ELSEVIER

PII: S0142-9612(97)00144-0

Biomaterials **18** (1997) 1573–1583

0142-9612/98/$19.00

REVIEW

Topographical control of cells

Adam Curtis and Chris Wilkinson
Centre for Cell Engineering, University of Glasgow

We review the literature on the reaction of cells to their surrounding topography. The topography may be that of surrounding cells, intercellular materials or biomaterials. The reactions include cell orientation, rates of movement, and activations of the cells. We concentrate on those papers where quantitative measurements of the reactions have been made and largely ignore those on subjective impressions. A wide range of topographies are considered but special attention is given to results on groove-ridge topographies. The question of whether the cells are reacting to the topography directly or to patterned substratum chemistry formed on the topography is discussed. The review ends with a summary of the types of prosthesis where advantage has been taken of the ability to fabricate topography. © 1998 Elsevier Science Limited. All rights reserved

Keywords: Topography, substratum.

Received 6 January 1997; accepted 6 July 1997

This review reports on recent work on the reaction of cells to the topography of the substratum. These reactions occur in development and natural regeneration of tissue where extracellular material or other cells provide the topography. They also occur whenever we insert a material structure into tissue or expose it to crawling or settling cells.

When biomaterials are fabricated into a test structure or a prosthetic device it is likely that topography will be imposed on the surface of the material, either deliberately or by accident. Sometimes this happens without the knowledge of the fabricator. Surfaces of biomaterials are rarely flat at a molecular level: such surfaces only occur after cleavage of single crystal materials in particular planes. It is improbable that a molecularly smooth surface will be provided, except in the rare case of certain mica surfaces. This review explores the effects that topography has on cells, how it is measured and defined and how we may take advantage of topography, especially for therapeutic purposes. Recently we have published another review on cellular reactions to topography, which concentrates on cell motility reactions and also incidentally covers the early history of the subject. Readers primarily interested in those topics are directed to Curtis and Wilkinson[1].

FABRICATION OF TOPOGRAPHY

Precise fabrication

In an integrated circuit, a silicon wafer is taken and a precise pattern of metal lines, holes and dopant atoms created on or in it. This general technology can be used to pattern a wide range of biocompatible materials.

The starting point is to define a pattern in a radiation-sensitive material, a resist. This pattern is then transferred into or onto the substrate. The resolution required in the final structure defines the type of radiation used to expose the resist. For details of 1 μm or more laterally, UV light is used, while if 100 nm or less features are required, it is necessary to resort to electron beam lithography where a scanning and focused electron beam is used to expose the resist. After exposure, the resist is developed to leave a relief pattern: typically the resist is a polymeric thin film. Some resists are negative, others positive depending on whether the exposure to radiation hardens or softens the resist's reaction to the developer. The pattern can be transferred to the substrate by etching as the resist precludes etching in the areas covered in resist. Alternatively, the resist can be used as a stencil and additional material added to the substrate.

The choice of substrate is important. As cells typically have low optical contrast, transmission optical microscopy in the phase contrast mode is normally used for observation. However, glass does not etch well and so fused silica is a good choice for the substratum for experiments on the behaviour of cells on patterned surfaces consisting of grooves and ridges. The patterned substrates used in the work of Wojciak-Stothard *et al.*[2] were made by the following steps. First a photo-mask—a plate—was made which carries the desired pattern in a metal coating which absorbs UV. The mask itself is usually made using electron beam lithography and can be purchased commercially. Photoresist is coated onto the cleaned substratum and exposed to UV light

Correspondence to Professor A.S.G. Curtis: Centre for Cell Engineering, Joseph Black Building, University of Glasgow G12 8QQ, UK. Tel: +44 141 330 5147. Fax: +44 141 330 4501. email A.Curtis@bio.gla.ac.uk.

through the photomask and the resist is developed to leave a relief pattern. As in modern integrated circuit technology, a positive photo-resist is used in which the action of light is to change the resist so that it is removed by the developer. The substratum is now ready for etching. If wet chemical etching in say hydrofluoric acid is used, the action of the etch is isotropic and sloping walls result. However, etching with the assistance of reactive ions directed by an electric field vertically onto a horizontal substratum gives, under the correct conditions, vertical sided structures. In the case of the substrates mentioned above, the etching took place in an rf discharge of CHF_3. After the etching is completed (the depth can be monitored using optical reflectrometry), the resist is removed in a strong solvent. It is best at this point to return the substratum to the etching machine and etch the whole sample for a little longer, which does not change the shape of the grooves, but does ensure that all surface have the same chemistry. An example is shown in *Figure 1*.

It can be desirable for some purposes to use polymeric substrata. In these materials it is possible to use a more direct method of pattern transfer, casting or embossing. The first step is to move a mould in a solid material. To cast a replica, a solution of desired polymer and a solvent is allowed to dry on top of the mould and then the polymeric film is stripped off and used. In embossing the mould is pressed into the polymeric material which has been heated to soften it. Casting is routinely used in transmission electron microscopy to form replicas of high fidelity and embossing is used to print pits of size roughly $0.3 \times 0.7\ \mu m$ in compact disk (CD) manufacture.

There are other methods of microfabrication which have been used to make structures to exercise living cells. Laser ablation can be used to remove materials —either on a point-to-point basis with a moving beam or through a mask. All serial methods of manufacture, in which the topography is formed point-by-point, are intrinsically slower than those in which the whole pattern is transferred at the same time—as in photolithography or casting.

It is relatively easy to make grooves which have vertical walls, a little more difficult to control the slope of the walls and much more difficult to make arbitrary shapes in three dimensions on a micron scale. An arbitrary profile to a groove implies having a similar shape in the resist, which implies varying the dosage of radiation to the resist point. This can be achieved in a electron beam machine, but requires the precise control of both the dosage and the resist development characteristics.

One of the main practical ends of studies of cell movement on structure is to be able to understand how cells migrate in the body in three-dimensional structures and to apply this in the production of devices for tissue repair. This of course requires topography extending appreciably in the third dimension on a scale larger than that which would just be occupied by a single layer of cells. Most studies have been carried out in collagen or fibrin gels oriented to some degree by flowing the system during gelling. Guido *et al.*[3] used intense magnetic fields to orient collagen as it gelled, but even in this case orientation was not well controlled in the *z* plane.

Table 1 Routes to unintentional topography

Machining with tools that cut repetitive lines.
Surface abrasion with too coarse an abrasive.
Casting or moulding from surfaces that have surface topography.
Surface flow patterns from moudling or embossing.
Improperly mixed polymer blends causing topography due to shrinkage or surface tension relationships while cooling from a melt.
Polymers with microdomains sensitive to differential swelling effects.

Accidental topography

We remind readers of the ways in which topography may be unintentionally and perhaps unknowingly imposed on a surface. These are summarized in *Table 1*.

