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Topographical control of cell behaviour

I. Simple step cues

P. CLARK[1], P. CONNOLLY[2], A. S. G. CURTIS[1], J. A. T. DOW[1] and C. D. W. WILKINSON[2]

Departments of Cell Biology[1], and Electrical and Electronic Engineering[2], University of Glasgow, Glasgow G12 8QQ, Scotland, UK

Summary

The photolithographic techniques of the microelectronics industry have allowed us to fabricate patterned plastic substrata to investigate contact guidance of animal tissue cells. The reactions of cells to single steps on a substratum were examined using time-lapse videorecording and scanning electron microscopy. BHK cells and chick embryonic neural cell processes exhibited gradual inhibition of crossing steps with a concomitant increase in alignment at steps dependent on increasing step height. Comparison of these cells' reactions, with those of chick heart fibroblasts and rabbit neutrophils, at a 5 μm step revealed that the influence of topography is also dependent on cell type, the neutrophils being relatively unaffected. The presence of an adhesive difference at a series of steps altered BHK cells' reactions such that the frequency of crossing was dependent on the direction of approach to a step. Although our data are consistent with Dunn & Heath's proposal (1976) that the inflexibility of the cytoskeleton of a moving cell's protrusion is the cellular property determining such reactions to topography, we have found that, on encountering a topographical feature, the response of a cell may be predictable on a probabilistic basis, i.e. the topographical feature reduces the probability of a cell making a successful protrusion and contact in a given direction, that even the largest features tested did not act as absolute barriers to cell locomotion since a small proportion of a population of cells were able to overcome them, and that other guidance cues could significantly alter a cell's response. Even in situations where it is not the primary cue in directing cell locomotion, topographical control may be an important factor during morphogenesis since it must, at the very least, influence the efficiency of other cues.

Key words: cell behaviour, contact guidance, photolithography, neurone guidance.

Introduction

Directed cell movement is a phenomenon generally recognized as being crucial in the development of multicellular organisms and, therefore, an understanding of its control will be essential to an understanding of basic morphogenetic processes. Since there have been many suggestions that topographical reactions of cells may play a role in embryogenesis, regeneration and wound healing (Dunn, 1982; Trinkaus, 1985; Wood & Thorogood, 1984) we decided to investigate the reactions of cells to very simple topographical cues.

It has long been known that substrate topography can affect cell morphology and cell behaviour, see Dunn (1982) for review. The early work on 'contact guidance' (Weiss, 1945, 1958) showed that cells aligned and migrated along fibres and grooves and was interpreted in terms of the molecular orientation of the substrate. Later, (Curtis & Varde, 1964) it was shown that the cells probably reacted to the topographical features such as curvature rather than to any molecular orientation. Dunn & Heath (1976) demonstrated that cells would not extend around external angles when the angle was only 17° greater than a horizontal plane: they attributed this to the inflexibility of the cytoskeleton. On the other hand, it was suggested (O'Hara & Buck, 1979) that the orientation of cells on multiple topographical features, such as a parallel-grooved substratum, is due to the fact that cells tend to span the grooves and the ridges have the effect of orienting adhesion plaques (focal adhesions).

Recently several groups have begun to employ microfabrication techniques to obtain grooved and ridged substrata for the study of cell behaviour, which

can be made with more precision than has previously been available to biologists (Brunette, 1986; Brunette, Kenner & Gould, 1983; Dunn & Brown, 1986). In the present study, we have examined the reactions of cells to a single step feature in an otherwise planar substratum. We consider this approach essential if a thorough understanding of the reaction of cells to topography is to be achieved, because it provides a simpler system whose size can easily be manipulated, and because it is the basic component of the more complex multiple parallel-grooved substratum, which has been subject to extensive study (Brunette, 1986; Brunette *et al.* 1983; Curtis & Varde, 1964; Dunn, 1982; Rovensky, Slavnaja & Vasiliev, 1971; Weiss, 1945). Patterns of adhesiveness will orient cells (Harris, 1973) and may contribute to cells' responses to topographical features. The substratum fabrication techniques we have employed have allowed us to examine the effect of topography in both the presence and absence of such an adhesive difference. As it might also be expected that responses will vary between cell types, we have examined the reactions of four different cell types to an identical topographical feature.

Materials and methods

Substratum patterning

Sheet Perspex (ICI) (2 mm thick) was cut to an appropriate size and the surface patterned using photolithography. The plastic was coated with 100 nm of aluminium in an Edwards vacuum coating unit at 4×10^{-5} Torr. This aluminium surface was then spin coated with positive photoresist (AZ1340J, Shipley Chemicals, UK). After baking at 80°C for 30 min the photoresist was exposed to u.v. through a chrome mask of the desired micropattern. Exposed resist was removed by development in AZ developer (Shipley Chemicals, UK) and the exposed aluminium removed with 80% conc. phosphoric acid/5% conc. nitric acid/15% water aluminium etch. Residual resist was removed by blanket exposure to u.v. followed by development. The pattern was then transferred to the plastic by exposure to an oxygen plasma in a reactive ion etch system (System 80, Plasma Technology, UK), base pressure 2 m Torr, oxygen pressure 100 m Torr. For a given oxygen pressure and RF power the etch rate is linear with time and the wall profile in reactive ion etching is vertical. The patterned aluminium is removed with aluminium etch exposing the patterned plastic. The plastic patterns then were re-exposed to the oxygen plasma so that the whole surface was uniformly treated.

