Attachment of Human Gingival Fibroblasts to Periodontally Involved Root Surface Following Scaling and/or Etching Procedures: A Scanning Electron Microscopy Study

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INTRODUCTION

This study evaluated, in vitro, fibroblast attachment to periodontally involved root surfaces which were either root planed or acid/chelated by different agents. Specimens were divided into 3 groups of 12 specimens each. The root surfaces were root planed with a Gracey 7/8 curette, an EMS or an Amdent piezo-electric scaler and treated with saline, citric acid, tetracycline hydrochloride or EDTA to produce different surface textures. They were then cultured with fibroblasts for 72 h and examined by scanning electron microscopy. There was a significantly greater number of fibroblasts attached to specimens treated with citric acid, tetracycline and EDTA than to those root planed only. Furthermore, fibroblasts were more likely to attach to rough-surfaced than to smooth-surfaced specimens.

Key Words: fibroblasts, scaling, etching, SEM.

INTRODUCTION

The ultimate goal of periodontal therapy is the regeneration of periodontal support lost due to periodontitis. On the basis of the work by Urist (1) who described the potential of intramuscular demineralized dentin implants to induce new bone formation, Register (2) and Register and Burdick (3) reported enhancing the new attachment by acid. One of the most widely used...
adjuncts is citric acid, which primarily removes the smear layer (4). Other agents such as tetracycline (5) and EDTA (6) have been reported to favor new periodontal attachment. This process renders the root biologically compatible to the adjacent tissues.

Prior to any periodontal therapy, cementum should be removed from the root surface (7). However, Nyman et al. (8) reported that cementum removal is not a pre-requisite for new attachment. This inconsistency may be due to the inadequate removal of endotoxins (9).

Cogen et al. (10) observed that fibroblasts attached normally to instrumented periodontally diseased root surfaces, whereas little or no attachment was seen on uninstrumented root surfaces in vitro. In contrast, Adelson et al. (11) could not confirm that hypothesis. According to Lowenberg et al. (12), demineralization of the root surface may provide a more favorable substrate for enhanced and stronger cell attachment.

The purpose of this investigation was to investigate the correlation between the roughness of periodontally involved root surfaces and the ability of cultured fibroblasts to attach to these root surfaces treated with different scaling and etching procedures.

MATERIAL AND METHODS

Eighteen periodontally-diseased teeth, with the following criteria, were selected for use in this study: 1) no history of scaling or root planing in the previous 6 months, 2) proximal attachment loss of 5 mm or more, 3) no history of acute pain or swelling, 4) absence of caries. The teeth were individually placed in sterile, capped tubes containing saline and were processed immediately after extraction.

The teeth were cleaned of blood, saliva and irrigating solutions with a soft bristle toothbrush and deionized water. Only the diseased part of the root surface was used in this study. The anatomical crown was removed with a water cooled high speed bur. Each root surface was divided into two equal parts, one half of the specimen (experimental) was scaled with either hand instruments or one of the piezo-electric scalers. The other half was not instrumented and served as control. The teeth were randomly divided into three groups: Group 1: the experimental root surfaces were treated with a Gracey 7/8 curette (Hu-Friedy, Chicago, IL, USA) until a smooth root surface was obtained; Group 2: the root surfaces were treated with an EMS piezo-electric scaler (Nyon, Switzerland) using back-and-forth strokes until a smooth root surface was obtained. The power setting was medium; Group 3: the experimental surfaces were treated as in group 2, except an Amdent piezo-electric scaler (Nynäshamn, Sweden) was used. Test and control areas were marked with a notch to ascertain the coronal direction.

Following scaling, two blocks, each measuring 3 x 4 mm, were obtained from each tooth. Each specimen was placed in a 96-well microliter Honeycomplate, (Labsystem, Helsinki, Finland). A total of 36 specimens representing 36 test/control surfaces were registered according to the root treatment performed. Each group had a total of 12 specimens. Three specimens from each group (I, II and III) were treated with saline, saturated citric acid (pH 1.0), saturated tetracycline
hydrochloride (pH 1.8) or 8% EDTA dissolved in PBS solution (pH 7.3). After 3 min, the solutions were removed by suction and the specimens rinsed with distilled water.

All specimens were placed at the bottom of a single, sterile 3.5 x 8 mm Petri dish with the external surface of the root facing upward. A suspension of human gingival fibroblasts containing 1 x 10^5 cells/ml propagated prior to use according to the method of Aleo (9) was added to each Petri dish so that the fluid level of the suspension totally immersed the root sections. The medium used for propagating and culturing the cells with the root sections was Dulbecco's Modified Eagle medium supplemented with 10% fetal calf serum, 100 IU penicillin/ml, 0.1 mg streptomycin/ml, and 2 mM glutamine. Primary cell cultures used for the test were from the third to the eighth passage. The root sections were incubated with cells for 72 h at 37°C. After incubation, the specimens were washed in Hank's balanced salt solution.

Specimens for SEM were rinsed two times in PBS at 37°C and fixed in 0.1 M cacodylate buffer containing 2.5% glutaraldehyde at room temperature for 45 min. This was followed by post-fixation in 1% OSO 4 in 0.1 M cacodylate buffer for 15 min, rinsing in water and staining in 2% aqueous uranyl acetate for 30 min. The specimens were dehydrated through a graded series of ethanol (35 to 100%) and then critical point dried. Each specimen was coated with gold palladium and examined by scanning electron microscopy (JEOL, Tokyo, Japan), at 15 Kv with a tilt angle of 0 to 40°.

