ORIENTATION OF CALVARIA AND PERIODONTAL LIGAMENT CELLS IN VITRO BY PAIRS OF DEMINERALIZED DENTINE PARTICLES

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SUMMARY

Rat calvaria cells (RC cells) and monkey periodontal ligament fibroblasts (MPL cells) were cultured for 4-6 days with demineralized dentine, acid-washed glass particles or untreated glass particles to determine whether the presence of these particles can affect the orientation of the cells. Encapsulation of dentine particles began as early as the first day in culture and occasional cells were attached to, and oriented between adjacent particles. Multilayers of cells formed along the periphery of the dentine particles and the more superficial of these cells projected outwards in a radial fashion. Oriented cell sheets were evident between dentine particles after 3 days in culture. Finally, the cell sheets tore away from the surface of the culture dish to give rise to thick multilayered cellular bridges between dentine particles. The nuclear orientation index (ratio of L/W) for each RC cell lying between 15 randomly selected pairs of each particle type in 4-day cultures was measured along 2 axes; the length represented by the shortest distance between the 2 particles and the width perpendicular to this axis. The nuclear orientation index was 1.52 for cells between dentine, 1.13 between acid-washed glass and 1.06 between untreated glass. Control measurements of cells associated with single particles were 1.06, 1.09 and 1.06 respectively. The frequency of cellular orientation occurring between dentine particles was significantly greater than that occurring between acid-washed glass particles (χ² > 0.001) which in turn was significantly greater than that for untreated glass particles (χ² > 0.001). The observations and results suggest that the development and maintenance of the observed cell orientation depends upon development and maintenance in the cell sheet of tensional forces oriented parallel to the axis between the dentine particles, and that this is related directly to the capacity of the cells to attach to the particles.

INTRODUCTION

Cell orientation in vitro is thought to be influenced by properties of the substrate and the ability of cells to recognize and adapt to these properties (Weiss, 1958, 1961). More recently, it has been shown that the orientation of cells can be affected by the failure of their microfilament bundles to follow certain surface curvatures (Dunn & Heath, 1976). Cells migrating in vitro have been shown to change their direction of travel and orientation through angles of between 30° and 60°, and it has been suggested that these changes are initiated by the centriole through the microtubules (Albrecht-Buehler, * Present address: Department of Oral Biology, Faculty of Dentistry, University of British Columbia, 2075 Wesbrook Mall, Vancouver, B.C. V6T 1W5, Canada.

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As cell growth proceeds, mutual constraints among cells direct their movement into parallel arrays, and lateral adhesion with slippage stabilizes these parallel arrays of cells in monolayers (Elsdale, 1968, 1973).

A number of experiments have shown that multilayers and sheets of cells can exist under tension. Cultures of mouse mammary cells significantly reduce the diameter of floating collagen gels (Emerman, Enami, Pitelka & Nandi, 1977; Emerman & Pitelka, 1977). Bell, Ivarsson & Merrill (1979) have shown that when a collagen lattice containing cells is attached at 4 points stress patterns are formed such that the cells become aligned between pairs of the points. Tension within cellular sheets formed by merged fibroblast outgrowths from two adjacent explants has been measured by James & Taylor (1969) and found to be $3.4 \times 10^4$ dynes/cm² ($3.4 \times 10^3$ N m⁻²).

The present investigation was initiated to determine whether particles of biological and non-biological origin can provide points of attachment for sheets of cells growing in vitro and, if so, whether tension leading to orientation of the cells will develop between the particles. Our results indicate that cells can become aligned between the particles and suggest that the maintenance of this orientation depends upon the attachment of the cells to the particles and the development of tension between them.