Cell culture

The basic culture medium in these experiments was the Glasgow Modification of Eagle's Minimal Essential Medium (GMEM) (Gibco, UK) supplemented with 3 mM-glutamate (Sigma Chemical Co., UK), 100 units ml^{-1} penicillin, 100 $\mu g\,ml^{-1}$ streptomycin (Flow Labs, UK) and 2·5 $\mu g\,ml^{-1}$ amphotericin-B (Gibco, UK). Further supplementation to GMEM depended on the cell type being cultured: for baby hamster kidney (BHK) cells, 10% calf serum (Gibco, UK), 10% tryptose phosphate broth (Gibco, UK), 20 mM-Hepes (Sigma, UK); for chick heart fibroblasts, 10% calf serum, 10% tryptose phosphate broth, 0·25% bicarbonate; for chick cortical nerve cells, 15% horse serum (Gibco, UK), 5% chick embryo extract (Flow Labs, UK), 0·25% bicarbonate.

BHK cells were routinely maintained in our laboratory and cell suspensions obtained by detachment of the cells with 0·05% trypsin (Difco, USA) in 0·2 mg ml^{-1} EDTA (BDH Chemicals Ltd, UK) after rinsing in Ca^{2+}-/Mg^{2+}-free 20 mM-Hepes-buffered Hank's basic salt solution (CMF). Trypsinization was then stopped by the addition of serum containing medium (GMEM + 10% calf serum + 10% tryptose phosphate broth + 20 mM-Hepes). Cells were washed by centrifugation, resuspended and triturated in this medium, then counted.

To obtain chick heart fibroblasts (CHF), chick hearts were dissected out, under sterile conditions, from 8-day chick embryos, minced into small fragments then washed in CMF. CMF was replaced by trypsin/EDTA mixture and the tissue fragments incubated at 37°C for 15 min. Trypsin/EDTA was replaced by serum containing medium (0·25% sodium hydrogen carbonate-buffered GMEM + 10% calf serum + 10% tryptose phosphate broth) and the cells suspended by trituration. Cells were washed, resuspended in this medium and counted. After seeding at high density in 25 ml tissue culture flasks and incubating at 37°C for 1 h, nonadherent cells (mainly cardiac myocytes) were poured off and the medium replaced. Cultures were incubated in a 5% CO_2/95% air atmosphere until required (usually within 2–3 days) at which time cell suspensions were obtained for seeding onto patterned substrata, by the same method as for BHK cells.

Chick cerebral hemisphere cells were obtained by sterile dissection of this tissue, taking care to remove the meninges, from 8-day chick embryos. The tissue was subsequently minced, washed in CMF, treated with trypsin/EDTA and incubated at 37°C for 20 min. The trypsin/EDTA was decanted off, serum containing medium (0·25% sodium hydrogen carbonate-buffered GMEM + 15% horse serum + 5% chick embryo extract) added and the tissue triturated and filtered through 100 μm Nitex mesh to remove large clumps of cells. The resulting cell suspension was centrifuged, resuspended in medium and cell concentration determined.

Rabbit peritoneal exudate neutrophils (PMN), prepared by standard methods, were kindly provided by Dr J. M. Lackie and his group. Cells were washed by centrifugation and resuspension in a 1:1 mixture of filtered peritoneal exudate and Hepes-buffered Hank's basic salt solution.

BHK cell adhesion assay

BHK cells, at a concentration of 10^6 cells in 6 ml of medium, were seeded onto Perspex samples in 6 cm Petri dishes (Nunc). After incubation at 37°C for 30 min, the samples were rinsed twice by gentle agitation in BSS and fixed in 4% formalin in phosphate-buffered saline. The number of adherent cells per field was determined in 25 fields for each

sample by examination under inverted phase optics (Leitz Diavert) at ×320. Statistical comparisons between samples were made using Student's *t*-test.

Recording and analysis

BHK and CHF cells were seeded onto patterned substrata at a density of $0{\cdot}2\times10^6$ in a 6 cm Petri dish. PMN were similarly seeded but at a density of 10^6 cells. The neural cells were seeded onto substrata that had been coated with poly-L-lysine (poly-L-lysine hydrobromide, M_r 4000–15 000, Sigma, UK) (adsorbed from a solution of 10 μg ml^{-1} at 37°C for 1 h) at a density of 10^6 in a 6 cm dish. These cell densities were low enough to allow an appreciable frequency of cell–step encounters, without cell–cell encounters being frequent enough to interfere with these reactions.

Cell movement was recorded using a time-lapse video-system consisting of a Leitz Diavert inverted phase-contrast microscope mounted with a Panasonic (Japan) WV-1450 CCTV camera with Newvicon pick-up tube which was connected to a Panasonic NV-8050 videocassette recorder. Recording speed was controlled by an external one-shot stimulator switching every 100 s, except in the case of PMN which were recorded at a time-lapse ratio of 1:70.

On playback, cells encountering the step were scored as to the direction from which they encounter (i.e. the upper or lower portion of the steps) and their reaction to it. Reactions to a step were noted as crossing (i.e. whole cell displacement from one surface of the step to the other), aligning (i.e. spreading and/or moving along the edge of the step at any point during the encounter, though a cell may have subsequently crossed or moved away from the step) and others (e.g. short-lived contact with the step). Controls for these experiments consisted of filming cells moving on an unpatterned, planar substratum and analysing their reactions to a straight line drawn at random on the videomonitor screen. This will give a measure of the reactions of moving cells at a straight line in the absence of any topographical feature. The reactions of growing processes of chick hemisphere cells did not require to be recorded because the process itself indicates the path taken by the motile tip. Cultures of hemisphere cells were examined at a time when significant process growth had occurred (usually at between 6 and 8 days in culture) by phase-contrast microscopy and processes encountering a step scored as to their origin from cell bodies on either the upper or lower portion of the pattern, and as to whether or not they crossed the step. In all cases, crossing and aligning reactions are expressed as a proportion of the total number of cells in a group encountering a step. For each step height and cell type (except chick hemisphere cells) three or four time-lapse recordings of 20–24 h (2 h for PMN) duration were made and subsequently analysed. The number of cells in the data groups ranges from 22 to 73 (mean 47 cells). Statistical comparisons between individual data groups were made by obtaining χ^2 from 2×2 contingency tables, applying Yeats' correction where required. Comparisons of groups of data were made by a method of combining 2×2 contingency tables (Snedecor & Cochran, 1967).