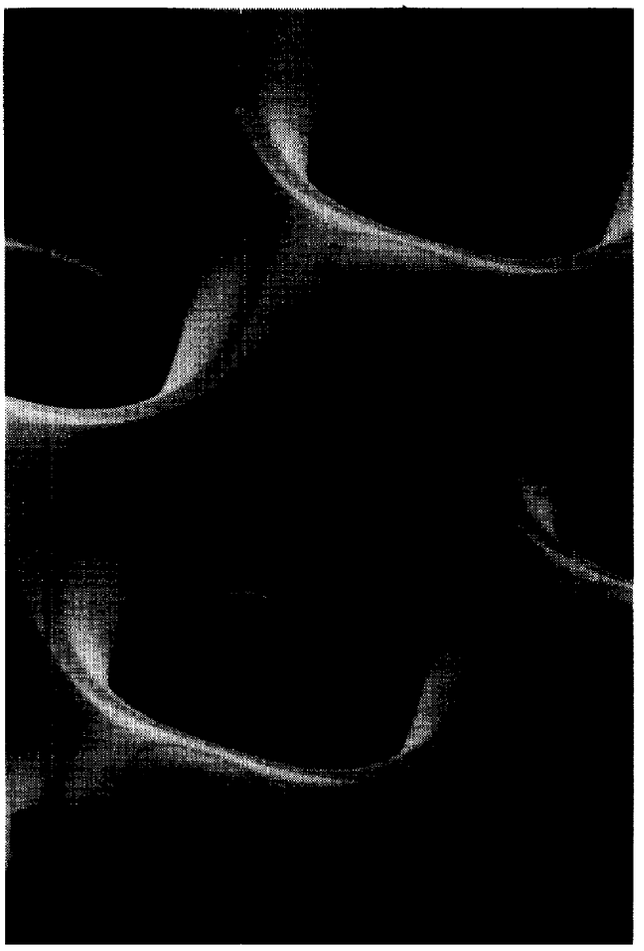
In practice, surfaces other than those derived by cleaving a perfect crystal or those obtained by cooling a melt under vibration-free conditions will have some type of topography on them. Even the 'perfect' surfaces just mentioned are likely to have topography of a few nm on them, but this may perhaps be insufficient to induce cell reaction (see under Effects of depth).

CHEMISTRY OR TOPOGRAPHY?

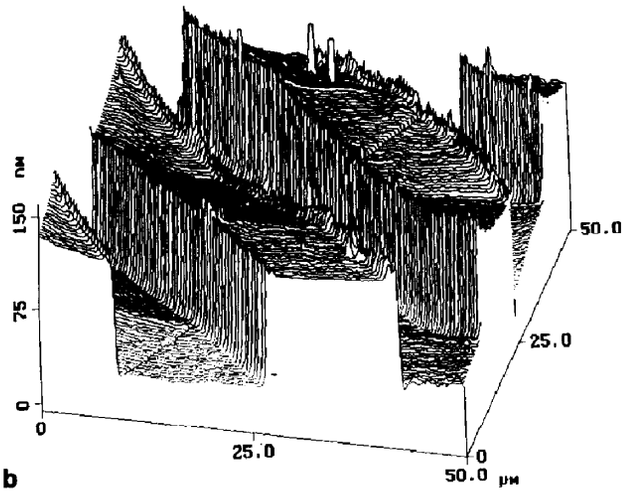
One question which has repeatedly occurred to those working in this field, as well as those standing by criticising it, is whether the methods of fabrication of topography actually produce chemical patterns on the surface. These in turn might result in the cellular reactions. Techniques of protein patterning, for example those introduced by Britland *et al.*[4], can also be used to produce patterns that guide cell movement, see for example Clark *et al.*[5] and Britland *et al.*[4,6]. Since these protein patterns have edges, it is possible to reverse the criticism and then ask whether cells can react to edges which may be a single molecule in height or are simply discontinuities in molecular ordering. This problem has been largely answered by Pritchard *et al.*[7] who used patterning systems to fill adjacent spaces with different proteins or silanes to level up the surface so that any step at the junction is less than a molecule in height. Cells still reacted adhesively to the patches of adhesive material. Nevertheless, there may be zones of disordered molecules at the junction and it is possible that it is these that the cells react to. Whichever explanation is correct it is a widespread observation that the moving or extending cells appear to be localized more frequently at the region of junction or discontinuity than would be expected if cell attachment to the patches of protein was random.

The work of Britland and colleagues (Britland[6]) is of especial interest since they used a chemical cue (laminin) oriented at right angles to a topographic one. When the grooves were 500 nm deep or less, the cells reacted chiefly to the chemical cue. On deeper grooves the topographic cue over-rode the chemical one and at $5\ \mu m$ depth the topographic effect oriented about 80% of the cells and the chemical one 7%.

Curtis *et al.*[8] devised a method for applying very



a



b

d

c

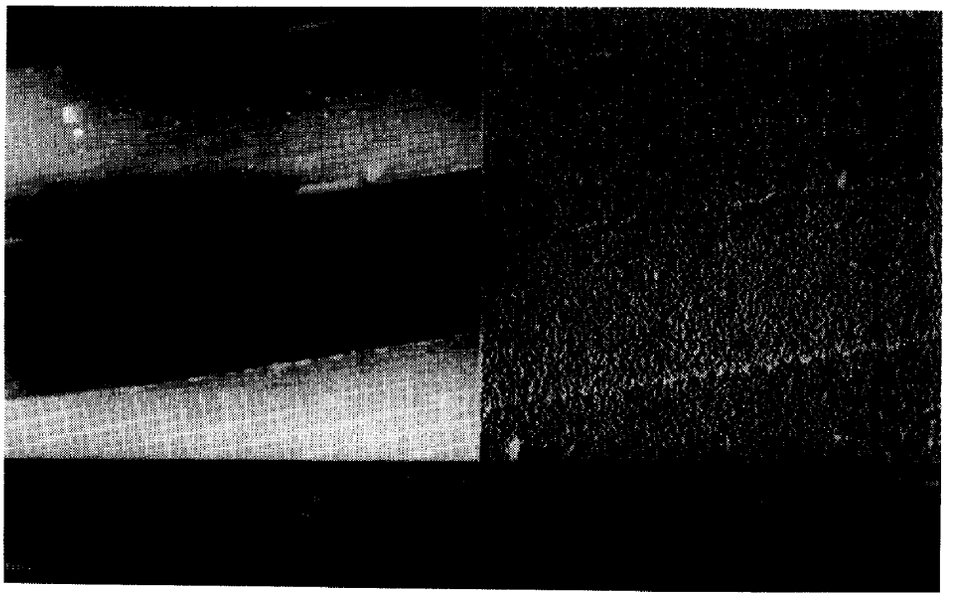


Figure 1 **a** Scanning electron micrograph of Silica topography formed by photolithography and reactive ion etching. This structure was used to demonstrate that cells prefer to 'ridge-walk', see also *Figure 5*. **b** AFM scans of 60 nm deep silica grooves showing precision obtainable with this type of fabrication. **c** High *z* resolution AFM scan of ridge top of freshly prepared topography also shown in **b**. **d** Protein adsorption from serum albumin solutions, fixed and dried. All AFM pictures in contact mode.