**MATERIALS AND METHODS**

**Preparation of dentine and glass particles**

Particles of demineralized dentine were prepared. The dentine was demineralized because cells have been shown to attach with less avidity to mineralized than to demineralized dentine (Boyko, Brunette & Melcher, 1980). Molar teeth were removed from porcine mandibles obtained from a local abattoir (Quality Packers, Toronto, Canada). The periodontal ligament was removed from the roots of the teeth by scraping and the teeth were then washed twice in phosphate-buffered saline (PBS). They were then demineralized in 0.6 N HCl for 24 h, neutralized with 2% NaHCO₃ for 3 h (Huggins & Urist, 1970), and stored in PBS. The crown and root apices were removed and discarded, after which the roots were bisected axially, the pulp removed and, while being viewed in a dissecting microscope, cut into transverse slices ~1 mm thick. Following removal of the cementum from each slice, the dentine was ground into particles with a mortar and pestle. The particles were then filtered through a 224-μm mesh tissue sieve (Cistron Corp., Elmsford, NJ.) and stored in PBS at 4 °C. Prior to use, the dentine particles were sterilized by washing in 5 changes of an antibiotic solution comprising 100 μg/ml Penicillin G (Sigma Chemical Co., St Louis, Mo.), 50 μg/ml Gentamicin sulphate (Sigma) and 30 μg/ml Fungizone (Flow Laboratories, McLean, Va) in a minimal essential medium (α MEM) (Flow Laboratories, Rockville, Md.) followed by storage overnight in the same solution. The particles were then transferred to α MEM and warmed to 37 °C in a waterbath before being added to the cells.

Cells adhere better to glass washed in acid than to unwashed glass (Paul, 1975). Glass coverslips (9 x 35 mm; Belco Glass; Vineland, N.J.) were ground into particles with a mortar and pestle, collected in PBS and filtered through a 234-μm mesh sieve. Some of these particles were cleaned for 24 h in a chronic-sulphuric acid cleaning solution (Fisher Scientific Co.; Fair Lawn, N.J.). The particles were stored and sterilized in the same manner as were the dentine particles.

**Cells and culture procedures**

Cells were isolated from newborn rat calvaria (RC cells) according to the procedure of Rao, Ng, Brunette & Heersche (1977). Cell suspensions were collected after 5 sequential incubations, each lasting 20 min, and all 5 populations of cells were pooled.
Fibroblast-like cells were isolated from monkey (Macaca fasicularis) periodontal ligament (MPL cells) by the explant procedure of Marmary, Brunette & Heersche (1976). The medium used in all cultures was α MEM with 15% foetal calf serum (FCS) and antibiotics (100 μg/ml Penicillin G, 50 μg/ml Gentamicin sulphate and 0.3 μg/ml Fungizone). All cells were grown at 37°C in 95% humidified air and 5% CO₂. The cells were subcultured using trypsin and by plating 1 : 3 in tissue culture flasks (Lux Scientific Corp., Newbury Park, Calif.) as described previously (Marmary et al. 1976). Cells used in the experiments were 2nd to 5th subculture RC cells and 9th to 15th subculture MPL cells.

Both RC and MPL cells were plated at a concentration of 5 x 10⁶ cells/dish in 60-mm dishes (Falcon 3002; Becton, Dickinson & Co.; Oxnard, Calif.) containing 5 ml medium, after which 3-4 drops of a suspension of demineralized dentine or glass particles in α MEM without FCS were added immediately with a Pasteur pipette. In some experiments the cells were permitted to reach confluency (3-4 days) before the addition of particles. The medium was changed every second or third day and cultures were maintained for up to 10 days. Cultures were observed and photographed daily using a Leitz Diavert phase-contrast microscope. Successive cultures were fixed daily with Bouin’s fixative, and were stained by either haematoxylin and eosin and mounted in situ in glycerol gelatin or by van Giesen’s method counterstained with haematoxylin after which they were dehydrated, carefully removed from the plastic dish, cleared and mounted on a glass slide.