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**RESULTS**

*Controls (non-scaled specimens)*

The untreated root surfaces were relatively smooth and free of collagen fibers, with spots of attached calculus. The root surfaces were not affected by saline, citric acid or EDTA (Figure 1).

*Gracey 7/8 scaled surfaces*

The smooth planed surfaces showed fibroblast clusters mainly in the notched areas (Figure 2). The etched or chelated specimens had a rougher surface with scattered fibroblasts. The surface was covered with a material suggestive of organic debris with occasional exposure of dentinal tubules (Figure 3).

*Amdent treated root surfaces*

The ultrasonic treated root surfaces were smooth, well planed but with no migration of fibroblasts. One specimen of the same group displayed a fibrous network resembling collagen fibers (Figure 4). When the roots were etched or chelated there was a significant increase in the number of attached fibroblasts. Most of the adherent cells were flat with prominent microvilli and filopodia forming a multilayer on the specimen surface (Figure 5). The root surface was not visible between the multiple layers of fibroblasts (Figure 6).
Piezon treated root surfaces

The appearance of the root surfaces were similar to the Amdent treated and etched or chelated root surfaces.

Figure 1. SEM photomicrograph of untreated root surface. Spots of attached calculus are visible (arrow). (Original magnification, 500X).

Figure 2. SEM photomicrograph of root surface scaled with a Gracey 7/8 curette. Presence of cell inhabiting crack areas (arrow). (Original magnification, 350X).
Figure 3. SEM photomicrograph of root surface scaled with a Gracey 7/8 curette and etched with EDTA. Fibroblast attached to the etched surface (arrow). (Original magnification, 1500X).

Figure 4. SEM photomicrograph of root surface ultrasonically treated with Amdent. Note the presence of a fibrous network (arrow) and the absence of cells. (Original magnification, 500X).

Figure 5. SEM photomicrograph ultrasonically scaled with Amdent and etched with tetracycline hydrochloride for 3 min. Note the well-spread presence of cells (arrow). (Original magnification, 200X).

Figure 6. SEM photomicrograph ultrasonically scaled with Amdent and etched with citric acid for 3 minutes. Note the spreading of cells (arrow) completely masking the root surfaces.
DISCUSSION

Several *in vitro* studies have suggested that root surface modifications by tetracycline (13) or citric acid (14) could improve the attachment and spreading of periodontal fibroblasts. Fibroblast attachment is a common method used to evaluate the biocompatibility of root surfaces (15).

This study was designed to investigate the correlation between the roughness of periodontally involved root surfaces and the ability of cultured fibroblasts to attach to them. Acid treatment of periodontally diseased human teeth has given both successful and unsuccessful results. The observations in this study confirm and extend previous work.

More cells were attached to demineralized surfaces with no difference between the various demineralizing agents in enhancing cell attachment. This suggests that cell attachment to the demineralized root can be enhanced by the detoxification of the root and also indicates that root planing removed substances that inhibited or prevented fibroblasts. Lucas et al. (16) suggested that endotoxins from periodontal pathogens penetrate the root surface and inhibit cellular attachment. In contrast, Fardal et al. (17) reported that periodontally diseased roots did not inhibit the initial attachment *in vitro*.

In this study, fibroblasts did not attach to periodontally involved roots; nor did they attach to ultrasonically treated root surfaces. This study suggests that the biochemical modifications of the dentinal tubules induced by conditioning with citric acid, tetracycline hydrochloride or EDTA are responsible for an increase in fibroblast attachment. These modifications could be either a direct consequence of root conditioning by the exposure of some of the extracellular matrix constituents acting on the attachment mechanism of fibroblasts or an indirect effect by the increased fixation on the demineralized root surface of biochemical factors.

A recent study by Vanheusden et al. (18) also confirmed that dentin conditioning by citric acid or by mynocyycline enhanced the reattachment of human gingival epithelial cells to the root, thus favoring faster periodontal healing; therefore influencing the behavior of periodontal fibroblasts, improving their attachment and spreading.

Cells binding to both dentinal and cementum surfaces and root planning alone permit diseased roots to interact with fibroblasts as well as normal roots. Fardal et al. (17) observed that periodontally diseased teeth incubated with human gingival fibroblasts showed equal attachment regardless of whether they were instrumented, uninstrumented or non-diseased. They also observed attachment of fibroblasts to calculus. It appears that fibroblast attachment to periodontally diseased root surfaces is not uncommon. However, this study did not confirm this nor did the study by Gamel et al. (19) which demonstrated that cell adherence was significantly higher in the healthy control group when compared to periodontally diseased treated control, a finding that confirms that cultured human PDL fibroblasts prefer not to adhere to periodontally
diseased treated root surfaces. In this study, fibroblasts did not adhere to periodontally involved teeth, were low in number in root planed root surfaces and more abundant when the root was etched or chelated.

This study would suggest that the direct cytotoxic nature on nonmicrobial root surface irritants may play an important role in the overall nature of root surface toxicity. However, Gamal et al. (20) found that the application of tetracycline hydrochloride on the tooth surface had little effect on cell adherence except to change their morphology.

Based on the results of this study, periodontal disease alters the biocompatibility of the root surface and thus, potentially renders it less likely to achieve tissue attachment. It has not been determined if the conditioned root can be rendered as biocompatible as a healthy root surface.

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REFERENCES