Scanning electron microscopy

Cells on patterned substrata were fixed in 2·5 % glutaraldehyde in phosphate-buffered saline (PBS) at 37°C for 15 min then transferred to 4°C for 12 h. After rinsing in phosphate-buffered saline, samples were postfixed in 1 % osmium tetroxide in distilled water for 1 h and subsequently rinsed in PBS. The samples were then rapidly quenched in liquid-nitrogen-cooled liquid propane and placed in a precooled stage in the vacuum chamber of a modified high-vacuum coating unit (Edwards High Vacuum, UK) and held at −80°C and at 10^{-6} Torr for 16 h. Rewarming was at a rate of 10°C h^{-1} to 25°C after which the samples were sputter coated with gold (approx. 20 nm thick) in a Polaron E5000 sputter coater (Polaron PLC, UK) and mounted for examination in a Philips SEM500.

Results

Properties of microfabricated structure surfaces

It became obvious in early experiments that the adhesion and spreading of cells was greater on Perspex surfaces exposed to the oxygen plasma during the dry etching step of fabrication, than on unexposed surfaces, and that the etching process itself had the effect of roughening these surfaces, the degree of roughening being dependent on etch depth. Scanning electron microscopy reveals that this roughening is slight for etching producing 1 μm steps but becomes progressively more pronounced, resulting in a carpet of spicules at greater etch depths (Fig. 1).

The effect of treatment of Perspex samples on BHK cell adhesion, 30 min after seeding is seen in Fig. 2. In all cases, adhesion to Perspex samples was less than for tissue culture plastic ($P<0{\cdot}001$). Exposure to aluminium etch does not alter the adhesiveness of Perspex but exposure to oxygen plasma results in a greater than tenfold increase ($P<0{\cdot}001$) over either untreated or aluminium-etch-treated samples. No statistically significant differences in adhesiveness were found between samples oxygen etched to 1, 5 or 10 μm. Though the difference in adhesion between etched and unetched plastic is large at 30 min after seeding, after longer periods cells on unetched plastic became fully spread and motile with little obvious difference from those on the more adhesive etched surface. It was for this reason that samples were blanket etched, as this will have the effect of abolishing the adhesive difference, though a series of experiments was carried out on samples prior to blanket etching to test what influence such a difference would make to cells' reactions to a topographical feature (see below).

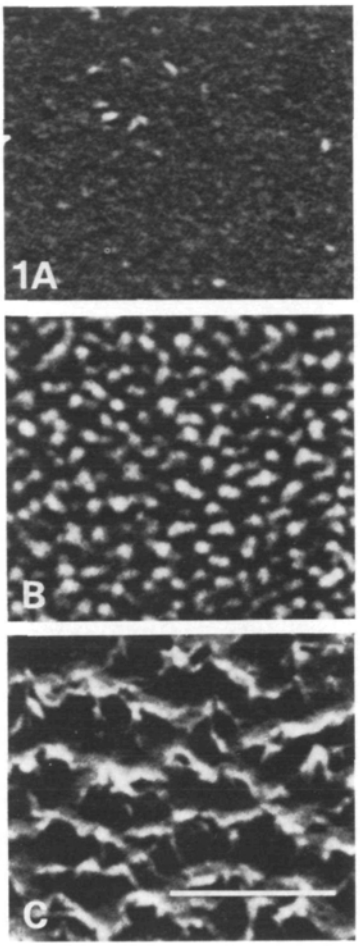

Fig. 1. Scanning electron micrographs of the oxygen-etched surfaces of Perspex. (A) 1 μm, (B) 5 μm and (C) 10 μm etch depth. Bar, 8 μm.

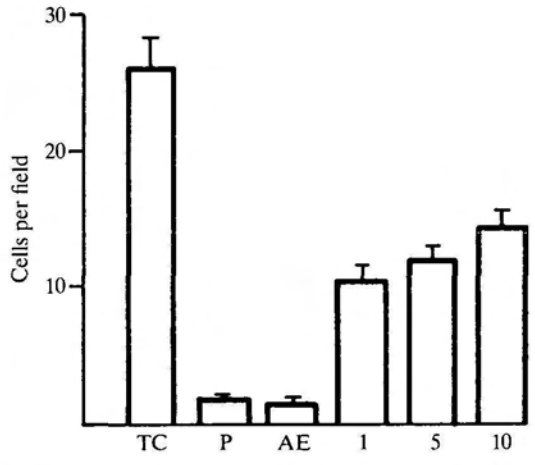

Fig. 2. The effect of various treatments of Perspex on the adhesion of BHK cells, as estimated by the degree of attachment 30 min after seeding. TC, tissue culture plastic; P, untreated Perspex; AE, Perspex exposed to aluminium etch; 1, 5 and 10, Perspex etched to 1, 5 and 10 μm respectively. Bars represent S.E.M.

