Immunohistochemical Investigation of Tracks Left by the Migration of Fibroblasts on Titanium Surfaces

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IMMUNOHISTOCHEMICAL INVESTIGATION OF TRACKS LEFT BY THE MIGRATION OF FIBROBLASTS ON TITANIUM SURFACES

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Abstract

Fibronectin, a major attachment protein, has been thought to be involved in pathway guidance, a process in which cells migrate along specific pathways within a tissue during development. Fibroblasts exhibit the phenomenon of contact guidance, the tendency of cells to be guided in their direction of migration by the shape of the substratum. The purpose of our study was to test the hypothesis that fibronectin tracks are deposited by fibroblasts moving on smooth and grooved titanium surfaces.

The study was carried out on human gingival fibroblasts which were plated onto both smooth and grooved titanium substrata using medium containing either serum or fibronectin-depleted serum. The migratory paths of the cells were determined by time-lapse photography using reflected-light differential-interference-contrast optics. Anti-fibronectin antibody, 1 nm gold particle conjugated secondary antibody, and silver enhancement techniques were applied to the cultured cells, and the specimens observed in a scanning electron microscope using backscattered detection. By correlating the paths of the cells with the location of the fibronectin-containing material, it could be demonstrated that cells left behind fibronectin tracks on both smooth and grooved titanium surfaces. Fibronectin tracks appeared to be deposited more abundantly by fibroblasts cultured in medium with 5% serum depleted in fibronectin than in complete, i.e., non-depleted, 5% serum. On the grooved titanium substratum, the tracks were found on the ridges as well as on the floors and walls of the grooves. The fibronectin tracks are aligned with the grooves so that they would be expected to reinforce the contact guidance produced by the substratum.

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Introduction

It is well established that fibronectin, a major attachment protein, plays an important role in the attachment of fibroblasts to their substratum (Hynes, 1985). Using cryosections of fibroblasts grown on gelatin *in vitro*, Chen and Singer (1982) demonstrated that fibronectin was localized between fibroblasts and the substratum. Fibronectin associated with the substratum has been thought to be involved in pathway guidance, a process in which cells migrate along specific pathways within a tissue during development (Newgreen and Thiery, 1980; Mayer *et al.*, 1981). Experimental evidence for this possibility is afforded by Halfter *et al.* (1988, 1990) who demonstrated that fibroblasts deposited extracellular matrix components along their migration trail on dishes coated with extracellular matrix and/or poly-lysine. It is, however, not known whether similar tracks are laid down on biomaterials, such as titanium, that are used in dental implants.

Dental implants contact soft connective tissue, epithelium and bone. If fibronectin were deposited on the implant surface, it would be expected that the cell adhesion to the implant would be affected. Cell adhesion is also influenced by topography of the substratum. Contact guidance, sometimes called topographic guidance, refers to the tendency of cells to be guided by the shape of the substratum (Weiss, 1958). The phenomenon has been observed in several types of cells on titanium surfaces including fibroblasts (Brunette, 1986a), epithelial cells (Brunette *et al.*, 1983; Brunette, 1986b), and osteoblasts (Brunette *et al.*, 1991). Brunette *et al.* (1983) proposed the use of contact guidance to inhibit epithelial down growth on implants that penetrate a stratified epithelium. The migration of epithelium down the surface of such implants can ultimately lead to their exfoliation; percutaneous devices, for example, have a short lifetime, estimated at three months in the absence of heroic measures (Von Recum, 1984).

Placing horizontal grooves on titanium-coated implants inhibits epithelial downgrowth by the mechanism of contact guidance acting directly on the epithelial cells, and/or indirectly by orienting connective tissue cells attached to the implant (Chehroudi *et al.*, 1989,

1992). However the orientation of extracellular matrix components on the substrata is unknown. The purpose of this study was to test the hypothesis that fibronectin tracks are deposited by fibroblasts moving on smooth and grooved titanium surfaces *in vitro*.

Materials and Methods

Cell culture

Fibroblasts from outgrowths of human gingiva were isolated and subcultured as described previously (Brunette *et al.*, 1976). Briefly, the cells or explants were cultured on tissue-culture plastic (Falcon, Cockeysville, Maryland) in alpha minimal essential medium (MEM) (Terry Fox Media Lab, Vancouver, B.C.) supplemented with antibiotics [penicillin G (Sigma, St. Louis, Missouri) 100 μg/ml; gentamicin (Sigma) 50 μg/ml; amphotericin B (Gibco, Grand Island, NY) 3μg/ml], and 15% Calf Supreme serum (Gibco) at 37°C in a humidified atmosphere of 95% air 5% CO_2. Fibroblasts were removed from the growth surface by a trypsin solution [0.25% trypsin (Gibco) and 0.1% glucose dissolved in citrate-saline (pH 7.8)]. The cells were resuspended in medium containing either 5% Calf Supreme serum or fibronectin-depleted serum at a cell-population density of 10^5 cells/ml. A 10 μl drop of cell suspension was plated onto a corner of the substratum. The substratum was then placed in a sterile culture dish in an incubator for 1 hour before the dishes were flooded with additional medium. For time-lapse studies, observations began 2 hours after cell plating; other samples were fixed at varying intervals up to 24 hours. Fibronectin-depleted serum was prepared by passing Calf Supreme serum through a mini-column of gelatin sepharose 4B (Pharmacia Fine Chemicals). The depleted serum was assessed with enzyme-linked immuno-absorbent assay (ELISA). Ninety-six well plates (Falcon) were coated with medium containing either 5% serum or fibronectin-depleted serum. The same primary antibody that was used in the immunohistochemical study and secondary antibodies conjugated with alkaline phosphatase were employed. After development with p-nitrophenyl phosphate (Sigma) in diethanolamine buffer with 30 minutes incubation at 37°C, the optical densities were measured at 405 nm by a Titertek ELISA reader (Flow Laboratories, Mclean, Virginia).

