determined from photographic enlargements of four different randomly chosen fields on a bit pad (Summagraphics) linked to a BBC computer. The data were grouped in 10 degree sectors between 0 and 90°. BHK cells are normally elongated and their long axes are easily determined by eye. Spread single MDCK cells (i.e. not in contact with any other cells) are usually circular or polygonal and not normally obviously axially polarised so that any increase in elongation could be detected, though it is possible that smaller, subtler effects may be overlooked. Control data were taken from measurements made on cells on unpatterned Perspex areas adjacent to the grooved areas, the angle being measured relative to an arbitrary axis. Statistical comparisons between cells on different grooved structures, and between controls and grooved structures were made by determining \( \chi^2 \) from contingency tables, the level of significance being taken as \( P \leq 0.05 \). This method of comparing circular measures is valid for data grouped in this way (Batschelet, 1981). Multiple linear regression was used to examine the relative effects of pitch and depth for BHK cells.

Image analysis systems requiring direct digitisation of the image was not found to work well for the observation of cells on surfaces patterned with gratings. Even when the cells were heavily stained and examined under bright-field optics, the diffraction patterns from the deeper gratings distorted the images of the cells to such an extent that digitized images were obviously not true representations of their shape.

Results

BHK cells
Four examples of the radial distribution of BHK cells on four different substrata are shown in Fig. 1. The figure illustrates why the proportion of cells whose long axis makes an angle of \(<10^\circ\) with the direction of the grooves was used as a measure of cell alignment in this paper. The choice of an alignment criteria, of 0–45° for aligned and 45–90° for unaligned cells, as used by Ohara and Buck (1979) and McCartney and Buck (1981), can be seen to be inappropriate in this case, as it would ignore differences that were very obvious from comparisons of radial distributions (for example, when comparing a highly aligned population of cells where all cells lie between 0 and \(10^\circ\) with one where all cells lie between 0 and \(45^\circ\) but not between 0 and \(10^\circ\)). The index of alignment used in other studies (Dunn and Heath, 1976; Fisher andTickle, 1981 and Brunette, 1986a,b) could not be employed easily as the optical properties of the deeper grooves made such measurements impossible. It can be seen in Fig. 1 that the cells on the control substratum were randomly distributed but that on the grooved substrata a monomodal response (i.e. alignment shift in one direction only) was observed. This was found to be the case for all of the grooved substrata tested.

**Fig. 1.** Graphs of the radial distribution of the long axes of BHK cells on various topographies.
The random distribution of the elongated, fibroblast-like BHK cells on a control (planar) substratum can be seen in Fig. 2A. Cells on shallow (0.3 \( \mu m \) deep) grooves show some degree of alignment but in many cases their long axes cross the grooves and their lamellar regions conform intimately to the underlying grooves.

Fig. 2. Scanning electron micrographs of BHK cells on various substrata. (A) Planar substrata; (B) 6 \( \mu m \) pitch, 0.3 \( \mu m \) deep grooves; (C) 6 \( \mu m \) pitch, 2 \( \mu m \) deep grooves; (D) 12 \( \mu m \) pitch, 2 \( \mu m \) deep grooves; (E) 6 \( \mu m \) pitch, 0.3 \( \mu m \) deep grooves, arrows indicate where lamellar regions conform to substratum shape; (F) 6 \( \mu m \) pitch, 2 \( \mu m \) deep, arrow indicates ruffling growth cone-like leading lamellar structure. Scale bars; A, B and C, 120 \( \mu m \); D, 54 \( \mu m \); E and F, 27 \( \mu m \).
alignment to the direction of the grooves within 10° of groove direction over the total number of cells, and the calculated proportion.

The epithelial properties of MDCK cells allow these cells to form stable contacts on collision, which result in the formation of monolayer colonies. It became obvious that those cells that were part of colonies reacted differently than those that were still isolated single cells. For this reason, they were considered separately.