Effect of step height on cell behaviour

As step height increases, there is a decrease in the frequency of crossing of those BHK cells that encounter a step, irrespective of their direction of approach (Fig. 3A). For cells approaching steps from the lower surface, all step heights significantly decreased crossing frequency ($P < 0{\cdot}025$ for 1 μm step; $P < 0{\cdot}001$ for steps 3 μm and above). The frequency of crossing of cells approaching from the upper surface was not significantly different from controls at 1 μm steps, but was decreased for step heights of 3 μm and greater ($P < 0{\cdot}001$). No difference was found between the frequencies of ascent and descent when compared over the range of step heights tested (excluding controls). The degree of alignment of these cells (Fig. 3B) at steps, mirrors that of crossing, where the proportion of cells aligning increases with step height, until, at the largest step heights (10 and 18 μm), few cells encountering failed to align. All step heights, for cells at both the upper and lower edges of the steps, significantly increased the degree of alignment ($P < 0{\cdot}001$), and again, no difference was found between the reactions of cells approaching from the

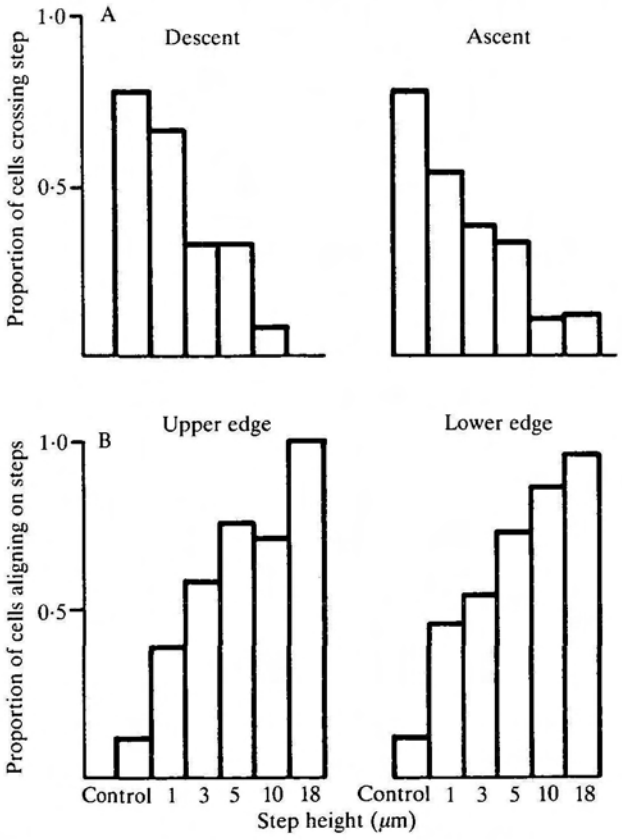

Fig. 3. The reactions of BHK cells to step features of various heights. (A) The proportion of cells encountering a step from the upper surface that cross (Descent) and the same for those from the lower surface (Ascent). (B) The proportion of cells aligning at either upper or lower edges of steps.

upper and lower surfaces. Scanning electron microscopy shows that BHK cells straddle a 1 μm step with little obvious distortion (Fig. 4A,B) and that an aligning cell may be in contact with both the upper and lower surfaces (Fig. 4A). Occasionally, flattened cell protrusions are intimately in contact with a step such that the underlying topography can be discerned (Fig. 4B). At 10 μm steps the majority of cells are aligned at both the upper and lower surfaces (Fig. 4C,D). Cells at the lower surface are also seen to be in contact with the step wall, and the underlying spicules of the lower surface appear to distort the more flattened areas of the cells (Fig. 4C,D).

When chick embryo hemisphere cells were seeded onto poly-L-lysine-coated Perspex steps of 1, 3 and 5 μm the frequency of crossing of growing processes, for both ascent and descent, could be seen to decline steadily with increasing step height (Fig. 5). In all cases the frequency of ascent was lower than for descent, but was not statistically significant when tested over the range of step heights.

Examination by scanning electron microscopy shows that many of the growing tips of neural cell processes fail to cross when they encounter a 4 μm step and follow the step edge (Fig. 6). This alignment of the direction of growth occurs at both the upper and lower edges of steps, though the path taken by a process often results in its doubling back (Fig. 6B). The growing tip of a neural process may, after encountering a step from the lower surface, move along the step wall for some distance (Fig. 6C). The growth of processes from cells that have settled on a 7 μm wide, 2 μm deep groove can be seen to be entirely confined to the groove over large distances, alternately encountering each wall of the groove (Fig. 6D,E).

Though it is not possible, on morphological grounds alone, to be certain that the cells producing