Quantitative procedures

RC cells and dentine or glass particles or MPL cells and dentine or glass were cultured for 4 and 6 days respectively, after which they were fixed in Bouin’s fluid, stained with haematoxylin and eosin, and mounted in situ with glycerol gelatin. Fifteen pairs of dentine particles, glass particles and acid-washed glass particles from RC cultures and an equal number of single particles were selected randomly and immediately photographed. To select pairs of particles or single particles, a culture dish was placed under the microscope and the first pair or single particle to come into view was selected. The pairs of particles were centrally positioned for photography while the single particles were placed in the lower left corner of the viewing frame. Prints were enlarged to 8 x 10 in. (20.3 x 25.4 cm). Each test photograph (pair of particles) was paired with the control photograph (single particle) taken from the same culture dish. A line representing the shortest distance between the pair of particles was drawn and used as an axis. A line was drawn on either side of the axis and parallel to it at a distance of 2.5 cm, and two lines were drawn at right angles to the axis to intersect with the 2 ends of the axis so enclosing a rectangular area (Fig. 1, p. 62). The length and width of each nucleus in focus within this rectangle was measured with a vernier caliper (Moore & Wright, Sheffield, England, no. 1140) graduated to 0.02 mm. Orientation was expressed as the ratio of L/W similar to the procedure of Dunn & Ebendal (1978) and this ratio was termed the nuclear orientation index (NOI). The axis in control photographs of single particles was drawn at an angle equal to a random number from 0° to 90° relative to the long edge of the photographic frame adjacent to the particle, and of a length equal to the axis on its corresponding test photograph (Fig. 2). The length and width of each nucleus in focus lying within 2.5 cm on either side of this line was measured and expressed as described previously.

To estimate the frequency of orientation between pairs, 500 pairs of particles from each of: (i) RC cells + dentine; (ii) RC cells + untreated glass; (iii) RC cells + acid-washed glass; (iv) MPL cells + dentine; and (v) MPL cells + untreated glass were observed in randomly selected culture dishes. The orientation of the cells was estimated as being either: (i) oriented parallel to the axis; (ii) slight orientation; (ii) no orientation; or (iv) oriented perpendicular to the axis.

Observations and Results

Development of orientation between dentine particles

RC and MPL cells formed multilayers after reaching confluency when grown on plastic. RC cells (Fig. 3) were selected for more critical study and for quantification
because they have a more rapid doubling rate and they form multilayers of more even density.

The orientation of most cells appeared random after 1 day in culture and was probably dependent upon the surface properties of the dish. Where a small number of cells was attached to a dentine particle many were oriented with their long axis approximately perpendicular to the surface of the particle. These cells were also frequently attached to the surface of the dish. Encapsulation of the particles began to occur once it had become evident that the cells associated with the particle had noticeably increased in number (Fig. 4). Occasional cells were oriented along an axis between adjacent particles (Figs. 5, 6).

After 2 days, most of the dentine particles were surrounded by a capsule of cells (Fig. 7). More rod-shaped particles appeared to be encapsulated than particles of other shapes. Cells attached to the sides and upper surface of particles were flattened and oriented in the same plane as the surface of the particle. Orientation of cells between some of the pairs of particles appeared to be random; however, the number of cells that were oriented between others appeared to have increased (Fig. 8).

After 3 days, the extent of encapsulation and the number of cells forming capsules had increased. The capsules surrounding larger particles were thicker and contained more cells than those surrounding smaller particles. The topography of the particle affected the orientation of the cells forming the capsule. Cellular orientation parallel to the surface of the particle was greater when a straight surface was present, and this was particularly evident in the case of rod-shaped particles. The more irregular or spherical the surface, the more irregular was the capsule and the more irregular was the orientation of its cells (Figs. 9, 10). The more superficial cells in these capsules projected outward in a radial fashion. The number of cells present between dentine particles and the extent of their orientation was much greater than after 2 days in culture (Fig. 11). Multilayering was prominent along the sides of the dentine particles and occasionally present between 2 adjacent particles. In areas where the number of dentine particles and the number of cells was greatest, and this usually occurred in the centre of the culture dish, sheets of cells frequently were seen to have torn away from the plastic. This resulted in the formation of a sheet or bridge of tissue extending between 2 or more dentine particles, leaving adjacent areas on one or both sides of the

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Fig. 1. Photomicrograph of RC cells between 2 dentine particles (d) illustrating the axis and rectangle used for the measurement of cellular orientation. The length and width of all visible nuclei within the rectangle were measured. The length of the axis as measured on the photograph was 189 mm and the orientation ratio of the cells in the rectangle was 1.81. Haematoxylin and eosin. × 300.