Substratum

Grooved substrata were prepared on n-type <100> silicon wafers by procedures that were originally developed for the fabrication of high-quality photomasks for solar cells (Camporese *et al.*, 1981). Four types of grooved substrata were used in this study: (a) 30-μm pitch comprising a 15-μm-wide ridge and a 15-μm-wide groove, 3 μm deep; (b) 20-μm pitch comprising a 10-μm-wide ridge and a 10-μm-wide groove, 3 μm deep; (c) 9-μm pitch comprising a 5-μm-wide ridge and a 4-μm-wide groove, 2.5 μm deep; and (d) 7-μm pitch comprising a 3-μm-wide ridge and a 4-μm-wide groove, 2.5 μm deep. Eight of each type of substrata

were coated with 50 nm titanium by a sputter coater (Randex 3140 Sputter system, California). The effects of the substrata on cell behaviour were assessed in a minimum of two separate experiments for each substrata. The titanium-coated substrata were cleaned by ultrasonication for 20 minutes in a detergent (7X, Flow) specifically formulated for tissue culture. After being rinsed 20 times with deionized, sterile water, the substrata were dried overnight in a tissue-culture laminar-flow hood and treated for 3 minutes in an argon-gas glow-discharge chamber (Doundoulakis, 1987; Baier and Meyer, 1988) fabricated according to the design of Aebi and Pollard (1987).

One randomly chosen sample from each type of substratum was inspected by scanning electron microscopy (SEM). Titanium surfaces prepared in this way have also been analyzed by X-ray photoelectron spectroscopy (XPS) at the National ESCA (electron spectroscopy for chemical analysis) and Surface Analysis Center for Biomedical Problems, University of Washington, Seattle, Washington and the Department of Chemistry, University of British Columbia.

Immunohistochemical procedure and SEM

The cells were fixed in 4% paraformaldehyde in 0.1 M cacodylate buffer at room temperature for 30 minutes. After rinsing with phosphate-buffered saline (PBS), the samples were immersed overnight at 4°C in more PBS containing 0.05 M NH_4Cl and 0.2% bovine serum albumin (BSA) in order to quench free aldehyde groups and also to block non-specific staining. The samples were then further incubated for 1 hour in PBS containing 0.2% BSA and 0.1% gelatin (IGSS IntenSE®M, Amersham, U.K.), to prevent background staining, and incubated with rabbit anti-human fibronectin (ICN, Costa Mesa, California) for 4 hours at room temperature. The samples were then rinsed with PBS and incubated for 2 hours at room temperature in goat anti-rabbit secondary antibody labelled with 1 nm gold particle. After rinsing in PBS again, a silver enhancement technique (IntenSE®M, Amersham, U.K.) was applied for 20 minutes at room temperature. The samples were then washed with deionized water, dehydrated in graded alcohols, critical-point dried with CO_2, and sputter-coated with carbon. The specimens were observed in a Cambridge S100 scanning electron microscope operated at an accelerating voltage of 25 kV at a working distance of 12 mm using secondary or back-scattered electrons (SE or BSE) to form an image.

Time-lapse studies

The grooved or control flat substrata were mounted on glass slides and placed in a Pentz chamber (Bachofer, Reutlingen, Germany) which was then placed in a stage incubator on a Zeiss inverted microscope equipped with reflected-light differential-interference-contrast optics. Using the X16 objective, photomicrographs were taken with technical pan film (Kodak 1024) at intervals of typically 2—3 hours, sufficiently frequent to follow the paths of individual cells.
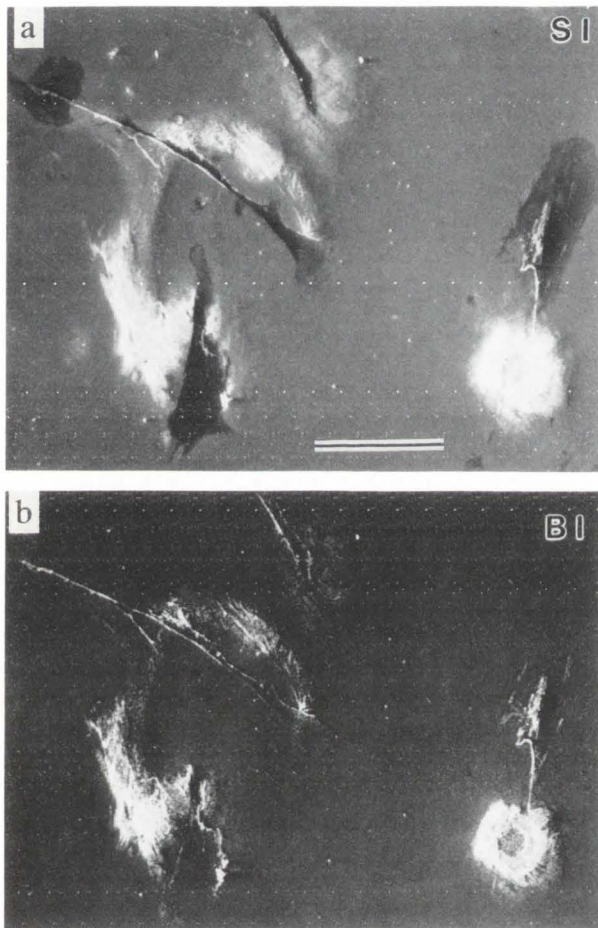
Figure 1. Scanning electron micrographs of fibroblasts cultured on titanium surfaces and immunostained for fibronectin followed by silver enhancement: (a) SE image (SI) and (b) BSE image (BI). Structures containing fibronectin appear white, but because the specimens are carbon-coated and the cells have not been treated with osmium, detailed cell morphology is not evident. Bar = 100 μm.