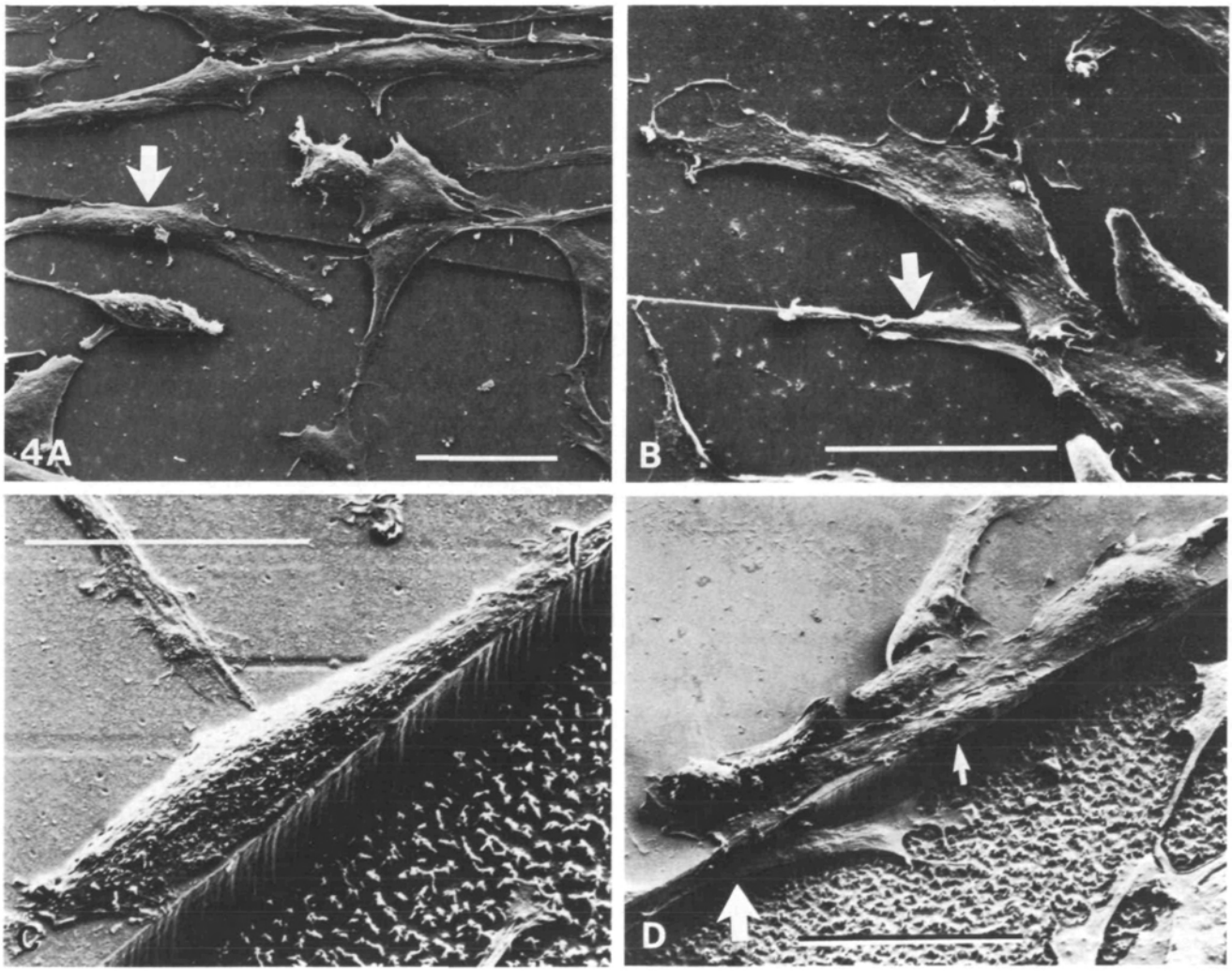

Fig. 4. Scanning electron microscopy of BHK cells on steps. A and B show cells at 1 μm steps, arrows indicating aligning cells in contact with both upper and lower surfaces. In C and D, cells at 10 μm steps are seen to align at both upper and lower surfaces, small arrow in D indicates the cell margin at the upper surface folded over the step edge, large arrow shows cell at lower surface in contact with the step wall. Bars, 40 μm.

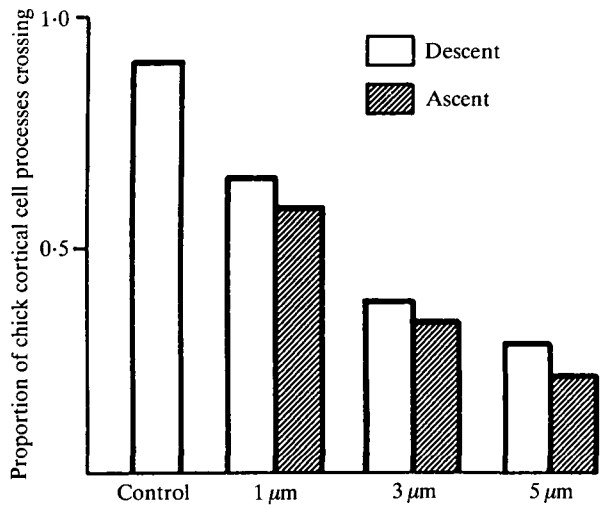

Fig. 5. The effect of step height on the direction of growth of chick embryo hemisphere neural cell processes. The proportion of processes encountering steps that cross from the upper surface (Descent) and lower surface (Ascent) are shown.

long processes are neuronal, previous studies have shown that there is a strong correlation between morphology (i.e. presence of processes) and specific neuronal staining under the culture conditions employed in this study (Sensenbrenner, Maderspach, Latzkovits & Jaros, 1978; Varon, Skaper, Barbin, Selak & Manthorpe, 1984).

Different cell types' reactions to an identical step

A series of experiments was carried out to compare the reaction of cells to a 5 μm blanket-etched step. In addition to the data already obtained for BHK cells and chick hemisphere cells, the crossing reactions of CHF cells and PMN were tested. In Fig. 7 it can be seen that although small differences exist, BHK, CHF and chick neural cells show similar responses to this feature. On the other hand, the frequency of crossing of PMN is more than twice that of the other cell types ($P < 0{\cdot}001$).