Fig. 2. Photomicrograph of a single dentine particle (d) and RC cells that were used as the control for Fig. 1. The orientation ratio was 1.11. The random axis was drawn at 8° as described in the Materials and methods section. Haematoxylin and eosin. × 300.

Fig. 3. Phase-contrast photomicrograph of a confluent culture of RC cells after 5 days in vitro. Note the multilayers. × 232.

Fig. 4. A dentine particle (d) with a small unorganized capsule of RC cells (arrowheads) from a 1-day culture stained with haematoxylin and eosin. × 438.
Cellular orientation by dentine particles

bridge free of cells. These cell-free areas have been termed 'tension spaces' (see Figs. 16, 17).

After 4 or more days in culture the radial configuration of the capsule had become more pronounced on spherical and irregular surfaces (Fig. 12). Many cells associated with these surfaces were perpendicular to the particle surface and were apparently migrating relative to the particle. Multilayers were extensive along the sides of particles and had become more frequent between particles (Figs. 13, 14). Orientation of cells between particles was usually greater where more cells were present and where spherical or triangular surfaces of the particles were opposed. The particles did not appear to influence the orientation of the cells between them if they were spaced at a distance greater than 400 \( \mu \text{m} \). Where 2 particles were less than 70 \( \mu \text{m} \) apart their capsules occasionally merged, and the cells between them were oriented perpendicular to an axis joining the particles. This occurred chiefly where 2 straight surfaces lay parallel and close to one another.

In areas where many particles were present, a given particle was frequently connected to several other particles in its proximity by oriented cellular connexions (Fig. 15). Thick cellular bridges comprising highly oriented multilayers and tension spaces (Fig. 16) were more numerous and involved more dentine particles and cells than was observed after shorter periods in culture. Cells from adjacent areas migrated into the tension spaces, but the orientation of these cells was random (Fig. 17). Chains involving as many as 5 dentine particles and cellular bridges were occasionally seen.

Effect of glass particles upon cell orientation

Many fewer cells, both RC and MPL cells, appeared to attach to the surfaces of unwashed glass particles than to dentine, and encapsulation of all sizes and shapes of the particles was far less frequently observed. The radial nature of capsules that was seen to surround spherical or irregular dentine particles was not obvious. Orientation of cells between particles was rare and cellular bridges and tension spaces were not observed.

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Fig. 5. One-day culture. RC cells are evenly distributed and some cells (arrowheads) are attached to dentine particles (d) and oriented perpendicular to the particle surface. Cells appear to connect 3 of the particles (arrows). Haematoxylin and eosin. \( \times 200 \).

Fig. 6. One-day culture. RC cells (arrows) extend between the surfaces of 2 dentine particles (d). Haematoxylin and eosin. \( \times 200 \).

Fig. 7. A rod-shaped dentine particle (d) possessing a capsule of RC cells oriented parallel to the surface of the particle in a 2-day culture. Note the cells on the surface of the particle that are out of focus (arrowheads). Haematoxylin and eosin. \( \times 438 \).

Fig. 8. The encapsulation of dentine particles (d) and the number of cells extending between the particles (arrow) appears to have increased after 2 days in culture. Haematoxylin and eosin. \( \times 200 \).

Fig. 9. After 3 days in culture a rod-shaped dentine particle (d) is surrounded by a more dense capsule of RC cells than after 2 days (compare with Fig. 7). The cells are oriented parallel to the surfaces of the dentine particle. Haematoxylin and eosin. \( \times 438 \).
Cellular orientation by dentine particles

The number of cells attaching to, and encapsulating exposed surfaces of glass particles appeared clearly to be increased by acid-washing. However, multiple oriented connexions between several adjacent particles were rare and poorly developed, and cellular bridges and tension spaces occurred infrequently.