Results

SEM observations of the test surfaces indicated that micromachined surfaces were replicated with high fidelity to the master pattern on the silicon wafers and that the titanium coating was smooth and continuous with no evidence of cracks. XPS analyses of the outer 5—10 nm of the test surfaces indicated that the major titanium class detected was Ti^{+4} (binding Energy = 459 eV). Another major element present was oxygen, which suggests that the Ti^{+4} probably exists as TiO_2. Other elements found on the implant surface included carbon and traces of nitrogen.

Fibronectin-depletion of serum medium

The amount of fibronectin remaining in the serum which had passed through the sepharose column was compared with control serum by ELISA. The fibronectin-depleted serum demonstrated approximately the same ELISA reading as normal serum that had been diluted 10 to 20 fold.
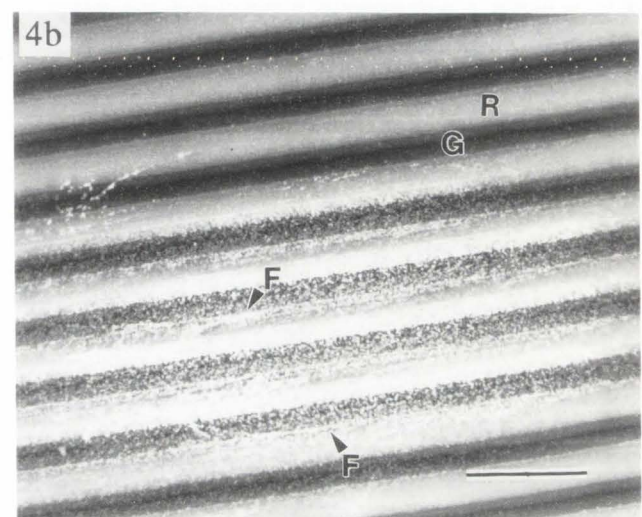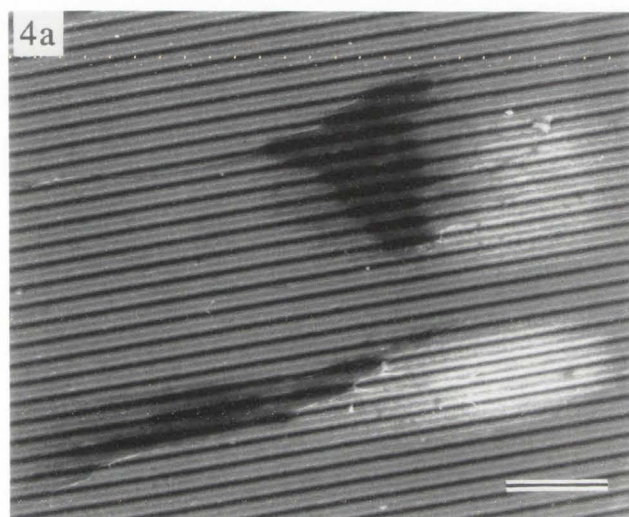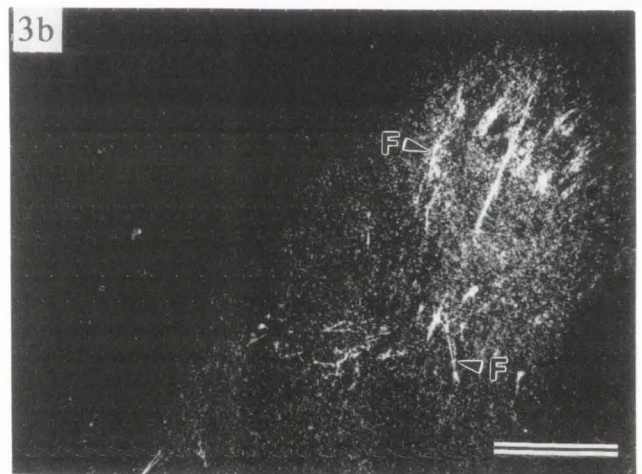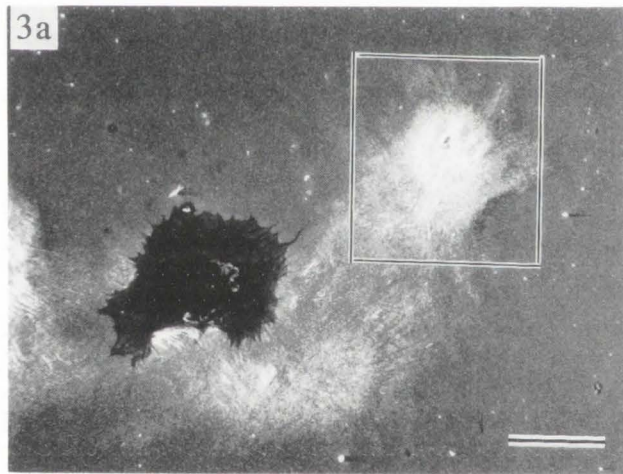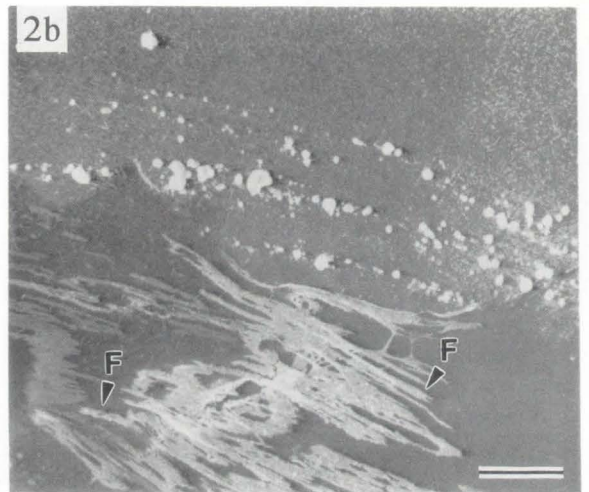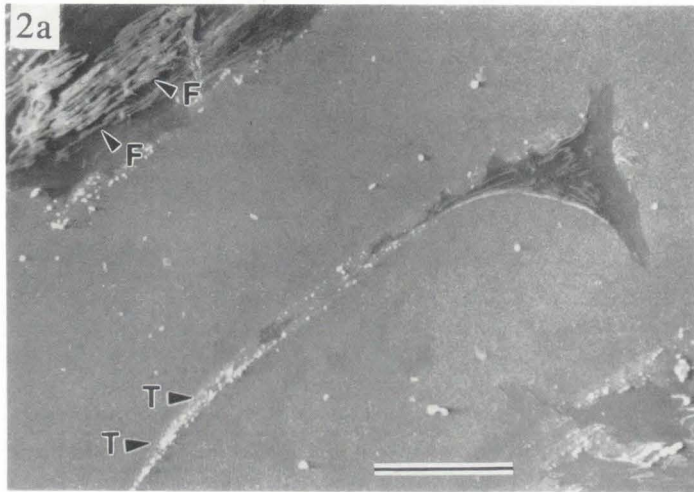
Scanning electron microscopy

