In Vitro Cytotoxicity of Periodontally Diseased Root Surfaces*

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THE PURPOSE OF this experiment was to develop a model system for controlled *in vitro* testing of root surface toxicity. Human gingival fibroblasts were placed into a depression created in the root surfaces of ten heat sterilized periodontally involved teeth, one half of which were root planed to remove all irritants. The teeth and fibroblasts were incubated in culture medium for 5 days, after which the cells were fixed and stained. Each tooth was examined for migration of cells onto the root planed and non-root planed areas of the tooth, and for evidence of any cytotoxic reaction. No difference in pattern of cell growth, cell migration, or cytotoxic reaction was discernible between root planed and non-root planed areas. Normal fibroblasts could be detected on the surfaces of dentin, calculus, periodontally involved cementum, and enamel. It appears that heat-sterilized periodontally-involved root surfaces, as well as calculus and calculus-laden enamel, were not toxic to human gingival fibroblasts in cell culture.

Basic to periodontal health is biologic compatibility between the adjoining hard and soft tissues of the periodontium. Microbial irritants bound to the tooth surface may render it cytotoxic to the adjacent tissues. If this is true, then a prerequisite in attempts to promote healing of the periodontium by procedures such as root planing, flap surgery, and debridement of intrabony defects would be removal of cytotoxic factors from the tooth surface and establishment of intimate contact between compatible soft and hard tissue walls of the pocket.

The dental literature contains many articles dealing with the toxicity of various dental materials. The biocompatibility of such substances has been assessed both *in vivo* and *in vitro*. A similar approach has been applied to study the biologic effects of root surface cytotoxicity. Morris implanted the roots of periodontally-involved and uninvolved teeth into the abdominal tissues of rats and found no difference in the reactions of the connective tissue to either type of root.[1] Hatfield and Baumhammers,[2] as well as Aleo, DeRenzis, and Farber[3] studied the effects of such roots in cell cultures. Cell culture has been used widely in medical toxicology and has great advantages over other means of biomaterial testing in that it separates the toxic reaction from the inflammatory reaction, thus allowing control over the test situation. Therefore, it provides higher statistical accuracy and is more sensitive than *in vivo* methods.[4]

The purpose of the present study was to establish a simple, controlled, and reproducible system for *in vitro* testing of root surface toxicity utilizing cell culture. Such a system could simplify testing of the efficacy of potential therapeutic agents and techniques in rendering the tooth surface compatible with the surrounding soft tissues.

MATERIALS AND METHODS

Preparation of Specimens

From a group of teeth extracted for periodontal reasons, ten were chosen whose soft tissue attachment was positioned at least one third the length of the root apically to the cementoenamel junction (CEJ). After extraction, the teeth were rinsed in sterile saline, and scrubbed with a toothbrush to remove remaining blood, gross plaque and other debris. They were then dried and stored in a sealed jar at room temperature until needed (1–3 weeks). All specimens were sectioned sagitally, separating each tooth into mesial and distal halves. Only the side with the greatest loss of attachment was retained for use in the experiment. A trough or reservoir about 2 mm in depth was cut into the outer surface of the root using a handpiece and a No. 6 round bur. The trough extended through the middle ¾ of the root's length and approximately ½ of its width (Figs. 1 and 2). The walls of the trough were sloped outwardly, providing a ramping effect. The entire reservoir was polished using an amalgam finishing bur, to create as smooth a surface as possible.