MDCK cells on a planar substratum became either extremely radially flattened (Fig. 4A) or partially spread such that their polarity could be determined due to their possession of a ruffling leading lamella (Fig. 4B); they were rarely if ever elongated to any degree. On shallow gratings (0.3 μm deep, 12 μm pitch) some single MDCK cells did become more elongated and aligned to the grating direction, however, many appeared unaffected except that, like BHK cells, lamellar regions intimately conformed to the grooves (Fig. 4C,D). By contrast, single MDCK cells on gratings 1.2 μm deep, 12 μm pitch, became uncharacteristically highly elongated and uniformly aligned to the direction of the grooves (Fig. 4E,F). These cells were relatively blunt as compared to aligned BHK cells, their ends being formed into spatulate lamellar strips (Fig. 4F).

Unlike cells on shallow grooves, these cells consistently spanned the grooves to the extent that on no occasion were MDCK cell processes ever found in the deeper grooves (Fig. 4E,F).

MDCK cell colonies on shallow grooves were little affected by them; colonies were roughly circular in plan, and lamellar regions of cells at the margins of colonies could be seen to be conforming to the shape of the underlying grooves (Fig. 5A). Colonies on deeper gratings were often elongated in the direction of the grating, though many of the cells appeared to be no different in shape from those on planar areas (Fig. 5B,C,D). Although, as stated above, single cells on these deeper grooved substrata are highly elongated and aligned and no cell was seen to have formed broad lamellipodia, cells at the margins of colonies on these

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<th>Pitch (μm)</th>
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<th>Alignment</th>
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<td>0.56</td>
<td>1.1</td>
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**Table 1.** Data of alignment of BHK cells on grooved substrata

**Fig. 3.** A 3-D representation of the comparative effects of grating pitch and groove depth on the alignment of BHK cells. X-axis is depth, decreasing towards observer.

**MDCK Cells**

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Fig. 4. Single MDCK cells on various substrata. (A) Spread cell on a control (planar) substratum; (B) partially spread cell with ruffling leading lamella; (C and D) Cells on 6 \( \mu \)m pitch, 0.3 \( \mu \)m deep grooves (inset in D is detail of lamellar region conforming to substratum; (E and F) cells on 6 \( \mu \)m pitch, 2 \( \mu \)m deep grooves, small arrow in F indicates spatulate lamellar structure, large arrow indicates where two such structures have made contact. Scale bars: A and B, 14 \( \mu \)m; C, 60 \( \mu \)m; D, 60 \( \mu \)m (inset, 30 \( \mu \)m); E, 60 \( \mu \)m; F, 30 \( \mu \)m.
Fig. 5. MDCK cell colonies on grooved substrata. (A) 6 μm pitch, 0.3 μm deep, arrow indicates lamellar region of marginal cell conforming to grooves. (B) 6 μm pitch, 2 μm deep, colonies are elongated. (C) 6 μm pitch, 2 μm deep, arrow indicates the region of contact of a pair of cells which have spread over the grooves. (D) Cells at the margins of a colony spread across and span the grooves, arrows indicate region of cell–cell contact. Scale bars; A, 30 μm; B, 60 μm; C, 120 μm; D, 30 μm.

same grooves often formed extensive flattened areas that spanned a number of grooves (Fig. 5C, D). In these cases, it appears that the cells may have attempted to conform to the substratum since slight sagging can be seen where the cells span grooves (Fig. 5D). The measured degree of alignment of single MDCK cells shows that, over the range tested, pitch has no effect, though it is likely that any differences in cell shape, such as the degree of elongation, will not be apparent in our measure. For pitches of 4, 6, 12 and 24 μm, where depth is constant (1.1 μm), no differences in the degree of alignment were found (Fig. 6). When MDCK cells were seeded onto gratings of identical pitch (12 μm), but different depths (0.20, 0.56, 1.10 and 1.90 μm), it can be seen that depth does affect alignment to a greater degree than for BHK cells (Fig. 7). Cells on the shallowest gratings (0.20 μm deep) appeared to be minimally affected whereas those on 0.56 μm deep structures are more than four times more aligned, though further increases in depth have only a small additional effect

Fig. 6. A comparison of the degree of alignment of MDCK cells and BHK cells on grooved substrata of varying pitch.