The fibroblasts appeared flatter after mild fixation in the 4% paraformaldehyde used in the immunohistochemical studies than after the glutaraldehyde fixation commonly used for the routine preparation of SEM samples. Positive staining was noted in the secondary electron image but it was not always easy to identify whether the staining represented silver deposition. Accordingly, backscattered electron (BSE) detection, where contrast depends on the atomic number (reviewed by DeNee and Abraham, 1976), was used to distinguish structures stained with silver (Figure 1). The disadvantage of using BSE detection was that cell morphology was not depicted because most of the surface was not stained with silver. Thus, both techniques, i.e., BSE and secondary electrons (SE), were used to obtain a comprehensive understanding of fibronectin deposition and its relationship to the cell and its substratum.

In cultures grown in 5% serum, the fibronectin was found predominantly on dorsal cell surfaces in a fibrillar form. In addition, dots of silver on the titanium surface were observed which looked like cell tracks in areas adjacent to cells (Figure 2a). However, the titanium surfaces had abundant background staining which was thought to be fibronectin deposition from the serum (Figure 2b). To lessen the background staining, cells were cultured in fibronectin-depleted serum medium. This had the expected effect of markedly reducing the background staining of the substratum, and it was observed that the silver staining was localized to limited areas associated with cells. Thus, a comparison of fibronectin-deposition on smooth and grooved surfaces could be carried out.

On smooth surfaces, the fibronectin-containing structures as identified by silver staining included small globules as well as fine fibrillar structures. The immunolabelling was most intense at the position where the cell attached initially (Figures 3a and 3b). Although some fibrils were continuous with the edge of the cell, most of the fibrils were clearly separated from the cells.

On grooved surfaces, tracks of fibronectin were oriented parallel to the long axis of the grooves (Figures 4 and 5). On surfaces with a 7-μm or 9-μm pitch, fibronectin deposits were apparently oriented with the grooves, but individual fibrils were less distinct (Figure 4a); fibronectin was found on the ridges as well as on the floors and walls of the grooves (Figure 4b). On substrata with a 30-μm pitch or 20-μm pitch, the fibrils containing fibronectin were commonly localized mainly on the bottom of the grooves rather than on the ridges (Figure 5). At areas of high population density, such as

Figure 2. Scanning electron micrographs at different magnifications (**a, b**) using SE imaging of cells grown in media containing 5% serum and subsequently immuno-stained with silver enhancement for fibronectin. The titanium substratum has absorbed significant amounts of fibronectin and thus appears white; whereas the cell, which is largely unstained, appears dark. There are, however, fibronectin-containing fibrils (F) on the dorsal surface of the cell and fibronectin-containing tracks (T). Bars = 50 μm (a) and 10 μm (b).

Figure 3. Scanning electron micrographs using BSE detection of cells cultured in fibronectin-depleted medium on a smooth titanium surface, and subsequently immuno-stained for fibronectin with silver enhancement. The fibronectin-containing structures showed no preferred orientation (**a**) but small globules as well as fine fibrils (F) were seen in BSE (**b**). Bars = 50 μm (a) and 25 μm (b).