Table 2 Materials used for topography that affect cells

Elements
Gold
Titanium (its surface oxidises) (Brunette *et al.*[47])
Silicon (its surface oxidises)
Carbon (diamond an diamond-like materials)
Inorganic compounds
Silica (Clark *et al.*[11–13])
Lithium niobate
Silicon nitride (Breckenridge *et al.*[48])
Polymers
Polymethylmethacrylate (Clark[13])
Silicones (den Braber *et al.*[15])
Epoxy (unspecified) (Chehroudi *et al.*[50])
Polydioxanone
Nylon (Curtis and Seehar[42])
Cellulose acetate (Curtis and Varde[22])
Polyimide (Breckenridge *et al.*[48])
Collagen
Fibrin (Weiss[18])

Table 3 Types of topography

This list contains structures that have been examined (with references) and those that apparently have not been examined yet.

Single cliffs (Clark *et al.*[13])
Converging/diverging cliffs
Groove/ridges
V-section (Curtis and Varde[22])
Rectangular section (many reports, e.g. Clark *et al.*[12])
Round
Multiple grooves (many reports especially Brunette *et al.*[47] and Clark *et al.*[11])
Branching (Ternaux *et al.*[51])
Discontinuous ridges
Spiral grooves (Dow *et al.*[29])
Dots
Spikes (Rovensky *et al.*[52])
Hill (Drumlin type topography (Dow *et al.*[27])
Pits
Tunnels and tubes (Sanford *et al.*[53] and Aebischer *et al.*[44])
Fibres (Weiss[18,54,55])
Cylinders (Rovensky *et al.*[52])
Mesh (Curtis and Seehar[42])
Random roughness (Lydon and Clay[20])

small areas of chemically specified substratum to a cell using a bead technique. Although these cues often modified or activated cells leading to increased neurite extension, they did not lead to a polarized extension or any other obvious reaction resembling a topographic one. Den Braber *et al.*[9] modified silicone grooved and plane surfaces by exposure to UV light, which affected surface energies (and thus presumably protein adsorption) but did not alter topographic reaction. This result suggests that surface chemistry plays a small role in topographic phenomena. It is also consistent with the findings that cells react to topography in a broader similar way on very many different materials bearing topographic features, see *Table 2*.

Any etch process could produce chemical patterning of the unmasked areas. This can be avoided by giving the structure a short 'blanket' etch over the whole surface after the topography has been formed. Nevertheless, it is possible that the near-vertical walls between ridge and groove might be affected in some way different from the ridges or grooves. It is very difficult to find out whether this is so or not because methods of examination such as ESCA or AFM are primarily for use on horizontal surfaces. Embossing methods might be expected to produce little chemical patterning, but it is just possible that regions of maximal flow, for instance the near-vertical 'cliff' joining ridge and groove, have chemical differences from the rest of the surface. Casting methods should be free of such defects provided cast and mould separate easily. We have demonstrated identical guidance on cast polydiovanone and polyurethane films.

Of course surrounding cells, as well as intercellular material, especially if already oriented, should provide guidance structures as Vesely *et al.*[10] pointed out, but in most cases it has been difficult to exclude other effects the cells might have. It should be remembered that close-packed equi-dimensioned cells will provide a regular quasi-hexagonal set of guidance channels.

RANGE OF PHENOMENA

These can be classified in terms of the types of topography on which reactions occur and also in terms of the effects on the cell. Since it is easy to observe the morphology of the cell, most of the data refers simply to morphology and its concomitant orientation.

Table 3 gives a list of the main types of topography that have been studied and some of the main references. Some of the names are derived from the terms used by geomorphologists.

Effects of depth

Few studies of the effects of topography depth on orientation, let alone movement, have been carried out. Clark *et al.*[11,12] and Wojciak-Stothard *et al.*[2] have shown that the situation is complex, varying with cell type as well as with several of the topographical measures. Cells react to a single cliff and increasingly so with increasing cliff height in the range 1–20 μm (Clark *et al.*[13]). Clark *et al.*[11,12] showed that on groove/ridge topography the extent of reaction is related to groove width as well to depth and probably also to the number of adjacent grooves. There is general evidence that the extent of orientation increases with groove depth up to about 25 μm from topographies of about 1 μm relief. Below this degree of relief results are less available, partly owing to the difficulty of quantifying the etch depth in earlier years and perhaps partly because of a naive belief that there would not be any effect on the cells. Wojciak-Stothard *et al.*[2,14] have discovered that P388D1 macrophage-like cells react down to dimensions at least as small as 44 nm. Other cell types so far studied do not appear to be as reactive, but epitena, epithelia, fibroblasts and endothelia react to depths as shallow as 70 nm. Such small topographies approach the dimensions of large molecules and in the very near future the excitement generated by the suspicion that the sensing mechanisms of cells for topography are perhaps as sensitive as those of the atomic force microscope may lead to further discoveries. On the deeper and narrower grooves cells may bridge from ridge to ridge, see *Figure 2*, so that the cells are effectively reacting only to features of the ridges, see Den braber[9,15].

Figure 2 Diagram (cross-sectional) of cells aligned on grooves showing how cells may bridge from one ridge to another. Major actin accumulations shown as black dots with finer actin filaments extending towards the centre of the lower side of the cell.

Groove width

When the grooves or ridges are appreciably wider than the cells effects on orientation are not very marked, although cells may align to one edge, see Clark *et al.*[13]. As the groove/ridge width is reduced to the width of the cells and less, effects on orientation become more marked. When more than two discontinuities (ridge-groove meetings) underlay a cell, lines of actin condensation mark each discontinuity[2]. Clark *et al.*[5] showed that BHK cells will react to groove/ridge topography with a pitch (repeat) of 260 nm. These grooves were relatively deep at 500 nm. No work has yet discovered the minimum width of topography to which a cell can react.

Orientation of the cells to the topography

Orientation can be very precise, see for example Wojciak-Stothard *et al.*[14]. Although some authors have measured orientation in terms of the number of orientations lying in various degree interval classes, in many cases it may be worthwhile measuring the angle to the orientation of the topography to obtain results with a good statistical background. 0° is usually taken as perfect alignment. Results should of course be stated as mean orientation and include a measure of variance and of kurtosis, lest the distribution be bimodal. Some authors have taken a small variance in the angle as a measure of orientation, but this metric is not sufficient as it does not tell you whether the orientation of the cells is parallel or at some other angle to the substratum orientation, see work by Nagata *et al.*[16] where an orthogonal orientation was found. In most cases orientation is so obvious and extreme that the cell is reduced to having at most two pseudopods, at opposite ends of the cell.

Clark *et al.*[11] pointed out that orientation reactions have a fairly large random component, especially on shallow grooves, so that it should be borne in mind that, along with many other biological reactions, there is a probabilistic component in this type of reaction.