The influence of an adhesive difference on topographical effects

A series of experiments was carried out on steps before oxygen etching. The crossing reactions of BHK cells to step features, where no blanket etching has been carried out (thus the upper surface is less adhesive than the wall and floor of steps), differ depending on the direction from which the feature is approached (Fig. 8A). The frequency of descent at 1 and 3 μm steps is unaffected when compared to the control, though step heights of 5 μm or greater significantly decrease this reaction ($P < 0{\cdot}001$). When the cells approach from the lower step surface (i.e. the oxygen-etched surface) all step heights decreased the frequency of ascent ($P < 0{\cdot}01$ for 1 μm step, and $P < 0{\cdot}001$ for steps 3 μm and greater) to the extent that cells rarely crossed the largest steps (Fig. 8A). Comparison of ascending and descending behaviours (excluding controls) reveals that over the range of step heights the frequency of ascent is significantly lower than descent ($P < 0{\cdot}001$). The degree of alignment at steps mirrors that of crossing (Fig. 8B). Alignment at both the upper and lower edges increases with step height until the majority of cells encountering steps of 5 μm or greater will align irrespective of the direction of approach. Although 1 μm steps did not significantly affect alignment at the upper edge but did at the lower edge ($P < 0{\cdot}001$), and 3 μm steps allowed significantly greater alignment at the lower edge than at the upper edge ($P < 0{\cdot}025$), over the range of step heights from 1 to 18 μm the trend of increasing alignment was not significantly different at the upper and lower edges. When comparisons were made between the trends in crossing of nonblanket-etched and blanket-etched steps, it was found that the probability of blanket etching resulting in an overall decrease in descent was $P = 0{\cdot}0508$, and for an overall increase in ascent, $P = 0{\cdot}064$, over the range of step heights. Although these probability values are slightly larger than our limit of statistical significance ($P \leqslant 0{\cdot}05$), they suggest that the difference between ascent and descent by cells at steps where an adhesive difference exists, is not due to a change in behaviour of cells approaching from only one or other of the directions.

Discussion

Our examination of the effect of a simple step topography on cell behaviour has led us to reconsider currently held hypotheses concerning the mechanism and influence of contact guidance of cells. A major, if not unexpected, finding of this study is that the degree to which a cell is impeded in its movement is dependent on the size of the feature that it encounters. More surprising is that the effect of increasing step height on a cell's ability to cross and align is a gradual one, until large step sizes are tested, and that the modification of an adhesive difference at a step alters cells' responses. Our data give a clear indication that the alteration of a cell's behaviour by a topographical feature follows a probabilistic pattern.

When a cell approaches a step it must encounter a 90° convex angle (the upper edge) and 90° concave angle (the lower edge), the order of encounter depending on the direction of approach. It has been shown (Dunn & Heath, 1976), using cylindrical and angled substrata, that the degree to which a fibroblastic cell's protrusions are required to bend to accommodate a substratum will profoundly affect the size of

such a protrusion if it is to make contact with the substratum (the larger the angle, the shorter the protrusion), that this in turn will affect the traction that can be exerted in that direction, and that angles of greater than 17° will result in complete inhibition of crossing. This same study suggested a mechanism by which this phenomenon could occur involving limitations of the flexibility of cytoskeletal elements at the leading lamella. The smaller step sizes, in this study, may present an obstacle very similar to the angled prisms of Dunn & Heath (1976), where protrusions from cells at the upper edge of a step can contact the lower surface and *vice versa*. For larger step sizes, it would seem unlikely that protrusions would be formed large enough to span the distance between the upper and lower surfaces, and that crossing will require contact with the step wall. Scanning electron microscopy did show that BHK cells at a 10 μm step were in contact with the step wall from either the upper or lower surfaces, the former requiring a 90° folding of the cell margin. This has also recently been observed for primary fibroblasts at the edges of microengineered silicon substrata and it was suggested that such features do not act as an absolute barrier to cell movement (Brunette, 1986). It is obvious that these cells are capable of considerable distortion in accommodating to substratum topography, but the low or negligible frequency of crossing and high degree of alignment suggest that such distortions are rare, unfavourable or unsuccessful in altering the direction of movement of the cells.

O'Hara & Buck (1979), after detailed examination of cells' reactions to various grooved substrata, proposed that the mechanism responsible for orientation of the cells was in fact the orientation of sites of focal adhesion, which could in turn influence the orientation of microfilament bundles within the cells. It is possible that a single edge at a step could affect the formation and orientation of an elongated focal adhesion, but in a series of preliminary experiments