Figure 4. Scanning electron micrographs at different magnifications (**a, b**) using BSE detection of cells cultured in fibronectin-depleted medium on a 7-9 μm pitch grooved titanium surface, and subsequently immuno-stained for fibronectin with silver enhancement. Aligned but somewhat less well defined fibronectin-containing fibrils (F) were seen on these surfaces. Bars = 50 μm (a) and 10 μm (b).

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where the cells were initially plated, fibrillar structures on the dorsal cell surfaces were observed. These cells were aligned with the grooves, and dorsal fibrils were aligned with the long axis of each cell (Figure 6).

Time-lapse experiments were carried out to determine whether or not the fibronectin localization on titanium surfaces represented tracks made by the cells. Two hours after plating, cells had spread extensively on titanium, and by six hours, cells had begun to migrate. By twelve hours, most cells had moved one or more cell diameters. By correlating the paths of the cells with the location of the fibronectin-containing fibrils, it could be demonstrated that cells left behind fibronectin tracks on the titanium surface (Figure 7).

On some substrata, cells were observed in areas where smooth and grooved surfaces met. In this junctional area, the fibronectin-containing fibrils on the smooth area were oriented parallel to the grooves (Figure 8). This was possibly the result of cells migrating onto the grooves from the smooth surface and continuing in the direction of the grooves. Fibronectin fibrils were not observed on the grooved surfaces on which cells had not been cultured but were in contact with medium containing 5% serum that had not been depleted of fibronectin. Therefore, the formation and alignment of fibrils of fibronectin was not a consequence of the physical properties of the grooved surface.
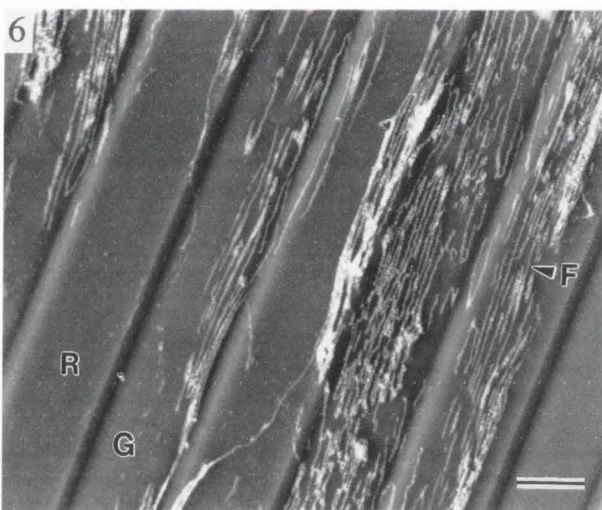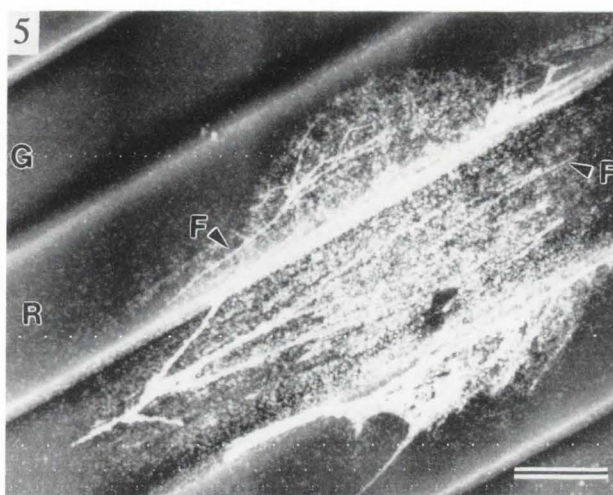

Figure 5. Scanning electron micrograph using BSE detection of cells cultured in fibronectin-depleted medium on a 30 μm pitch grooved titanium surface, and subsequently immunostained for fibronectin with silver enhancement. Fibronectin containing fibrils (F) were more prominent on the bottom of the grooves (G) than on the ridges (R), and were aligned in the direction of the grooves. Bar = 10 μm.

Figure 6. Scanning electron micrograph (using BSE) of cells on grooved surfaces in an area of high cell population density. The fibronectin fibrils (F) on dorsal cell surfaces were aligned in the direction of the grooves. G = grooves; R = ridges. Bar = 10 μm.