Extension

Neurite extension is stimulated by growing cells on grooves (see Curtis *et al.*[17]) for precise measurements, although this has been known qualitatively since Weiss[18] and Hoch *et al.*[19] demonstrated similar effects for the extension of fungal hyphae on a leaf and on microfabricated surfaces.

Table 4 lists the main types of reaction that have been observed.

Range of cell types responding

Cell types that have been shown to react to topography include those listed in *Table 5*. Quite a range of embryonic cell types have also been shown to react.

Table 4 Effects on cells of contacting topography

Orientation (many studies, especially Clark *et al.*[11, 12])
aligned many studies (e.g. Clark[11, 12] and den Braber *et al.*[15])
normal to topography (Nagata *et al.*[16])
Extension enhanced (Curtis *et al.*[17])
Accelerated movement (Curtis *et al.*[17])
Polarised movement (Dow *et al.*[29])
Capture (movement stopped at a particular feature)
Effects on adhesion
Activation of (Wojciak-Stothard[2])
Tyrosine phosphorylation
Actin polymerisation
Vinculin accumulation over the groove edge
Phagocytic activity
Fibronectin m-RNA expression (Chou *et al.*[37])

Table 5 Cell types reacting to topography

Chondrocytes
Endothelia
Epitena (Wojciak *et al.*[13])
Epithelia (Chehroudi *et al.*[49] and Clark *et al.*[12])
Fibroblasts (Curtis and Varde[22])
Leucocytes (Wilkinson *et al.*[56])
Lymphocytes (Haston *et al.*[57])
Macrophages (Wojciak-Stothard *et al.*[2])
Mesenchyme (Wood[58])
Neurons ((Weiss[54, 55]), Aebischer *et al.*)
Osteocytes (Vesely *et al.*[59])
Oligodendrocytes (Webb *et al.*[60])
Smooth muscle cells (Tranquillo *et al.*[61])
and some tumour cells (see Curtis *et al.*[17])
Fungi (Hoch *et al.*[19] and Sherwood *et al.*[62])

If claims of lack of reaction are made for cells of the above groups, such claims should be viewed with some scepticism because the exact dimensions chosen may have been inappropriate for the cell type. For instance, macrophage-like cells such as P388D1 cells react to steps as small as 30 nm in height (Wojciak-Stothard *et al.*[2]), whereas endothelia respond to steps of 100 nm height or greater. In addition, some cell types, such as epithelia, tend to show lack of reaction when the cells are in contact with each other, but react by alignment very strongly when isolated, see Clark *et al.*[12].

Roughness

The question of the reaction of cells to random roughness is an intriguing one. First there are problems at the apparently simple level of defining roughness. Second, the quantitation of roughness presents similar difficulties. Height, gradient and correlation length statistics need to be defined. There are many reports that rough, often grossly rough, surfaces aid cell adhesion, for example that of Lydon and Gray[20]. A method which circumvents this approach is to use structures of defined geometry, but on a nanometric scale. This has been achieved by electron beam lithography to define small x and y axis dimensions and extremely short etch times using reactive ion etching to define small z axis dimensions. The results are of course obtained with the nanometric grooves described above.

INVESTIGATING MOVEMENT

Although there are many studies in which explants have been placed on structures and cells have moved

out of the explants (e.g. Weiss[18]), not only have measurements of speed of movement not been made but it would have been almost meaningless to have done so. The reason for this is that when cells move in streams or sheets there is a large component of interaction between cells (contact inhibition of movement) in the reaction, see Abercrombie and Gitlin[21]. Curtis and Varde[22] found that contact inhibition was enhanced on both fibres and on groove/ridges compared with plane surface. Thus, collisions between cells moving on these types of structures might have a very marked effect on movement. Consequently, measurements designed to obtain a measure of the locomotory activity of single cells, uncomplicated by collision, must be carried out on very sparse populations. This condition was met in the work of Curtis *et al.*[17]. We suggest an alternative approach, namely that cell locomotion be measured on widely spaced groove/ridges ('race tracks') of defined dimension, where collision effects and random excursions across groove/ridge edges are largely suppressed.

Speed of movement

Cells moving on a flat surface show random walk with an element of persistence in all cases so far studied, in the absence of other stimuli such as chemical, electrical or other oriented signal gradients (Gail and Boone[23]). There is one exception known to us, that is the paper by Hartman *et al.*[24] who used the Markov methods described in the next paragraph. Cells moving on topographies such as grooves often show highly oriented movement and this frequently becomes effectively one-dimensional. The method of analysis introduced by Gail and Boone is based on treating the path and transit time along the path as a two-dimensional walk. It is very unclear as to how a two-dimensional walk can be compared with a one-dimensional one using the Gail and Boone approach. Friedl *et al.*[25] and Guido and Tranquillo[3] used comparable methods of analyses of cell movement in three-dimensional (3-D) collagen gels. In the latter study orientation of movement in relation to collagen fibril orientation was seen. Kuntz & Saltman[26] used a similar approach for estimating neutrophil movement in collagen type I gels supplemented with laminin, fibronectin or heparin. However, it should be borne in mind that Olson[27] showed that cells in collagen gels could generate relatively large forces which might tear the gel open to allow entry and movement by cells.

Curtis *et al.*[17] unaware of the paper by Hartman *et al.*[24] suggested that these difficulties can be resolved by treating a locomotion as a Markov-type process in which there are transitions between movement and arrest. A step is defined as a rectilinear movement at constant speed. Thus, we can compare 1-D, 2-D and even 3-D situations.

An unresolved question in all these investigations is whether the movement measured should be in the front of or the hind end of the cell, or of the nucleus or the centre of mass. The first and second of these give measurements which may be affected by cell extension or retraction, the third is easy but arbitrary and the centre of mass approach is difficult even if the phase stepping microscope is used to follow mass distribution (Dunn & Zicha[28]).

Polarization of movement

Although movement on grooved substrates is highly oriented, there is no evidence that it is polarized on rectilinear grooved substrates so that cells move only in one direction. This emerges from the study by Curtis *et al.*[17], although cells do make longer and more lasting runs in one direction before stopping or reversing. In the one case polarization was seen; this was the opening logarithmic spiral groove used by Dow *et al.*[29]. Cells migrated centrifugally, not along the groove, but by forming chords from one part of the groove edge to another, usually crossing from the inner side to the outer side of the groove further out on the spiral. No cells moved centripetally: those cells at or very close to the centre of the spiral were rounded and did not move at all.

While major lamellopodia are usually aligned to the topography, there are cases where microspikes are arranged orthogonally to the main locomotory organs, see *Figure 3*.

Extension of neurites by their growth cones is of course normally polarized and retraction events are usually interpreted as being due to the collapse of the cone.