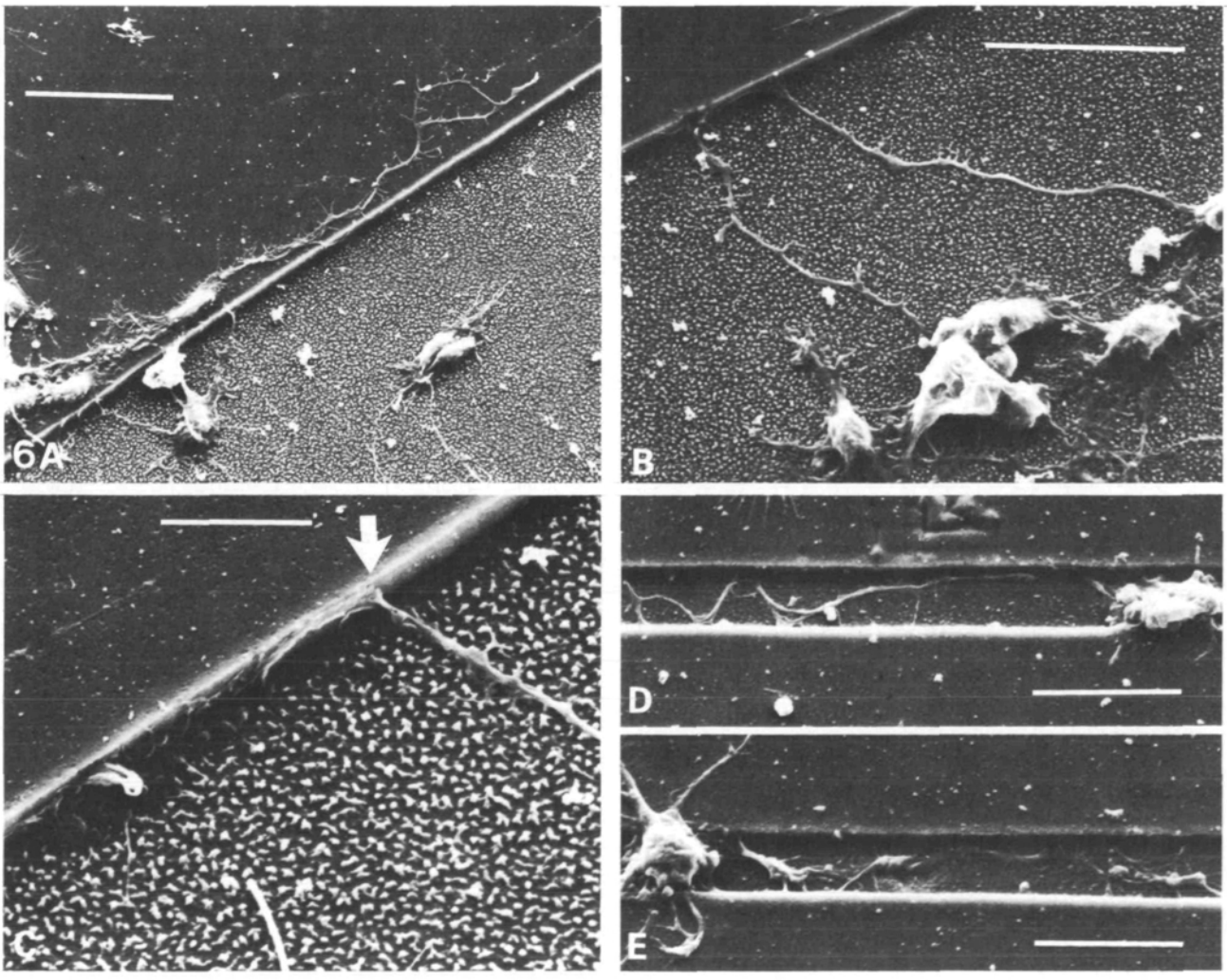

Fig. 6. Scanning electron microscopy of chick embryo hemisphere neural cells on poly-L-lysine-coated steps. (A–C), 4 μm steps; (D,E), 2 μm deep, 7 μm wide grooves. Arrow in (C) indicates the point of encounter of a growing process, the path of the growth cone having been deflected to the left. Bars, (A,B), 40 μm; (C), 10 μm; (D,E), 20 μm.

in our laboratory (unpublished observations), we have found that groove depth is an important, perhaps the most important, dimension of parallel-grooved substrata influencing the orientation of cells. It would seem unlikely that the formation of focal adhesions would be affected differently by different step heights.

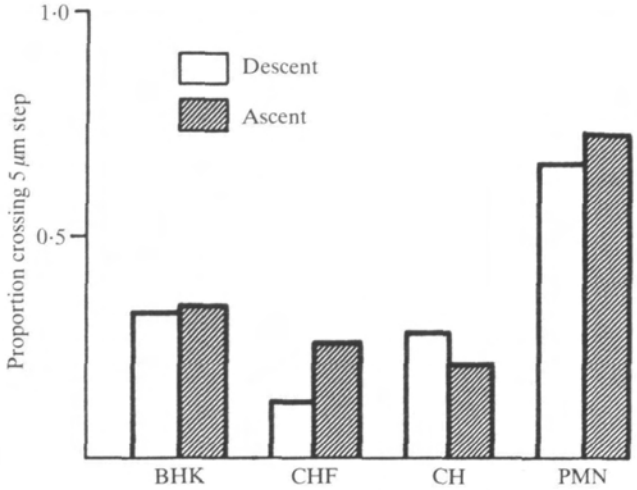

Fig. 7. The reactions of various cell types to a 5 μm blanket-etched step. Descent, the proportion of cells encountering from the upper surface that cross; Ascent, the proportion from the lower surface that cross. BHK, baby hamster kidney cells; CHF, chick heart fibroblasts; CH, chick embryo hemisphere neural cells; PMN, rabbit peritoneal exudate neutrophil leucocytes.

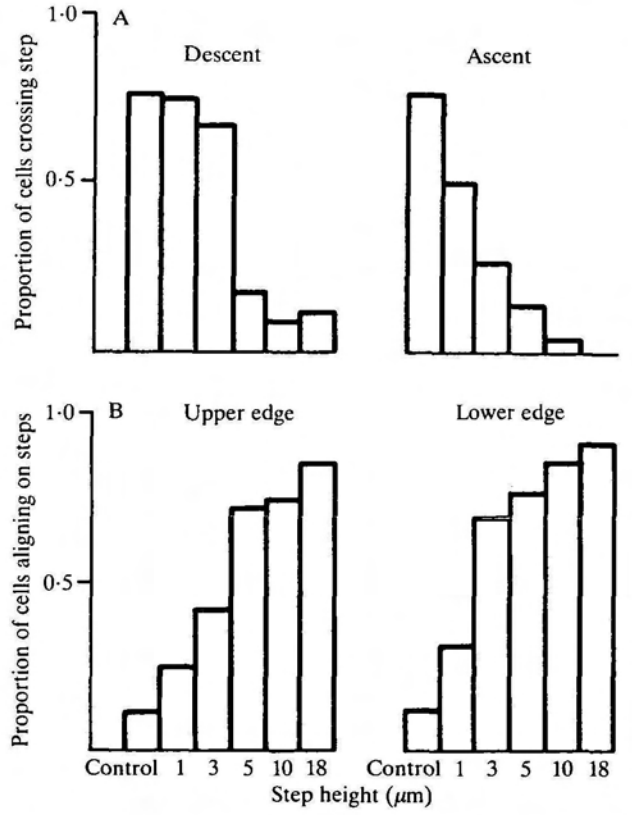

Fig. 8. The reactions of BHK cells to nonblanket-etched step features of various heights, i.e. the upper surface is less adhesive than the step wall and lower surface of the step. (A,B) as in legend to Fig. 3.