Discussion

Scanning electron microscopy using backscattered electrons was first applied by Becker and Sogard (1979) to cells and tissues affixed with heavy metals by means
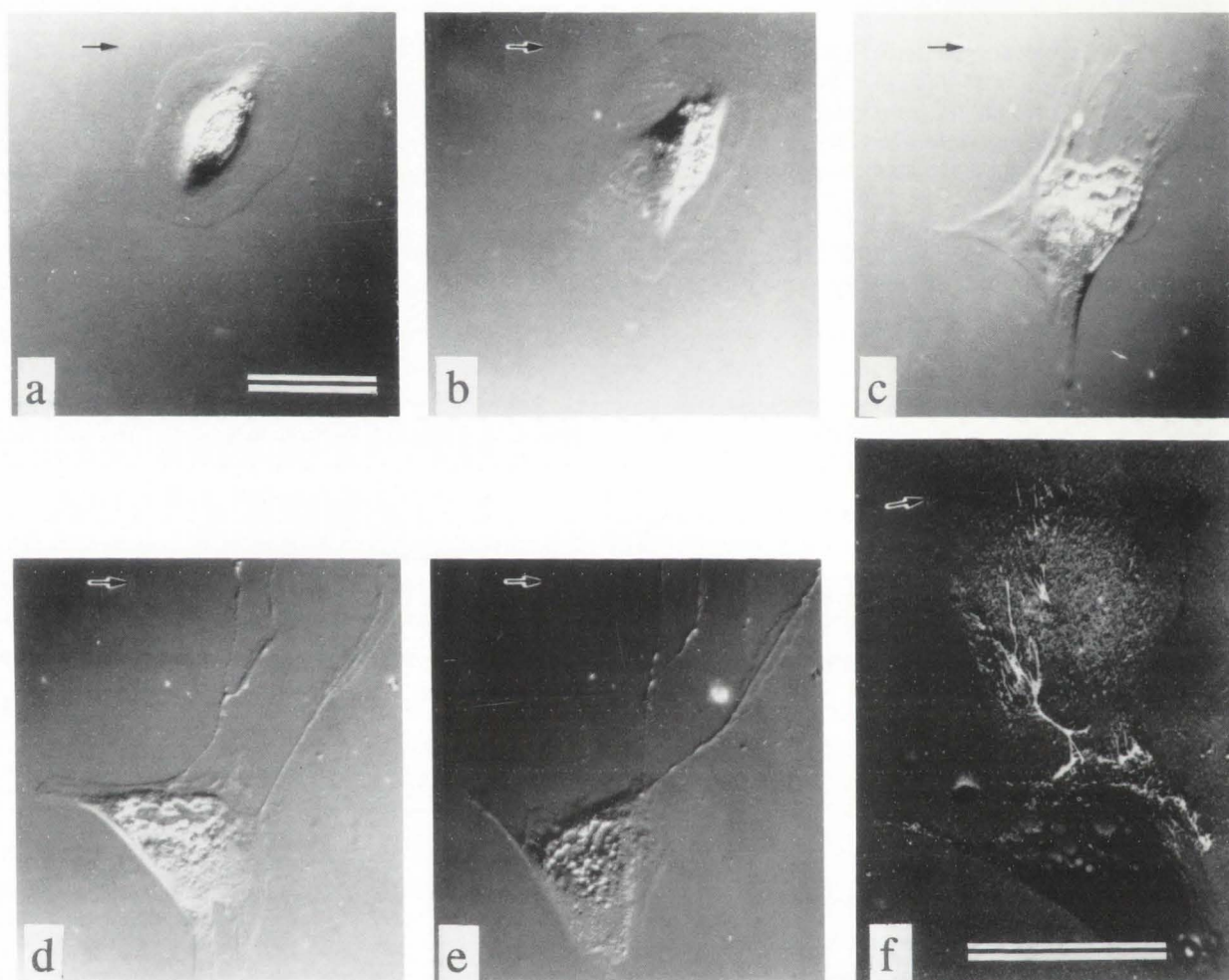
Figure 7. Time-lapse series of photomicrographs demonstrating that fibronectin tracks were made by the cells. Arrows indicate the same point on the titanium substratum in each picture. Photomicrographs using differential interference contrast optics were taken two (**a**), four (**b**), six (**c**), ten (**d**) and twelve hours (**e**) after cell plating. The same field after immunostaining (**f**) shows the fibronectin tracks left on the titanium by the cell. Bar = 100 μm (note Figures 7a to 7e are at the same magnification).

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of staining methods such as silver-reduction staining and DAB-osmium. Subsequently this technique was employed by de Harven *et al.* (1984) to detect colloidal gold-labelled cell surfaces. The method utilizes the difference in atomic number between cell components and gold to show regions containing gold marker particles in high contrast. Most commonly gold particles larger than 20 nm have been used in SEM studies, but such large particles have limited access to some sites. We utilized ultra small gold particles (1 nm) followed by silver enhancement on the hypothesis that the smaller particles would have better access to the fibronectin incorporated into fibrils.