(Fig. 7). The scanning electron microscopic examination of MDCK cells showed that almost all single cells were aligned to 1.2 μm deep grooves, whereas the maximum measured alignment of cells on the phase contrast optics samples suggests that approximately 10% were
Depth (um)

0.20 0.56 1.10 1.90

Fig. 7. A comparison of the effect of groove depth on the alignment of MDCK cells and BHK cells on grooved substrata of varying depth.

not aligned. These non-aligned cells were mainly poorly spread. The difference may be due to a combination of the larger sampling area of the latter set of data, and differences in the time of recovery from passaging into cell suspension, since these experiments (i.e. scanning electron microscopy and measured alignment) were undertaken at different times with different cell suspensions from our maintained stock.

Chick cerebral neurones

Cultured isolated chick embryo neurones, when seeded onto poly-L-lysine coated substrata of identical spacing (8 µm grooves, 20 µm ridges), but different depths (1 or 2 µm), exhibited markedly different reactions. The outgrowth of neurites appeared unaffected on the 1 µm patterns, the growth cones having crossed many grooves and ridges (Fig. 8A). On 2 µm deep patterns neurite outgrowth is markedly aligned to groove direction, though crossing over edges does occur (Fig. 8B).

Discussion

Reactions of cells to grooved substrata

The present study has examined two parameters of substrata with multiple topographical cues: grating pitch (repeat distance) and groove depth. The major findings are that although pitch can have a significant effect on cell alignment, the effect of depth is much larger, and increasing depth reduces and ultimately abolishes the smaller effect of pitch. It was also found that different cell types reacted to different degrees on identical substrata, and that the reaction of an epithelial cell type (MDCK) to these topographies was dependent on whether or not the cell was isolated or part of a colony.

It has been known for some time that various cell types react to multiple topographical cues in vitro (Dunn, 1982), though only recently have photolithographic microfabrication techniques been applied to address the problems of to what extent and by what mechanism topography influences animal tissue cell behaviour (Brunette, 1986a,b; Dunn and Brown, 1986; Clark et al. 1987; Wood, 1988). A number of studies have examined in some detail certain parameters of grooved substrata that alter cell shape and guide cell movement. Most attention has been given to altering the spacing of grooves and the intervening ridges. Brunette examined the orientation of fibroblasts (Brunette, 1986a) and epithelial cell colonies (Brunette, 1986b) on grooved substrata of varying repeat and depth but found relatively small differences in the strong degree of alignment. This was possibly due to the fact that most of the structures were deeper than those that caused maximum alignment in the present study and the spacing was often larger than the diameter of the cells themselves, so that cells may have reacted to these structures as single steps. This work did show that the shallow grooves tested (0.5 µm deep) were less effective in orienting epithelial cell colonies than deeper grooves, and that both fibroblasts and epithelial cells were capable of considerable distortion in conforming to both concave and convex topographies. Ohara and Buck (1979) found that larger pitched grooved substrata were less effective in aligning cells, though this was less
obvious in a later study (McCartney and Buck, 1981). Dunn and Brown (1986) showed that the shape change and alignment of fibroblasts on grooved substrata was most dependent on ridge width, alignment being inversely proportional to ridge width. Brunette (1986a) found that minor grooves that are formed at the bottom of features etched into silicon (110) were effective in orienting fibroblasts but this orientation is overridden by the larger patterned grooves. When the mesenchyme from the developing teleost fin was explanted onto various grooved quartz substrata, narrower grooves were less effective in aligning cells (Wood, 1988). Ohara and Buck (1979) found that 2 μm repeat grooves would align fibroblasts, but Dunn (1982) using grooves of similar spacing was unable to see such alignment and suggested that one possible reason for the discrepancy may be a difference in depth.