As previously mentioned, we feel that our data strongly indicate that a cell's reaction to a topographical feature is dependent on to what extent the feature alters its probability of making a successful protrusion and contact in a given direction, i.e. a cell's response to an anisotropic feature is anisotropic with regard to protrusive activity. The proposal that cytoskeletal inflexibility is the cellular property responsible for cells' reactions to topography (Dunn & Heath, 1976) is still, we believe, the most economical explanation of the phenomenon of contact guidance. Topographical perturbation of a cell's ability to make a protrusion in a given direction will be, in the first instance, mechanical. It will result in shortened processes, unable to exert sufficient traction, or could result, in fact, in areas of the cell whose microfilament organization is effectively isolated from that of the rest of the cell, e.g. where the cell margin is folded at 90° over a step edge. Protrusions made in directions where there is no topographical obstacle, e.g. along the long axis of a cylinder of small radius of curvature or parallel to a step, are more likely to result in polarization and movement in that direction than protrusions made over a curved surface or step. This is underlined by Dunn's observation (Dunn, 1982) that CHF cells adhering to spheres of sufficiently small radius of curvature (<50 μm) will remain rounded and relatively immotile. Such an isotropic topography may effectively trap a cell because it is unable to make successful protrusions in any direction.

We wish to emphasize that cells are able to overcome considerable topographical obstacles and that other guidance cues will modify a cell's response to such an obstacle. In this study, the presence of an adhesive difference at steps altered the reactions of BHK cells. It has been shown for human PMN, whose locomotion is contact guided in aligned three-dimensional gels (Haston, Shields & Wilkinson, 1983; Wilkinson, Shields & Haston, 1982), that in an aligned fibrin gel their response to a gradient of chemoattractant is influenced by the axis of alignment. Cells required to cross the axis of alignment will migrate towards the source of chemoattractant, but their response is impaired, whereas those moving along the axis exhibited an enhanced response (Wilkinson & Lackie, 1983). A chemotactic or adhesive cue may alter the reaction of a cell to a topographical obstacle by altering the probability of forming a successful protrusion or contact, thus antagonizing or enhancing the mechanical effect of the substratum.

Because a step may be considered to present two distinct topographical features, it might be expected that one or other of these features may be more important in eliciting a response from a cell. Concave anisotropic curvature was shown not to impose orientation on CHF cells, whereas equivalent convex curvature would do so (Dunn, 1982). Scanning electron microscopy in this study has shown that both BHK and neuronal cells approaching a step from the lower surface can make extensive contact with the step, suggesting that a 90° concave angle does not present a significant obstacle. This indicates that alignment at, and inability to cross, a step are mainly due to the 90° convex angle of the upper edge, irrespective of the direction of approach of a cell. A cell approaching from the lower surface will not require to distort in order to make contact with the step wall, whereas at the upper edge 90° bending would be necessary. This cannot be said to be the entire answer since cells approaching the lower edge of larger steps appear to align without contact at the upper edge.

The possibility that the microtopographical differences at step features (i.e. the differences in roughness) may contribute to influencing cells' reactions must be considered. We believe that this is unlikely to be significant in view of the fact that for blanket-etched samples no differences were found in ascending and descending behaviours even though the lower surfaces were in all cases more roughened than the upper surfaces.

The relative inability of a 5 μm step to perturb the locomotion of PMN, compared to the other cell types examined, must reflect properties inherent to that particular cell type. PMN do not form focal adhesions (Lackie, 1982), nor do they exert significant traction (Harris, 1982). In an isotropic concentration of chemoattractant they are capable of rapidly reversing their polarity (Keller, Zimmermann, Schmitt & Cottier, 1985), indeed their polarization is not anchorage dependent and they exhibit persistence of locomotion (Shields & Haston, 1985). It is only possible to speculate as to which, if any, of these properties may contribute to their relative 'agility'. Comparisons with growth cones of chick hemisphere neurites would indicate that the inability to form focal contacts or exert significant traction at the substratum locally are not important, since these properties are shared by both cell types (Abercrombie, 1982; Keller *et al.* 1985; Lackie, 1982). The simplest explanation may be that PMN lack a rigid cytoskeleton, having the ability to adapt their highly labile and flexible organization to external stimuli (Keller *et al.* 1985). It would seem that persistence of locomotion in stimulated PMN, which is believed to be due to asymmetry of chemotactic factor receptor distribution at the cell surface in both the presence and absence of a gradient (Shields & Haston, 1985; Sullivan, Daukas & Zigmond, 1984), is a stronger factor in determining the direction of locomotion than is a mechanical cue of this kind.

The extent to which topography is important as a morphogenetic cue during the development and growth of multicellular organisms is difficult to determine. The systems available for study often will not allow any one factor to be considered independently, though the use of natural substrata, *in vitro* (Dunn, 1982; Dunn & Ebendal, 1978; Ebendal, 1976; Haston *et al.* 1983; Wilkinson *et al.* 1982) and, more recently, simple *in vivo* systems (Wood & Thorogood, 1984) have lent further credence to contact guidance being worthy of the consideration of developmental biologists, and our work suggests that topography will, at the very least, affect the efficiency of directed locomotion due to other cues. Artificial substrata, such as those used in our work, will allow topographical factors to be studied in relative isolation and may eventually provide simple means, as illustrated by the fact that neurites can be guided by a single groove only 2 μm deep, of building specific patterns of cells to study novel cell interactions.

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