When dentine or glass particles were added to confluent cultures that had been grown for 4 days and the 2 then maintained together for a further 4 days, encapsulation of particles and orientation between particles was less frequently observed than in cultures where particles and cells were added together. Small cellular bridges and tension spaces occurred only between dentine particles and only in those regions where large numbers of both particles and cells were present.

Quantitation of cellular orientation

The precision of the measurement procedure for determining width and length of cell nuclei in relation to the axis between particles was tested by measuring the length and width of 5 randomly selected cells on 20 occasions. The results are presented in Table 1. The average coefficient of variation for the 5 cells was 5.0%.

Table 1. Precision of the measurement procedure

<table>
<thead>
<tr>
<th>Cell no.</th>
<th>Mean* L/W</th>
<th>S.D.</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.762</td>
<td>±0.035</td>
<td>5.6</td>
</tr>
<tr>
<td>2</td>
<td>0.826</td>
<td>±0.042</td>
<td>9.0</td>
</tr>
<tr>
<td>3</td>
<td>1.015</td>
<td>±0.035</td>
<td>3.6</td>
</tr>
<tr>
<td>4</td>
<td>1.021</td>
<td>±0.041</td>
<td>3.4</td>
</tr>
<tr>
<td>5</td>
<td>0.946</td>
<td>±0.035</td>
<td>4.3</td>
</tr>
</tbody>
</table>

* Mean of 20 measurements of each cell.

Average coefficient of variation for the 5 cells = 5.0%.

Fig. 10. After 3 days in culture the RC cells surrounding an irregularly shaped dentine particle (d) form an irregular capsule. Note the cells on the surface of the particle that are out of focus. Haematoxylin and eosin. × 438.

Fig. 11. An oriented sheet of cells (arrows) extends between the dentine particles (d) after 3 days in culture. Haematoxylin and eosin. × 300.

Fig. 12. Phase-contrast photomicrograph of a single dentine particle (d) surrounded by a capsule of RC cells in a 4-day culture. The cells surrounding the particle are multilayered and some appear to be oriented outwards in a radial fashion (arrowheads). × 232.

Fig. 13. Phase-contrast photomicrograph of a 4-day culture showing orientation of RC cells between 2 dentine particles (d). The capsules of both particles (arrowheads) are radial and their leading edges merge between the particles to form a continuous sheet (arrow). × 232.

Fig. 14. Phase-contrast photomicrograph of oriented RC cells between dentine particles (d) in a 4-day culture. The cellular capsules surrounding these particles are smaller than those present in Fig. 13 but an oriented, multilayered sheet is visible (arrows). × 232.
Cellular orientation by dentine particles

Measurements of 2350 cells found between 15 pairs of dentine particles and adjacent to 15 single dentine particles showed that the orientation of RC cells along the axis joining adjacent dentine particles was significantly greater than that along a random axis drawn from the unopposed surface of a single dentine particle \( (P < 0.0005); \) Table 2. Because the data were not normally distributed, a non-parametric median test using a \( 2 \times 2 \) contingency table was constructed and was found also to be highly significant \( (\chi^2 > 0.0005) \). Differences between the test and control measurements of RC cells cultured with untreated glass particles were not significant using the same statistical tests. The difference between test and control measurements for RC cells and acid-washed glass was significant at the \( P < 0.05 \) level.

The influence of the distance between particles upon cellular orientation was found to be weak or not significant. The correlation value \( (r) \) for the effects of distance between dentine particles upon orientation of RC cells was \( r = 0.26 \). That for acid-washed glass particles was \( r = -0.03 \) and the value for untreated glass particles was \( r = -0.18 \). The size of the particles, the contours of their opposing surfaces and the

<table>
<thead>
<tr>
<th></th>
<th>NOI(^\dagger)</th>
<th>No. of cells(\ddagger)</th>
<th>NOI(^\ddagger)</th>
<th>No. of cells(\ddagger)</th>
<th>Mean distance between pairs, (\mu m)(\S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dentine</td>
<td>1.92 ± 0.030*</td>
<td>1172</td>
<td>1.06 ± 0.022*</td>
<td>1178</td>
<td>249 ± 78</td>
</tr>
<tr>
<td>Acid-washed glass</td>
<td>1.13 ± 0.020**</td>
<td>1004</td>
<td>1.09 ± 0.021**</td>
<td>834</td>
<td>178 ± 54</td>
</tr>
<tr>
<td>Untreated glass</td>
<td>1.06 ± 0.020</td>
<td>835</td>
<td>1.06 ± 0.018</td>
<td>846</td>
<td>154 ± 40</td>
</tr>
</tbody>
</table>