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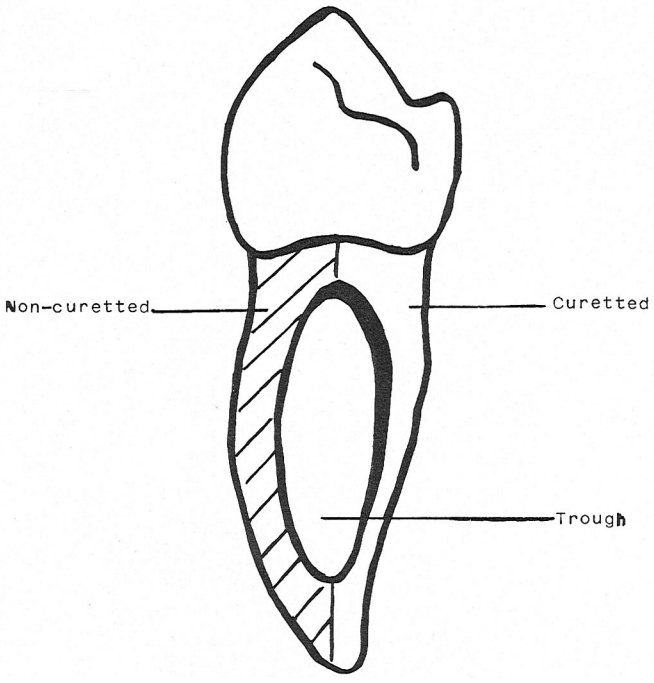

Figure 1. *Sketch of tooth preparation.*

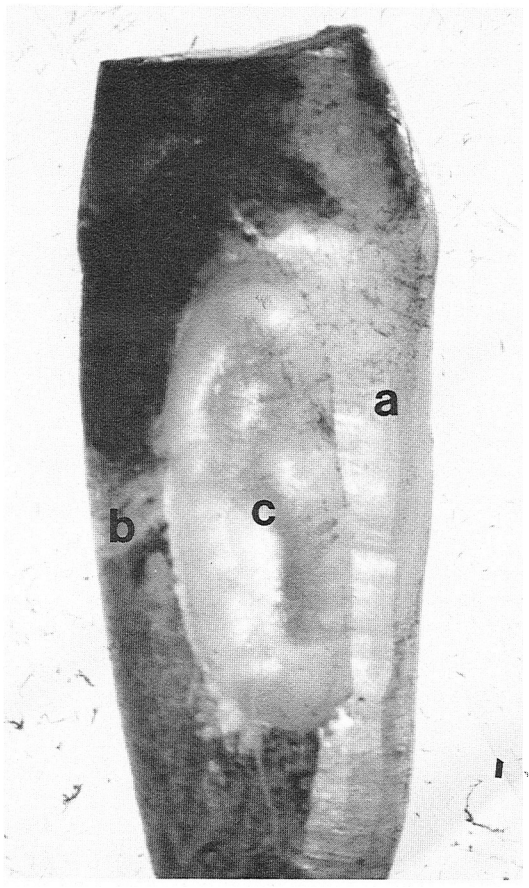

Figure 2. *Experimental tooth design: a, curetted half; b, noncuretted half; c, trough.*

The right half of the root length was scaled and planed, using a sharp Columbia No. 13–14 curette. An attempt was made to remove all the cementum and produce a surface that was both visually and tactually smooth (as determined using a No. 3 explorer). The left half of the root length was not scaled or root planed. All teeth were subsequently placed in glass vials, sealed and sterilized with dry heat for 50 minutes at 125°C.

Preparation of Cells

Human gingival fibroblasts in early passages were maintained at 37°C in a humidified atmosphere of 5% CO_2–95% air in 75 cm^2 plastic flasks.* The cells were fed every 3 days with 15 ml of growth media consisting of supplemented Eagle's minimum essential medium containing 15% heat inactivated fetal calf serum, as well as penicillin (1,250 units/10 ml), streptomycin (1,250 mcg/10 ml), and mycostatin (500 units/10 ml).

Experimental Procedure

The ten sterilized teeth were removed from their vials and placed in 35 mm plastic tissue culture dishes* with their troughs facing up. Using a Pasteur pipette, approximately 8.3×10^4 cells in 0.1 ml supplemented medium was pipetted into the troughs of eight teeth. Supplemented medium without cells was added to two teeth. These served as controls.