Locomotory behaviour

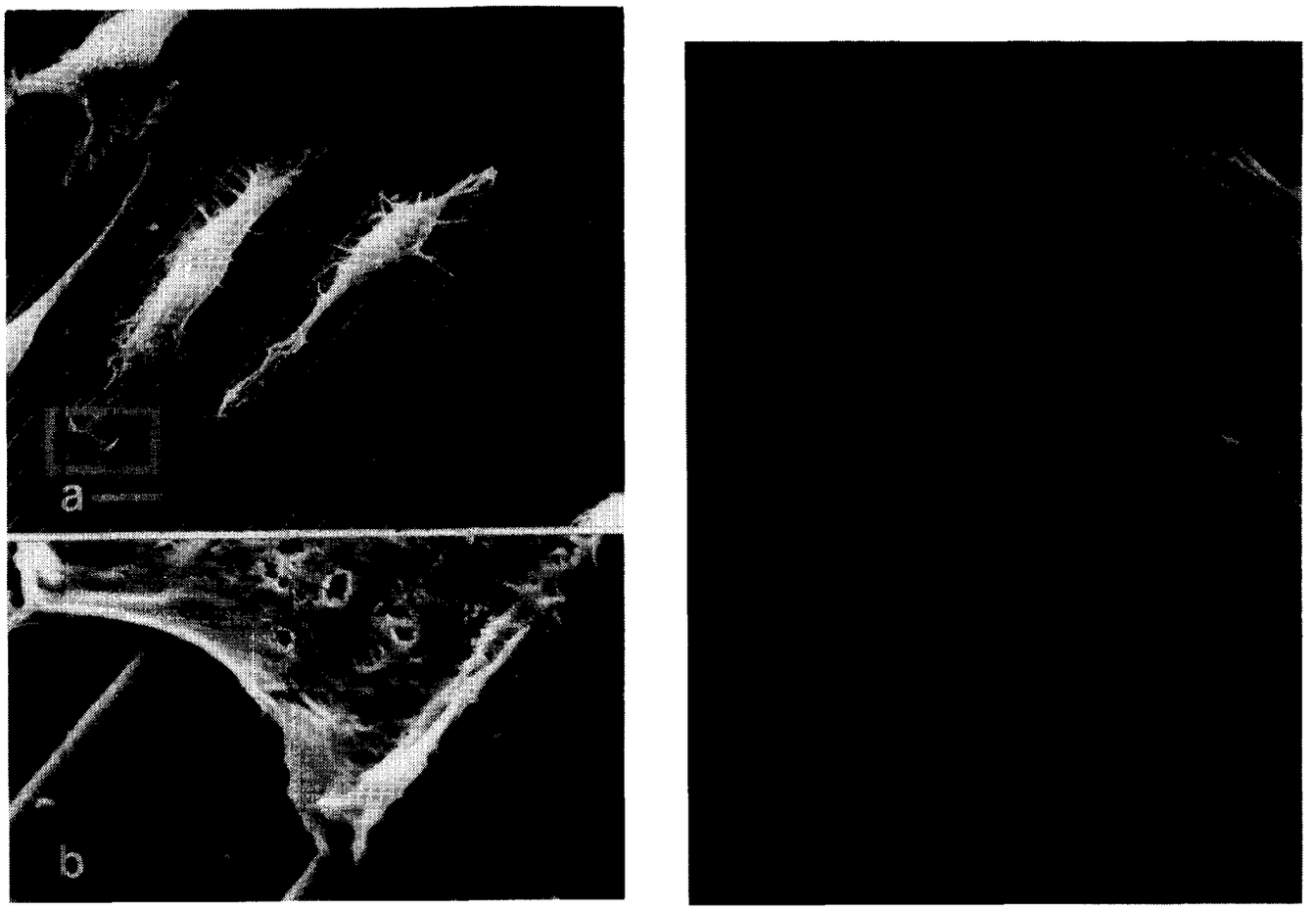
Although contact guidance has been distinguished from contact inhibition of movement it should be appreciated that cells migrating on a guiding structure may still exhibit considerable contact inhibition of movement. Curtis and Varde[22] found that chick-heart fibroblasts moving on 12.5 or 25 μm wide groove/ridges showed more contact inhibition than that found on planar surfaces. It increased for groove/ridge structures as the structures became narrower and decreased on fibres as they became narrower.

Mechanisms of movement

There is no reason to suggest that the locomotion of cells on narrow guidance paths is different in mechanism from that on planar surfaces. The sensitivity of movement to agents that disrupt the cytoskeleton is similar on grooves as on planar surfaces (Wojciak-Stothard *et al.*[2]).

It is, however, noteworthy that the cells on grooves or ridges often show very narrow lamellopodia (or growth cones in the case of neurons). Thus, those in grooves or on ridges must exert relatively greater tractive effort per unit area than those on a planar surface. Britland *et al.*[6] have recently demonstrated that interference reflection microscopy reveals focal contact on relatively wide grooves/ridges. The actin cytoskeleton in cells (fibroblasts, endothelia and macrophages) reacting to topography is organized in a way which we believe to be appropriate for movement. Focal contacts (Meyle *et al.*[30,31]) and vinculin (Wojciak-Stothard *et al.*[2]) are aligned over or close to the groove/ridge contact.

Although Oakley and Brunette[32] claimed that the first cytoskeletal event in the reaction of cells to



a **b**

Figure 3 P388D1 macrophage cells aligning on 88 nm high ridges. Note microspikes at right angles to the groove. This type of cell became highly motile on this type of substrate. Note also the attachment of the cell to the ridge-edge discontinuity.

grooves was a microtubular one, this seems unlikely, partly because Wojciak-Stothard *et al.*[2] found that actin-based events occurred at times shorter than those used by Oakley and Brunette and partly because, as Mitchison and Cramer[33] observe, cells such as keratinocytes and neutrophils move without microtubule involvement. These cell types react to grooves. In addition, the association of alignment with focal contacts and vinculin is consistent with an actin-based explanation rather than a microtubular one.

Neurite extension is believed to take place by the same mechanisms as movement in other cell types (see for example Smith[24]). No special study of locomotion on structures that might affect movement appears to have been made, but it is of interest that Hammarback *et al.*[35] showed that the microspikes produced by neurons can be extended across appreciable distances to locate on surfaces suitable for attachment. Consequently, a growth cone might be able to bridge over otherwise unsuitable topographies.

It is rather unclear how cells move in 3-D systems such as collagen, but Lackie[36] has suggested that some cells move not so much by adhesion to the substratum fibres but by 'hand-jamming' like a climber and using the purchase obtained by distorting themselves around fibres and pulling on the hold so formed. On the other hand, many cells have receptors for collagen or for fibronectin which in turn binds to collagen.

ACTIVATION OF CELLS BY REACTION TO TOPOGRAPHY

The changes in cytoskeletal condensation produced by contact of a cell with topography, see Oakley and Brunette[32] and Wojciak *et al.*[2], were shown by the later group to occur rapidly (in less than 5 min for a macrophage-like cell), although some cell types react more slowly. Such fast changes raise the possibility that cell activation occurs as a result of the contact with topography. Wojciak-Stothard[2,14] investigated this and showed that tyrosine phosphorylation was stimulated (*Figure 4*) over the discontinuities. The cells became more active in phagocytosis. Chou *et al.*[37] have shown, using Northern blotting, that expression of m-RNA for fibronectin is stimulated by contact with a grooved topography.