* Significant at \( P < 0.0005 \).
** Significant at \( P < 0.05 \).
\( \dagger \) Mean of the ratios of length/width of each measured cell ± 95% confidence limits.
\( \ddagger \) Total number of cells measured in 15 photographs.
\( \S \) Mean distance between pairs of particles ± S.D.

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**Table 2. Effect of dentine and glass particles upon nuclear orientation index (NOI) of RC cells**

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Fig. 15. One dentine particle can form oriented cellular connexions with several other particles. Particles 1 and 3 are attached, by a cellular connexion. Connexions between particles 2 and 3 and 2 and 4 are less well oriented, while connexions between particles 4 and 5 and 6 are rudimentary. No cellular orientation exists between particles 3 and 4. Four-day culture. Haematoxylin and eosin. × 58.

Fig. 16. RC cells have contracted to form an oriented cellular bridge leaving tension spaces \((t)\) between particles of dentine \((d)\). The cellular bridge appears to be under tension because of its concave surfaces. Four-day culture. Haematoxylin and eosin. × 142.

Fig. 17. Cellular bridges (arrows) comprising multilayers of oriented cells between dentine particles \((d)\) link 3 particles in a chain. The cellular bridges appear to be under tension because of their concave surfaces. The tension spaces \((t)\) are occupied by un-oriented cells that appear to have migrated into them. Four-day culture. Haematoxylin and eosin. × 75.
The frequency of orientation was determined by classifying the orientation between 500 pairs of particles in each of 5 different combinations of cell and particle types (Table 3). Orientation parallel to the axis was considered to occur when the superficial layer of cells was oriented along an axis between the 2 particles and the cellular sheet merged with the radial capsule of both particles (Fig. 15, particles 1 and 3). Slight orientation was considered to occur where a multilayer was not obvious, but most cells were aligned along an axis between the particles (Fig. 15, between particles 5 and 6). No orientation was considered to occur when the particles had no apparent effect upon cellular orientation (Fig. 15, between particles 3 and 4). Orientation perpendicular to the axis resulted from heavy parallel encapsulation of the surfaces of closely opposed particles, where the capsules merged with each other. This usually occurred between 2 parallel surfaces of rod-shaped particles. The observed frequency of orientation was significantly greater between dentine particles than between glass particles irrespective of cell type ($\chi^2 > 0.001$). Cellular orientation following acid-washing of glass particles was also significantly different from that caused by leaving the glass untreated ($\chi^2 > 0.001$). No significant difference was observed between the orientation of RC cells and MPL cells between dentine particles.

**DISCUSSION**

Since the orientation of the nucleus is considered to be the same as that of the entire cell (Weiss & Garber, 1952; Margolis, Samoilov, Vasiliev & Gelfand, 1975) only nuclear measurements were used in this study to determine the orientation of cells. The repeated measurements performed upon each of 5 cells (Table 1) showed an average coefficient of variation of 5.0. The range for the 3 series of controls as well as the nuclear orientation index for untreated glass particles all lie within this level. Only the results for cellular orientation between dentine particles lie significantly outside this range. In an unoriented population of cells the ratios of the lengths and widths of...
the cells should approximate 1:oo. The nuclear orientation index obtained from the 3
sets of control photographs were 1:06, 1:09 and 1:06 from totals of 1178, 834 and 846
cells respectively. There was no significant difference between these figures, sub-
stantiating the precision of the measurement procedure and the randomization method.
The difference between these figures and the theoretical ratio of 1:oo could result from
the tendency of cells to form radially oriented capsules.