The teeth were then carefully placed into the incubator and left for 2 hours so that the fibroblasts would have an opportunity to settle and adhere to the floor of the trough. After this time, all the dishes were removed from the incubator and medium containing the antibiotics was added gently to the dishes until the level of medium just covered the tooth. All the dishes were then returned to the incubator. After 5 days, the teeth were removed, fixed and stained with Giemsa stain, using the method described by Fox.[5] All teeth were observed for fibroblast adherence, using a stereomicroscope.† The number of cells adhering to the bases of the troughs as well as the relative number of cells migrating from the trough onto the curetted and noncuretted areas of the tooth were noted.

RESULTS

A distinct pattern of cell growth which would indicate a cytotoxic effect of periodontally-involved areas to cells was not evident. Nor did the fibroblasts migrate in a "stream" up from the trough and out onto the root surface. However, healthy cultured fibroblast growth was observed on the surface of the dentin (Fig. 3), calculus (Fig. 4), periodontally-involved cementum (Fig. 4), and enamel, both scaled and nonscaled (Fig. 3). A wide variation of cell growth existed from specimen to specimen. Not all sections exhibited fibroblasts on each type of surface.

Deep staining of calculus and attached periodontal fibers masked visualization of the lightly blue stained cytoplasm of the gingival fibroblasts. Hence, it was dif-

* Corning Glass Works, Corning, NY.
† Zeiss Stereomicroscope IV, Carl Zeiss, Inc., New York, NY.

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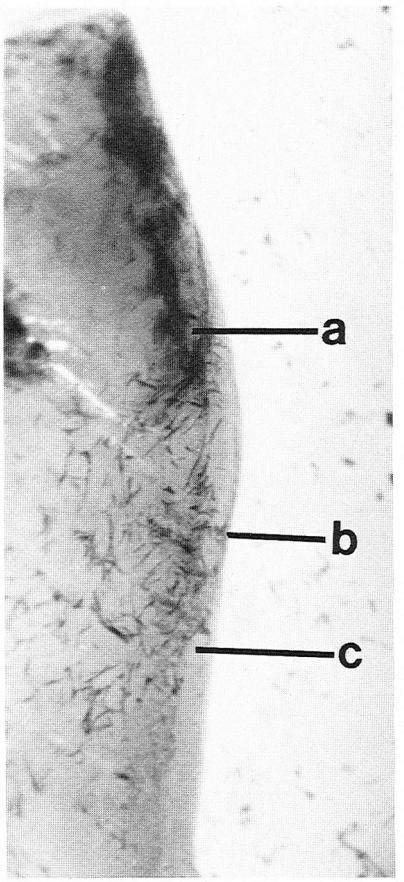

Figure 3. *Growth of fibroblasts on: a, enamel; b, cementoenamel junction; c, dentin (scaled root surface).*

Figure 4. *Growth of fibroblasts on calculus and periodontally involved cementum.*

ficult to determine with certainty whether the cells grew on calculus, calculus-laden cementum, and/or the remains of the periodontal ligament. However, in a few sections a clear distinction could be made between the fibroblasts and the calculus upon which they were positioned.

Overall, there appeared to be more cells growing on the curetted than on the noncuretted surfaces. This might be related to a more gradual incline towards the curetted side created by the root planing. Control specimens showed no evidence, either coronally or apically to the coronal extent of the attachment, of gingival fibroblast adhesion or growth. Cells of the periodontal ligament did not exhibit a distinctive structure under the stereomicroscope.

DISCUSSION

In view of the publications by Hatfield and Baumhammers,[2] and Aleo, DeRenzis and Farber,[3] it was assumed that a system capable of testing the biocompatibility of the tooth surface for cultured cell growth could be attained. The present system was developed after the testing of a number of different models.[6] This model eliminates the necessity for fibroblasts to migrate from the plastic surface of a culture dish onto a tooth surface. It also allows for a comparison of two experimental surfaces (treated and untreated). However, because of the deep staining of the periodontal ligament, it was difficult to distinguish cultured cell growth in periodontally uninvolved areas.

A diploid human/gingival fibroblast cell line derived from an explant of human gingival tissue was used to more closely reflect the sensitivity and metabolism of fibroblasts *in vivo*.