SENSING OF TOPOGRAPHY

In the Introduction we described some of the earlier theories proposed to account for the existence of topographical reactions of cells and the reasons why those theories were more or less inadequate. Curtis and Clark[28] put forward the idea that cells react to discontinuities. They based this on the observation of the positions of cells reacting to topography and also on the phenomenon of 'ridge-walking' seen on the

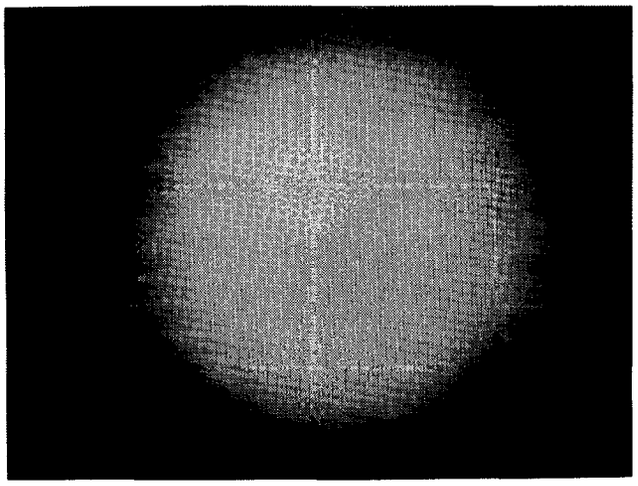


Figure 4 Tyrosine phosphorylation over grooves. P338D1 macrophage adhering to substratum with 70 nm deep grooves, 2 micron wide. Fluorescent antibody against phosphotyrosine. Confocal scanning microscope picture 20 min after plating out of cells. Note the staining is over the discontinuities.

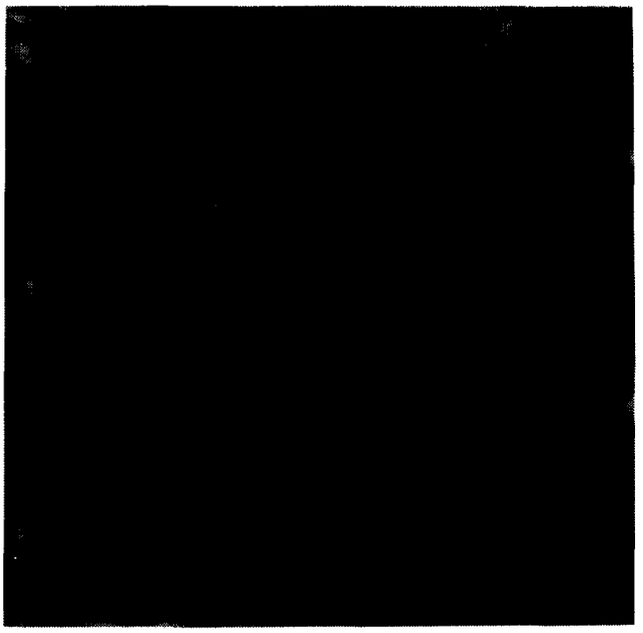


Figure 5 Ridge-walking. BHK fibroblasts on hills 2 mm high and 20 mm spacing from peak to peak. Note that cells are aligned to discontinuities (ridges) joining peaks.

'hill' topography (*Figure 1* and *Figure 5*) where cells align and move along the sharp intersections of hill slopes, not descending to flatter areas even though the ridges are themselves concave as they run from one 'peak' to another.

Although this idea seems to have attractions it should not be accepted uncritically. Dunn[39] has criticized it in terms of the theory he and Heath proposed (Dunn and Heath[40]). In any event the ideas of reaction to discontinuity merely moves the problem to the question of how cells sense discontinuities. Furthermore, it begs the question what is a discontinuity. A working definition might be that from the biological point of view a discontinuity has a radius of curvature less than the average length of a pseudopodium or of the distance part of the sensing elements that control movement. Although atomic force microscopy (*Figure 6*) shows that the groove edges can appear sharp at a 0.2 nm vertical resolution, at some magnification they would appear to be irregular or perhaps rounded. In addition we suspect that many topographies are less precise than that illustrated in *Figure 1*.

One idea which we find attractive is that the cells stretch themselves on the substratum and that stretch receptors are thereby activated. This would require firm adhesions at two or more points and cytoskeletal activity to tense the cell. As cells conform or attach to topography some receptors would be subject to variable degrees of deformation or even compression. Regions of activation might lead to cytoskeletal organization. Concave surfaces would lead to compression, while convex surfaces would cause tension. Clearly experiments on which various shapes are presented to

Figure 6 AFM picture of epitenon cells on polyhydroxybutyrate substratum. Note alignment of cell to the surface topography seen in the deflection picture (right-hand side).

the free surfaces of cells are needed. The concept is consistent with the theory of reaction to discontinuity. The evidence of Wojciak-Stothard[2] that tyrosine phosphorylation, cytoskeletal changes and activation of phagocytosis occurs as a fairly fast reaction to topography suggests that signal transduction pathways have been activated by contact and this in turn is consistent with a signal–reception event such as stretch reception. Differences in receptor-density and degree of anchoring to the cytoskeleton could account for differences in the reaction of different cell types to topography.

USE OF TOPOGRAPHIC GUIDANCE IN THERAPY

Jenkins *et al.*[41], working soon after the first commercial production of carbon fibre, used it to sew up a snapped ligament. Results were successful because, although the carbon fibre shaded within a few weeks, a cellular infiltrate had migrated along the fibres and rebuilt a tendon. This procedure was not so successful with other ligament repairs. Possibly the wrong diameter of fibre has been used since we now know that fibre diameter could have been of considerable importance. During the following two decades rough surfaces were applied to osseous prostheses with the concept that this would aid cell adhesion, but no logical approach to defining such surfaces appears to have been used. During the same period woven and non-woven fibre meshes, often of biodegradable materials, were used to provide supports for tissue repair but in principle Curtis and Seehar[42] had already shown that this would normally lead to circumferentially arranged clusters of cells unlike structures normally seen in tissues.

It is only recently that approaches to the use of topographies in prostheses have begun to be based on the results of quantitative experiments with cells. There is much activity in the field and although little has been published at the time of writing, much may be expected in the near future. A number of attempts to use conduits (i.e. tubes) to aid nerve repair after injury have been tried and these are reviewed by Doolabh *et al.*[43] and Aebischer *et al.*[44]. We have patented two approaches in which microfabricated topography is used to control cell orientation with the declared intention that such materials would be made of biodegradable polymers[45,45]. Collagen materials of course provide a measure, at times may be a large measure, of fibre orientation to guide cells. Brunette *et al.*[47] fabricated microgrooves on the surface of tooth crowns in the hope that this would deter fibroblasts from invading the gingiva. We have deliberately ignored those reports which have used fibrous material to guide cells in which no attention was paid to fibre diameter, because it is clear that quantitative analysis is needed in order to make the best designs of prosthesis.