Halfter *et al.* (1988, 1990) showed fibronectin deposition along the pathways of migrating fibroblasts by immunofluorescence microscopy. Fibronectin trails were detected on poly-lysine/fibronectin or poly-lysine/laminin-coated plastic, but trails were detected to a much lesser extent on substrata coated with poly-lysine alone or uncoated plastic or glass surfaces. Thus, the presence of fibronectin trails is substratum-dependent and must be examined for each substratum of interest. In these experiments on titanium surfaces, fibronectin tracks were abundant; they were, however, relatively shorter than the tracks reported on other surfaces (Halfter *et al.*, 1988, 1990).
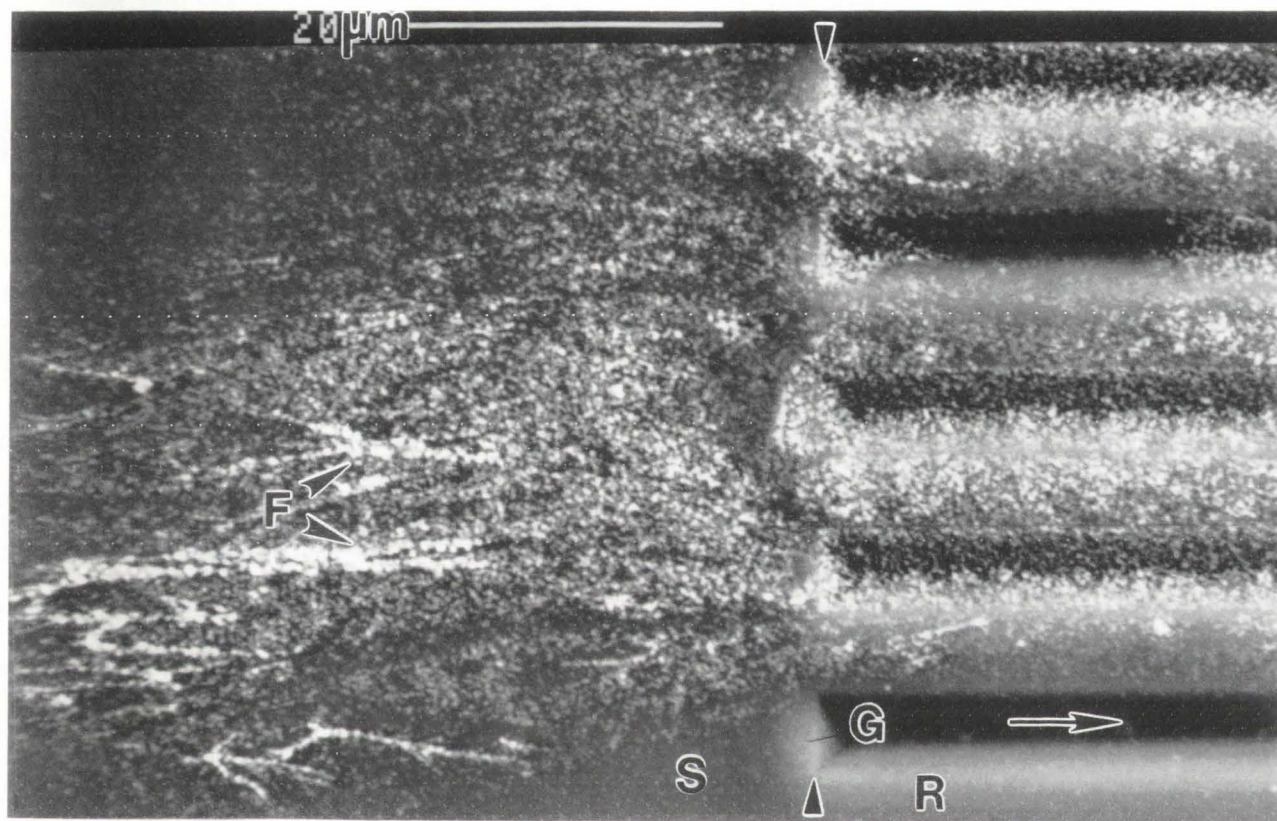
Figure 8. Backscattered electron micrograph of cells cultured on a titanium surface at the junction area between smooth (S) and grooved surfaces (G = grooves; R = ridges) and subsequently immunostained with silver enhancement for fibronectin. Arrow indicates the direction of cell movement. The fibrillar structures (F) in the portion of the cell on the smooth surface were aligned in the direction of the grooves.

Using an *in vitro* system, Fletcher *et al.* (1979) provided evidence that the attachment of the cells to titanium was stronger than to other substrata. But strength of attachment does not imply speed of movement; Harris (1982) has shown that rapidly moving cells such as leukocytes exert only weak tractional forces. It is possible that the reason the fibronectin tracks in our study were relatively short was that the cells adhere tightly to the substratum and move slowly, thus leading to shorter tracks.

The distribution of fibronectin on the cell surface depends on the condition of the culture. For example, Mautner and Hynes (1977) showed that the fibronectin was localized on dorsal cell surfaces with cells grown in 5% serum medium, whereas in 0.3% serum fibronectin was localized beneath cells but not found on their dorsal surfaces. In addition, they reported that fibronectin footprints were seen only when cells that had been kept in 0.3% serum were removed from their substratum but that footprints were not evident for cells cultured in 5% serum. One problem with varying serum concentration is that the cells are exposed not only to different levels

of fibronectin but also to different levels of all the other components of serum. To clarify the role of fibronectin in the medium, we compared cells cultured in normal serum with cells cultured in fibronectin-depleted serum prepared by absorption of fibronectin with gelatin-sepharose. Fibronectin tracks appeared to be deposited more abundantly by fibroblasts cultured in medium with 5% serum depleted in fibronectin than in complete, i.e., non-depleted, serum. These results suggest that higher concentrations of exogenous fibronectin may inhibit the accumulation of fibronectin underneath the cells, and the subsequent production of fibronectin-containing trails of fibrils.

It is well established that fibroblasts become oriented with the grooves produced by microfabrication techniques (Brunette, 1986a, 1988; Dunn, 1986; Clark, 1987, 1990; Wood, 1988). Moreover, the control of cell orientation is hierarchical, with larger grooves having precedence over smaller grooves (Brunette, 1986a). The mechanism underlying such phenomena is unclear, although the mechanical properties of cytoskeletal elements such as microfilaments (Dunn and Heath, 1976) or

focal contacts (O'Hara and Buck, 1979) appear to be involved. Cytoskeletal elements have also been thought to be involved with the distribution of fibronectin on cell surfaces. Hynes and Destree (1978) reported that fibronectin fibrils on the dorsal surfaces of fibroblasts corresponded to microfilament bundles in the cytoplasm. Similarly, Toyoizumi *et al.* (1991) observed that vinculin, a component of focal contacts, and α-actin were localized in regions of F-actin where filopodia and lamellipodia attached to fibronectin lines placed on the substrata. Fibronectin fibrils on the cell surface probably reflect the distribution of the underlying cytoskeletal elements in the cell. On grooved surfaces, the cytoskeletal elements are aligned with the cell which is oriented by the grooves.