The frequency of orientation of cells between pairs of dentine particles was sig-
nificantly greater than that observed between pairs of acid-washed glass particles
($\chi^2 > 0.001$) substantiating the results of the nuclear measurements. The frequency
of orientation of cells between pairs of acid-washed glass particles was also signi-
nificantly greater than those between untreated glass particles ($\chi^2 > 0.001$).

No significant difference was observed between the response of RC and MPL cells
to dentine particles. However, the response of MPL cells to untreated glass was
significantly different from that of RC cells. The reason for this difference is unknown.

This study has demonstrated that pairs of demineralized dentine particles, but not
glass particles, can regularly initiate and maintain cellular orientation between them
_in vitro_. Demineralized dentine particles probably provide exposed collagen fibrils to
which cells can attach (Boyko et al. 1980). The demineralized matrix of bone may be a
local mitogen for connective tissue cells (Rath & Reddi, 1979) and it is possible that the
collagen of demineralized dentine surfaces may act in a similar manner. Collagen has
also been suggested to be chemotactic for fibroblasts (Postlethwaite, Seyer & Kang,
1978). While no measurements were taken to determine whether the collagen of
dentine particles was chemotactic for the cells used in this study, the presence of cells
oriented perpendicular to particle surfaces after 1 day in culture and the large radial
capsules surrounding the dentine particles in older cultures suggest the possibility
that this may be the case. Further, collagen appears to provide a hospitable surface for
cell attachment (Ehrmann & Gey, 1956; Klebe, 1974; Boyko et al. 1980), probably
through the medium of fibronectin (Yamada & Olden, 1978). Consequently, attach-
ment of cells to, and encapsulation of dentine particles can be expected to have been
facilitated by demineralization of the dentine. Untreated glass particles present to the
cells the original surfaces of the coverslips as well as fracture surfaces that resulted
from the preparation of the particles. Thus, preparation of these particles probably
results in several surfaces being exposed to cells which possess material not removed
during routine washing procedures and which could interfere with cellular adhesiv-
ness. Overnight washing with chromic-sulphuric acid should have removed all traces
of grease and other foreign material from the surfaces of the glass particles, thereby
increasing their 'wettability' and so making them more adhesive to cells (Paul,
1975).

It is conceivable that when cells attach to and encapsulate hospitable surfaces of
particles, tensional forces that are generated by the cells in sheets that extend between
pairs of particles are transmitted to the particles. This could result in the tensional
forces and cells becoming oriented parallel to an axis extending along the shortest
distance between the particles. Where particles are not distributed in pairs, or are too
far apart, the tensional forces and cells will be randomly directed because of the lack of
fixed points between which the forces can be concentrated. The concept of tensional forces developing between particles receives support from first, the demonstration by James & Taylor (1969) of tensional forces in sheets of cells joining pairs of explants; second, from the alignment of cells embedded in a collagen gel in vitro between points to which the periphery of the gel has been attached (Bell et al. 1979); and third, from the observation in the present study that tears may appear in the cell sheet on both sides of the axis between pairs of particles, with contraction of the sheet to form a concave-oriented cellular bridge between the particles leaving cell-free spaces on each side of the bridge.

It has been established that the orientation of motile cells may be influenced by the orientation of cells with which they come in contact (Elsdale, 1968; Elsdale & Bard, 1972; Erickson, 1978). Although we have not attempted to determine the effect of this mechanism of orientation in our cultures, it is possible that it may have contributed to the alignment of cells at the periphery of the oriented sheets. The physical characteristics of the substrate are also known to influence the orientation of cultured cells (Weiss & Garber, 1952).

In conclusion, we believe that our observations suggest a mechanism that could operate in vivo to orient cells and perhaps fibres in connective tissue structures that are attached to mineralized tissue at both ends, in particular ligaments, the periodontal ligament and the transeptal fibres between teeth. It could also play a role in the orientation of cells and perhaps fibres in tendons and in the free gingival fibres that extend between bone and soft tissue structures. The findings also raise the possibility that orientation of cells precedes orientation of collagen fibres in development of these structures.

REFERENCES

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