From the previous literature it was expected that the extent of migration and growth of fibroblasts onto untreated periodontally diseased surfaces would be considerably less than surfaces treated with root planing or periodontally noninvolved root surfaces. Further, cell growth on calculus was not expected. The results of the present experiment did not agree with published findings.[3] This may be due to two reasons: First is the apparent inability of fibroblasts to migrate up inclined planes. Fibroblasts did not seem to have the capacity to migrate from the floor of the trough and out onto the tooth surface. Leirskar and Helgeland[7] have reported that few, if any, fibroblasts are able to climb from glass onto 1-mm thick disks composed of nontoxic dental materials. On the other hand, in the present experiment many cells were seen on the lateral walls of the trough as well as outside of it, on cementum, enamel, and calculus. It seems likely that instead of migrating, individual cells were able to reach these surfaces by landing or floating

onto them when the fibroblasts were added to the teeth. Once on these comparatively flat surfaces, the cells were apparently able to grow and multiply (as discerned from the patterns which the cells formed on these surfaces). Secondly, it is questionable whether endotoxins are able to produce cytotoxicity in culture conditions. It has been reported that endotoxin in root surfaces is cytotoxic to cells in pure cultures.[8] However, in the present experiment, the fibroblasts grew on noncuretted periodontally involved surfaces. While it has been reported that endotoxin has a direct cytotoxic effect on L929 cells in culture,[9] many investigators believe that endotoxins produce their toxic effects indirectly, i.e. via the immune system.[10-15] Levine and coworkers have written that "... endotoxins produce their effects by activating serum components, notably the complement system, to produce biologically active substances such as anaphylotoxin and have no direct effect on growth or metabolism of mammalian epithelial or fibroblast cells in culture"[16]

Furthermore, Neiders and Weiss[17] found that, while endotoxins were not toxic to cells in culture, they did reduce their ability to adhere to glass surfaces when concentrations of 10 μg/ml or greater were added to the media. Jones[18] found that in specimens taken from 50 periodontally-involved roots that consisted of a section 1 mm deep, extending from the CEJ apically for 5 mm and covering the entire proximal width of the root surface, there was only an average of 0.147 μg of endotoxin. Thus, endotoxins appear to be present in amounts much smaller than necessary to effect cell adhesion. The temperature and duration of sterilization used in this experiment probably would not have neutralized the endotoxins. Endotoxin inactivation reportedly requires 250°C for 30 minutes[19] or 140°C for 7 hours.[20]

In the present study cell growth was greater on the curetted than on the noncuretted surfaces. Rather than cytotoxic inhibition, this phenomena probably can be attributed to the difficulty of visualizing cells on the noncuretted tooth structure due to the deep staining of these surfaces which masks the lightly staining cytoplasm of the fibroblasts. Another reason is the more gradual slope to the curetted half of the trough created by extensive root planing which could bias the accumulation of cells. In any case, the density of fibroblast growth seen by other researchers[3] could not be duplicated in the present study even though far longer incubation periods were used.

The present study appears to indicate certain limitations in the testing of root surface cytotoxicity *in vitro*. First, at present there does not seem to be any way to quantitate variations in the growth of cells on tooth surfaces accurately. This is partly due to an inability to standardize the surface areas of the specimens. The wide variation in tooth shape, curvature of the root surface, and surface topography make precise standardization nearly impossible. Another limitation with *in vitro* testing is the need to sterilize the specimens so that they can be placed in a cell culture without producing microbial infection. This destroys the primary root surface irritant i.e., bacterial plaque. Thus, even if fibroblasts do grow on noncuretted but sterilized root surfaces, without viable bacteria it would not be a true reflection of the *in vivo* situation.

While the methodology employed by Aleo and coworkers[3] was not duplicated in the present investigation, the results indicate that cultured human gingival fibroblasts do not adhere preferentially to the root surfaces of instrumented and noninstrumented periodontally diseased teeth. No evidence of a cytotoxic reaction from root surface irritants was observed. No intracellular changes could be detected with the stereomicroscope. However, a scanning electron microscope was not used in this study, so that definitive statements cannot be made regarding intracellular changes.