REFERENCES

1. Curtis, A.S.G., Wilkinson, C.D.W. and Wilby, J., In *Motion Analysis of Lining Cells*, ed. D. Soll.
2. Wojciak-Stothard, B., Curtis, A., Monaghan, W., Macdonald, K. and Wilkinson, C., Guidance and activation of murine macrophages by nanometric scale topography. *Cell Res.*, 1996, **223**, 426–435.
3. Guido, S. and Tranquillo, R.T., A methodology for the systematic and quantitative study of cell contact guidance in oriented collagen gels. Correlation of fibroblast orientation and cell birefringence. *J. Cell Sci.*, 1993, **105**, 317–331.
4. Britland, S., Clark, P., Connolly, P. and Moores, G., Micropatterned substratum adhesiveness: A model for morphogenetic cues controlling cell behaviour. *Exp. Cell Res.*, 1992, **198**, 124–129.
5. Clark, P., Connolly, P. and Moores, G.R., Cell guidance by micropatterned adhesiveness *in vitro*. *J. Cell Sci.*, 1992, **103, 287–292.**
6. Britland, S., Perridge, C., Denyer, M., Morgan, H., Curtis, A. and Wilkinson, C., Morphogenetic guidance cues can interact synergistically and hierarchically in steering nerve cell growth. *Exp. Biol. Online–EBO*, 1996, 1:2.
7. Pritchard, D.I., Morgan, H. and Cooper, J.M., Microscale patterning of biological molecules. *Angew. Chem.*, 1995, **34**, 91–93.
8. Curtis, A., Quinn, R. and McGrath, M., Activation of neuron adhesion and neurite extension by tethered neurotrophins and adhesion molecules. *Exp. Biol. Online*, 1996, 1(1).
9. den Braber, E.T., Deruijter, J.E., Smits, H.T.J., Ginsel, L.A., Vonrecum, A.F. and Jansen, J.A., Effect of parallel surface microgrooves and surface energy on cell growth. *J. Biomed. Mater. Res.*, 1995, **29**, 511–518.
10. Vesely, P., Boyde, A. and Jones, S., Behaviour of osteoclasts *in vitro*–contact behaviour of osteoclasts with osteoblast-like cells and networking of osteoclasts for 3D orientation. *J. Anat.*, 1992, **181**, 277–291.
11. Clark, P., Connolly, P., Curtis, A.S.G., Dow, J.A.T. and Wilkinson, C.D.W., Topographical control of cell behaviour II. Multiple grooved substrata. *Development*, 1990, **108**, 635–644.
12. Clark, P., Connolly, P., Curtis, A.S.G., Dow, J.A.T. and Wilkinson, C.D.W., Cell guidance by ultrafine topography *in vitro*. *J. Cell Sci.*, 1991, **99**, 73–77.
13. Clark, P., Connolly, P., Curtis, A.S.G., Dow, J.A.T. and Wilkinson, C.D.W., Topographical control of cell behaviour. I. Simple step cues. *Development*, 1987, **99**, 439–448.
14. Wojciak, B., Crossan, J., Curtis, A.S.G. and Wilkinson, C.D.W., Grooved substrata facilitate *in vitro* healing of completely divided flexor tendons. *J. Mat. Sci. Mat. Medicine*, 1995, **6**, 266–271.
15. den Braber, E.T., Deruijter, J.E., Smits, H.T., Ginsel, L.A., Vonrecum, A.F. and Jansen, J.A., Quantitatbe analysis of fibroblast morphology on microgrooved surfaces with different dimensions. *Biomaterials*, in press.
16. Nagata, I., Kawana, A. and Nakatsuji, N., Perpendicular contact guidance of CNS neuroblasts on artificial microstructures. *Development*, 1993, **117**, 401–408.
17. Curtis, A., Wilkinson, C. and Wojciak-Stothard, B., Accelerating cell movement. *J. Cellular Eng.*, 1995, **1**, 35–38.
18. Weiss, P., *In vitro* experiments on the factors determining the course of the outgrowing nerve fiber. *J. Exp. Zool.*, 1934, **68**, 393–418.
19. Hoch, H.C., Staples, R.C., Whithead, B., Comeau, J. and Wolf, E.D., Signalling for growth orientation and cell differentiation by surface topography in Uromyces. *Science*, 1987, **235**, 1659.
20. Lydon, M.J. and Clay, C.S., Substratum topography and cell traction on sulphuric acid treated bacteriological-grade plastic. *Cell Biol. Internl. Rep.*, 1985, **9**, 911–921.