It is also established that the migration of fibroblasts can be directed by parallel lines of fibronectin placed on plastic culture dishes (Turner *et al.*, 1983; Toyoizumi *et al.*, 1991). It is possible that, as suggested by Nakatsuji and Johnson (1984), the fibrillar network containing fibronectin could guide cell migration by contact guidance *in vivo*. Our results demonstrated that the fibronectin tracks produced by fibroblasts were aligned with the grooves. It would be expected that the fibronectin tracks would reinforce the contact guidance effected by the grooves. It may be the combined effects of grooves and fibronectin tracks that are instrumental in inhibiting downgrowth on implants which have grooves on their surfaces (Chehroudi *et al.*, 1989, 1992).

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Discussion with Reviewers

J.A. Jensen: Is the method used to prepare fibronectin depleted serum only selective for fibronectin or will other proteins be removed?

Authors: The gelatin sepharose 4B column would be expected to remove other proteins to a limited extent. It is not, therefore, absolutely specific but it does result in the production of serum that is depleted in fibronectin relative to most other serum components and has thus been commonly used to prepare fibronectin-depleted serum [Horowitz B, Chang MY (1989). In: *Fibronectin*, Mosher DF (ed.), 441). Indeed the manufacturer (Pharmacia) states that the gel's major application is the purification of fibronectin. In a related project on the control of fibronectin gene expression being done by Dr. Lee Chou at Univ. British Columbia (UBC), we have labelled all proteins secreted by cells growing on grooved titanium substrata, passed the medium through gelatin sepharose columns, and run the retained fraction on polyacrylamide gels. Our results indicated that fibronectin was the predominant protein bound to sepharose 4B with the 72k gelatinase being a minor component; no other bands were seen.

J.A. Jensen: Continuity of titanium coating can only be determined by transmission electron microscopy (TEM), especially for the coating present in the grooves. Have the authors attempted to quantify this?

Authors: No, we have not attempted to quantify the continuity of the titanium coating. It would be difficult to use TEM to quantify the continuity of the titanium layer because in these experiments the underlying substratum was silicon which of course does not section well. In any case, when we have sectioned Ti-coated epoxy specimens, we have found that the coating is continuous with the exception of some small tears which we interpret to be artifactual as a result of sectioning.

A. Nanci: How can you explain the higher accumulation of fibronectin underneath the cells in fibronectin depleted medium?

Authors: At present we have no definitive answer to this question. Currently our group at UBC is investigating the distribution of fibronectin intracellularly as well as the control of fibronectin gene expression by surface topography, and we hope to have more insight into this question in the future.

A. Nanci: How will other substrate parameters like surface chemistry, surface energy state, and mechanical substrate properties, interfere with the final cellular behaviour?

Authors: We make no claim that migration is influenced only by preferential adsorption of fibronectin in relation to surface microgeometry. In fact, we take great care in the preparation of our surfaces so that such factors as surface energy are controlled. Thus, for example, we glow-discharge treat our surfaces prior to culturing cells on them. The point being made in the paper, however, is that aligned fibronectin-containing fibrils are deposited on grooved surfaces and such aligned fibrils would be expected to affect cell behaviour.

A. Nanci: What is the relation of this research to other experiments which demonstrated that only very small surface grooves and pitches (1-2 μm) influence cell behaviour?

Authors: There is a large literature dating back to the early work of Paul Weiss, using such substrata as fish scales and scratches in glass, to modern workers, using polishing pastes and micromachining techniques, that demonstrates that grooves of dimensions varying from less than a micrometer to tens of micrometers can align cells. For any one application, however, a particular size of groove might be most effective.

R. Todescan, Jr.: Were there clear differences for the different grooved substrata?

Authors: We have not observed any dramatic differences in the distribution of fibronectin fibrils on the different grooved substrata although there was a tendency for fibronectin-containing fibrils to be less prominent on the narrower grooves.

R. Todescan, Jr.: Could the authors comment on their results in relation to other reports in the literature, such as Halfter *et al.* in which fibronectin-depleted medium was not used?

Authors: Halfter **et al**. did not use fibronectin depleted serum so their results are not directly comparable to ours. Clearly, deposition of fibronectin is altered in cells cultured in fibronectin-depleted medium as described in this paper. In addition, in preliminary experiments, Dr. T. Goto of our (UBC) laboratory has found that the intracellular distribution of fibronectin is also altered. The mechanisms underlying these changes remain to be elucidated.

J.P. Heath: Why do the cells move so slowly on titanium?

Authors: The cells are probably moving slowly on the titanium because they attach strongly to it. As noted by Harris (1982, text reference): "In the world of a tissue cell you can move yourself further and faster by pulling very lightly against your surroundings." In addition, these experiments were done shortly after plating so that the cells first had to spread before they began migrating, so that the net distance travelled was probably less than would have been observed for cells that were actively migrating at the start of the experiment. Another effect of starting the experiment with freshly plated cells is that the most intense labelling was observed at the site of initial attachment probably because the cells reside longer at that location than elsewhere because of the time taken to spread and polarize.

J.P. Heath: Why is the intensity of silver label on the dorsal fibronectin fibrils so different from that on the substratum?

Authors: The intensity of silver label is probably greater on the dorsal fibronectin fibrils because they contain a greater proportion of fibronectin than the material left on the substratum which would be expected to contain type I collagen and other extracellular matrix materials.