The *in vitro* model system that was employed in the present experiment may have little bearing on the *in vivo* relationship between the tooth and soft tissue. Periodontally diseased root surfaces may not be cytotoxic in the absence of the immune system (which pure cultures lack) and/or bacterial plaque. Nonetheless, as both of these elements are found *in vivo*, there seems to be little significance to any study that examines root surface cytotoxicity without taking these important factors into consideration. This study would suggest that the direct cytotoxic nature of nonmicrobial root surface irritants may play little, if any, part in the overall nature of root surface cytotoxicity.

CONCLUSIONS

Within the scope of this study it appears that: (1) Sterilized root surfaces exposed by periodontal disease, as well as calculus and calculus laden enamel were not toxic to human gingival fibroblasts in cell culture. (2) Human gingival fibroblasts did not adhere selectively to the sterilized root surfaces of instrumented versus noninstrumented periodontally diseased teeth *in vitro*. (3) Human gingival fibroblasts do not seem to be able to migrate up inclined tooth surfaces *in vitro*. (4) *In vitro* sterile model systems may have little in common with the *in vivo* relationship between tooth and soft tissue.

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Abstracts

Probing Force and the Relationship of the Probe Tip to the Periodontal Tissues

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J Clin Periodont **6:** 106, April, 1979.

The relationship of the probe to the periodontal fibers and the alveolar bone using different probing forces and whether a plateau in pocket depth measurement is reached with increased probing forces, was studied with two groups of patients. In the first group there were 21 molar teeth, scheduled for extraction, and in the second group 33 teeth located in areas scheduled for periodontal surgery. All were given initial preparation and evaluated using a new bleeding index, the Periodontal Pocket Bleeding Index (P.B.B.I.) which showed that both the extraction and the surgical group were statistically similar enough to conduct this survey. In the extraction group, pocket depth was measured with a probe 0.63 mm in cross section at the tip, from a diamond bur cut in each tooth using increased probe forces of 0.50, 0.75, 1.00, and 1.25 N. After extraction the teeth were again measured from the reference marks to the coronal portion of the connective tissue attachment and compared with the pocket depth level determined prior to extraction. It was found that the mean pocket depth increased with increased probing force and that a force of 0.75 N gave the most accurate representation of pocket depth. This was later confirmed with varying pocket depths in two sub-groups. In the second group (the surgical group) 33 teeth were measured from restorative margins on the teeth to the bottom of the pocket level and probed with the same increasing forces 0.50, 0.75, 1.00 and 1.25. These measures were compared with measurements taken during surgery. At 1.25 N a plateau existed in that increased forces no longer were observed to indicate increasing pocket depth. With a probing force of 0.75 N the tip of the probe was shown to be located at the most coronal intact connective tissue fibers. *Department of Periodontology, Faculty of Dentistry, University of Amsterdam, Louwesweg 1, Amsterdam, The Netherlands.*

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Recession: A 4-Year Longitudinal Study After Free Gingival Grafts

Rateitschak, K. H., Egli, U., and Fringeli, G.
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Sixteen free gingival grafts were placed according to the technique of Sullivan and Atkins (1968) around 42 teeth in 12 patients, to prevent progression of gingival recession. Changes in the keratinized gingiva, degree of recession, size of the transplant itself, width of wound extension, and depth of the vestibule were evaluated at 1, 6, 12, 24, and 48 months. Although no control group was used, healing 1 month after the surgical procedure revealed no change in degree of recession or sulcus depth. An average shrinkage of 25% of the transplant was found after 1 month, and loss of wound extension width was seen up to 6 months after surgery until mucosa reached the apical border of graft. The deepened vestibule created by the extension phase relapsed nearly to the presurgical dimension after 1 month, but over the following 47-month period regained a deeper position. *Zahnarztliches Institut der Universitat Basel, Petersplatz 14, Basel, Switzerland.*

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