21. Abercrombie, M. and Gitlin, G., The locomotory behaviour of small groups of fibroblasts. *Proc. R. Soc. B*, 1965, **162**, 289–302.
22. Curtis, A. S. G. and Varde, M., Control of cell behaviour — topological factors. *J. Natl. Cancer Inst.*, 1964, **33**, 15–26.
23. Gail, M. H. and Boone, C. W., Cell substrate adhesivity. A determinant of cell motility. *Exp. Cell Res.*, 1972, **70**, 33–40.
24. Hartman, R. S., Lau, K., Chou, W. and Coates, T. D., The fundamental motor of the human neutrophil is not random: evidence for local non-Markov movement in neutrophils. Biophys. J., 1994, **67**, 2535–2545.
25. Friedl, P., Noble, P. B., Shields, E. D. and Zanker, K. S., Locomotor phenotypes of unstimulated CD45RA high and CD45rO high CD4+ and CD8+ lymphocytes in three-dimensional collagen lattices. *Immunology*, 1994, **82**, 617–624.
26. Kuntz, R. M. and Saltzman, W. M., Neutrophil motility in extracellular matrix gels: mesh size and adhesion affect spread of migration. *Biophys. J.*, 1997, **72**, 1472–1480.
27. Olson, A. D., Contraction of collagen gels by intestinal epithelial cells depends on microfilament function. *Dig. Disease Sci.*, 1993, **38**, 388–395.
28. Dunn, G. A. and Zicha, D., Dynamics of fibroblast spreading. *J. Cell Sci.*, 1995, **108**, 1238–1249.
29. Dow, J. A. T., Clark, P., Connolly, P., Curtis, A. S. G. and Wilkinson, C. D. W., Novel methods for the guidance and monitoring of single cells and simple networks in culture. *J. Cell Sci. Suppl.*, 1987, **8**, 55–79.
30. Meyle, J., Gultig, K., Brich, M., Hammerle, H. and Nisch, W., Contact guidance of fibroblasts on biomaterial surfaces. *Mat. Medicine*, 1994, **5**, 463–466.
31. Meyle, J., Gultig, K. and Nisch, W., Variation in contact guidance by human cells on a microstructured surface. *J. Biomed. Mat. Res.*, 1995, **29**, 81–88.
32. Oakley, C. and Brunette, D. M., The sequence of alignment of microtubules, focal contacts and actin filaments in fibroblasts spreading on smooth and grooved titanium substrata. *J. Cell Sci.*, 1993, **106**, 343–354.
33. Mitchison, T. J. and Cramer, L. P., Actin-based cell motility and locomotion. *Cell*, 1996, **84**, 371–379.
34. Smith, C. L., Cytoskeletal movements and substrate interactions during initiation of neurite outgrowth by sympathetic neurons *in vitro*. *J. Neurosci.*, 1994, **14**, 384–398.
35. Hammarback, J. A., McCarthy, J. B., Palm, S. L., Furcht, L. T. and Letourneau, P. C., Growth cone guidance by substrate-bound laminin pathways is correlated with neuron-to-pathway adhesivity. *Dev. Biol.*, 1988, **126**, 29–39.
36. Lackie, J. M., Adhesion and locomotion of neutrophil leukocytes on 2-D substrata and in 3-D matrices. In *White Cell Mechanics: Basic Science and Clinical Aspects*, ed. H. J. Meiselman, M. A. Lichtman and P. L. LaCelle. A.R. Liss, New York, 1984, pp. 237–254.
37. Chou, L., Firth, J. D., Uitto, V.-J. and Brunette, D. M. D., Substratum surface topography alters cell shape and regulates fibronectin mRNA level, mRNA stability, secretion and assembly in human fibroblasts. *J. Cell Sci.*, 1995, **108**, 1563–1573.
38. Curtis, A. S. G. and Clark, P., The effects of topographic and mechanical properties of materials on cell behavior. *CRC Rev. Biocompatibility*, 1990, **5**, 343–362.
39. Dunn, G. A., How do cells respond to ultrafine surface contours? *Bioessays*, 1991, **13**, 541–543.
40. Dunn, G. A. and Heath, J. P., A new hypothesis of contact guidance in tissue cells. *Exp. Cell Res.*, 1976, **101**, 1–14.
41. Jenkins, D. H. R., Forster, J. W., McKibbn, B. and Ralis, Z. A., Induction of tendon and ligament formation by carbon implants. *J. Bone Joint Surg.*, 1977, **59B**, 53–57.
42. Curtis, A. S. G. and Seehar, G. M., The control of cell division by tension or diffusion. *Nature*, 1978, **274**, 52–53.
43. Doolabh, V. B., Hertl, M. C. and MacKinnon, S. E., The role of conduits in nerve repair. A review. *Rev. Neurosc.*, 1996, **7**, 47–84.
44. Aebischer, P., Guenard, V. and Valentini, R. F., The morphology of regenerating peripheral nerves is modulated by the surface microgeometry of polymeric guidance channels. *Brain Res.*, 1990, **531**, 211–218.
45. Curtis, A. S. G. and Wilkinson, C. D. W., Biomechanical structures. European Patent Bull. 1985/25, 19 June 1995, 84308230.6, 1985.
46. Curtis, A. S. G. and Wilkinson, C. D. W., Wound healing material. Int.PCT. WO95/22305, 1995.
47. Brunette, D. M., Kenner, G. S. and Gould, T. R. L., Titanium surface orient growth and migration of cells from human gingival explants. *J. Dental Res.*, 1983, **62**, 1045–1048.
48. Breckenridge, I. J., Wilson, R. J. A., Connolly, P., Curtis, A. S. G., Dow, J. A. T., Blackshaw, S. E. and Wilkinson, C. D. W., Advantages of using microfabricated extracellular electrodes for *in vitro* neuronal recording. *J. Neurosci. Res.*, 1995, **42**, 266–276.
49. Chehroudi, B., Gould, T. R. and Brunette, D. M., Effects of a grooved epoxy substratum on epithelial cell behavior *in vitro* and *in vivo*. *J. Biomed. Mat. Res.*, 1988, **22**, 459–473.
50. Chehroudi, B. and Brunette, D. M., Effects of surface topography on cell behavior. In *Encyclopedic Handbook of Biomaterials and Bioengineering*, ed. D. L. Wise, D. J. Trantolo, D. E. Altobelli, M. J. Yaszemski, J. D. Gresser and E. R. Schwartz. Part S. Materials. New York, pp. 813–842.
51. Ternaux, J.-P., Wilson, R., Dow, J., Curtis, A. S. G., Clark, P., Portalier, P. and Moores, J., Dendritic processing — using microstructures to solve a hitherto intractable neurophysiological problem. *Med. Biol. Eng. Comput.*, 1992, **30**, CE37–CE41.
52. Rovensky, Y. A. and Samoilov, V. I., Morphogenetic response of cultured normal and transformed fibroblasts, and epitheliocytes, to a cylindrical substratum surface. Possible role for the actin filament bundle pattern. *J. Cell Sci.*, 1994, **107**, 1255–1263.
53. Sanford, K. K., Earle, W. E. and Likely, G. D., The growth *in vitro* of single isolated tissue cells. *J. Natl. Cancer Inst.*, 1948, **9**, 229–246.
54. Weiss, P., *In vitro* experiments on the factors determining the course of the outgrowing nerve fiber. *J. Exp. Zool.*, 1934, **68**, 393–418.
55. Weiss, P., Experiments on cell and axon orientation *in vitro*: the role of colloidal exudates in tissue organization. *J. Exp. Zool.*, 1945, **100**, 353–386.
56. Wilkinson, P. C., Shields, J. M. and Haston, W. S., Contact guidance of human neutrophil leukocytes. *Exp. Cell Res.*, 1982, **140**, 55–62.
57. Haston, W. S., Shields, J. and Wilkinson, P. C., Lymphocyte locomotion and attachment on two-dimensional surfaces and in three-dimensional matrices. *J. Cell Biol.*, 1982, **92**, 747–752.
58. Wood, A., Contact guidance on microfabricated substrata: the response of teleost fin mesenchyme cells to repeating topographical patterns. *J. Cell Sci.*, 1988, **90**, 667–681.
59. Vesely, P., Boyde, A. and Jones, S. J., Behaviour of osteoclasts *in vitro*: contact behaviour of osteoclasts with osteoblast-like cells and networking of osteoclasts for 3D orientation. *J. Anat.*, 1992, **181**, 277–291.

60. Webb, A., Clark, P., Skepper, J., Compston, A. and Wood, A., Guidance of oligodendrocytes and their progenitors by substratum topography. *J. Cell Sci.*, 1995, **108**, 2747–2760.
61. Tranquillo, R.T., Girton, T.S., Bromberek, B.A., Triebes, T.G. and Mooradan, D.L., Magnetically orientated tissue-equivalent tubes—application to a circumferentially orientated media equivalent. *Biomaterials*, 1996, **17**, 349–357.
62. Sherwood, J., Gow, N.A.R., Gooday, G.W., Gregory, D.W. and Marshall, D., Contact sensing in Candida Albicans—a possible aid to epithelial penetration. *J. Med. Veterinary Mycol.*, 1992, **30**, 461–469.