The variety of spacings and depths of grooved substrata (unknown in some cases) and of cell types, make direct comparisons difficult, but in general it would seem that, as was the case in the present work, the degree of alignment of cells is inversely proportional to repeat distance. The present work would suggest that the effect of spacing of a topographical feature is of minor importance at depths of more than 2 μm, though our earlier work on cells reaction to single steps (Clark et al. 1987) indicates that a repeated feature has a much larger effect than could be predicted from a cell’s reaction to a single feature of the same magnitude, eg. approximately 30 % of BHK cells would be expected to align at a 1 μm step, whereas for grating structures 1 μm deep with pitches ranging from 4–24 μm, alignment ranged from 80 % to 50 %. A grooved substratum could be considered to be a closely packed array of single steps. It would be interesting to know at which density these single steps began to have the effect of a grooved substratum. Intuitively, it would seem likely that the maximum spacing would be one where a cell could frequently be in contact with more than one step edge. This would vary with cell size.

Possible mechanisms of topographical guidance

The mechanism by which grooved substrata affect cell behaviour is unclear. Differences in the adhesiveness of grooves (Weiss, 1959) or greater area for contact within grooves (Trinkaus, 1969) have been eliminated as possibilities (Dunn, 1982). Ohara and Buck (1979) found that cells tended to bridge grooves and suggested that the confinement of focal adhesions to the intervening ridges caused their alignment, thus influencing the alignment of the whole cell. Dunn and Brown (1986) showed that chick heart fibroblasts were able to form focal adhesions both on ridges and in grooves that were 0.69 μm deep, and they suggested that contacts formed in grooves may be less effective in allowing traction to be exerted. Our examination by scanning electron microscopy of BHK cells on grooves of different depths showed that the lamellar regions of the cells conformed to the shallow grooves whereas on deeper grooves where cells were highly aligned, long narrow processes could be found both on ridges and in grooves. Isolated MDCK cells also conformed to shallow grooves, but aligned cells on deeper grooved substrata always bridged. We also observed that MDCK cells at the margins of colonies on deeper grooved substrata bridged over the grooves but were not in any way aligned. This would suggest that the confinement of cell–substratum contact to the intervening ridges does not necessarily result in alignment. Indeed, many of Ohara and Buck’s (1979) original micrographs show bridging in the absence of alignment. Wood (1988) found that alignment of teleost fin mesenchyme cells was actually reduced by the cells’ ability to bridge over narrower grooves. Clark et al. (1987) emphasised the probabilistic nature of the response of cells to a single topographical feature, suggesting that such a feature would reduce the probability of a cell making a successful protrusion and/or contact in a given direction. The reduction in probability could be due to a number of factors such as Dunn and Heath’s (1976) proposal that the relative inflexibility of microfilament bundles in a cell protrusion does not allow significant bending, so that shortening of a protrusion is required to accommodate topography thus reducing the traction that can be exerted in that direction. Brunette (1986a,b) and Clark et al. (1987) have shown that cells can distort to accommodate large angles but it would seem unlikely that such distorted processes could oppose the traction exerted by a protrusion and adhesion in another direction, though this will happen with a certain degree of probability. The fact that altering feature spacing (Dunn and Brown, 1986), and spacing and depth of grooved substrata in the present study, has a gradual effect on cell alignment (ie. alignment is generally inversely proportional to spacing and proportional to depth) is suggestive of a probabilistic response, though simple extrapolation from the response to single features (Clark et al. 1987) would be unwise. It may be that cells align to grooved substrata simply in order to minimise cytoskeletal distortion, though, because MDCK cells can bridge deeper grooves, and therefore the cytoskeleton is not distorted to any degree, other factors must be involved. It is possible that BHK cells and MDCK cells are aligned through different mechanisms. Single MDCK cells seem to be more susceptible to substratum guidance than BHK cells. On deeper grooves, BHK cells are frequently in contact with the grooves whereas MDCK cells were always observed to be spanning grooves. The difference in the reactions of isolated MDCK cells and those in colonies may be due to a number of factors. Lateral adhesions between these cells may allow them to exert forces on one another that override the alignment by the substratum, though pairs of cells were observed to be much more spread in directions perpendicular to groove direction than would be expected if they were simply pulling or pushing on one another. Also, as already stated, cells at the margins of some colonies appeared to be little affected by the substratum and bridge over many grooves. The phenomenon of contact-induced spreading of epithelial cells (Middleton, 1977, 1982) may play a part in, or be entirely responsible, for the differences between iso-
lated MDCK cells and those in colonies, though little is known of the changes in adhesion or cytoskeleton that result from contact.

Consideration of the properties of cells that are less affected by substratum topography may be useful in understanding the phenomenon. There appears to be some correlation between the degree of organisation of a cell’s cytoskeleton and its susceptibility to topographical guidance. Transformed cells, which generally lack microfilament organisation, are less susceptible to topographical guidance on glass fibres (Fisher and Tickle, 1981) and grooved substrata (Rovensky et al. 1971; McCartney and Buck, 1981). Neutrophil leucocytes, whose cytoskeletal organisation is highly labile, were unresponsive to single steps to which other cell types would react strongly (Clark et al. 1987), and preliminary observations from our laboratory, in conjunction with Dr J. M. Lackie, suggest that they are largely unaffected by grooved substrata. Although the foregoing evidence indicates that the organisation of the cytoskeleton is a major factor in topographical guidance, it must also be noted that the less responsive cell types are also generally less adhesive for most substrata, though a possible exception to this could be contact-induced spreading of epithelial cells where the cells are less responsive even though increased spreading would seem more likely to be coincident with an increase in adhesiveness than with a decrease.

Dunn and Brown (1986) suggest that marginal expansion of fibroblasts across grooves is inhibited, but partially compensated for by marginal expansion along grooves. Probabilistically, it could be stated that protrusions made along the directions of the grooves are more frequently ‘successful’ than those made in any other direction, the probability of ‘success’ being dependant on edge density and depth. The most likely property of cells determining protrusion success would, we believe, be cytoskeletal flexibility, protrusions made ‘against the grain’ being less able to form stable lamellipodia that can exert traction. Clark et al. (1987) suggested that where a cell is distorted to accommodate topography, microfilament disorganisation may result (Dunn and Heath, 1976) and lead to regions of cytoskeleton being isolated from each other. If this occurred, then protrusions would be unable to exert traction on the whole cell. The success of a protrusion will result in the axial polarisation and/or translocation of the cell in the direction of its formation.

Topographical guidance in nature
Although many cells can easily overcome a single topographical feature, multiple cues appear to elicit a much stronger response. This may in turn be amplified by population density for contact-inhibited cells. Preliminary observations suggest that, on grooved substrata, BHK cell alignment increases with population density, i.e. partial alignment of a cell population is increased by the unsuccessful protrusions of cell–cell contact. Multiple topographical cues have been implicated as being an important guidance cue in a number of developmental processes. The shape of cellular substrata are believed to be capable of influencing cell migration (De Haan, 1963; Wylie et al. 1979). Oriented extracellular matrix material (ECM) will influence cell shape and locomotion in vitro (Dunn, 1982; Dunn and Ebendal, 1978; Ebendal, 1976; Haston et al. 1983; Wilkinson and Lackie, 1983), and is thought to do so in vivo: for example, the orientation of fibroblasts during corneal development (Bard and Higginson, 1977), the migration of mesenchyme in the developing teleost fin (Wood and Thorogood, 1984, 1987), mesoderm migration during gastrulation (Nakatsuji and Johnson, 1984), and in early neural crest cell migration in the axolotl (Lofberg and Ahlfors, 1978; Lofberg et al. 1980) and quail (Newgreen, 1989). In this last example, the alignment of ECM around the neural tube may in fact oppose a possible guidance effect of neural tube curvature (Lofberg and Ahlfors, 1978), and may inhibit cell migration posteriorly which could result from contact inhibition of locomotion and population pressure. Since multiple topographical cues of relatively small magnitude can cause major shape changes and influence the direction of locomotion of cells, topographical control may be a far more important and widespread factor in the natural control of tissue cell behaviour than is often realised.

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