Effect of Citric Acid Treatment on Initial Attachment of Human Periodontal Ligament Fibroblast-like Cells (in vitro)

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Abstract: The purpose of this study was to assess the initial attachment of human periodontal ligament fibroblast-like cells to periodontally affected root surfaces after demineralization with citric acid (pH 1, 3 min., 37°C), in vitro. Fourteen roots were obtained from 12 patients whose teeth were freshly extracted. The roots were prepared so that a comparison could be made between the initial attachments to citric acid demineralized (test group) and non–acid–treated (control group) instrumented diseased cementum (scaled and root planed), dentin and nondiseased cementum. The adherence of the fibroblast-like cells was determined by LM using an ocular grid system and orientation was evaluated by SEM. Results indicated that initial attachment was observed favorably in citric acid treated groups but with no significant difference.

Key words: Human periodontal ligament fibroblast-like cell, Initial attachment, Citric acid, Root surface
Introduction

Creation of new connective tissue attachment to diseased root surfaces has become one of the primary goals of periodontal therapy. Consequently, an important aspect of periodontal treatment is the removal of injurious factors from the root surfaces. To accomplish this goal in clinical entity, various mechanical methods have been suggested, from washing of root surface to total removal of cementum.

Another approach to detoxifying root surfaces other than mechanical removal of cementum involves use of acids. Recent studies, mainly in dogs, have suggested that partial demineralization of planed root surfaces with citric acid during periodontal surgery enhances formation of new cementum. In vitro studies have also indicated that gingival fibroblasts migrate, attach and become oriented to partially demineralized root surfaces to a significantly greater extent as compared to nontreated mineralized root surfaces.

Other in vitro studies have suggested that dentin collagen exposed as a result of surface demineralization of roots may make the root surface more hospitable for cell attachment and growth. On the other hand, recent studies related to the formation of a new connective tissue attachment have recommended a role for gingival fibroblasts but the vast majority of evidence suggests that cells from the periodontal ligament are the only ones that have the capacity to regenerate the periodontium.

In this study, our aim was to investigate the initial attachment of human periodontal ligament fibroblast-like cells to citric acid demineralized (test group) and non-acid-treated (control group) instrumented diseased cementum (scaled and root planed), dentin and nondiseased cementum.

Materials and Methods

1. Preparation of root surfaces and experimental procedure

A total of 14 caries free periodontally involved single rooted teeth with attachment loss of 6 mm or more, were obtained from patients aged 43 to 50 years old, with no history of systematic diseases, and had no antibiotic treatments and root preparations including prophylaxis, within 6 months prior to extraction. Following an atraumatic extraction, the crown was excised with a diamond disk slightly below the cemento-enamel junction. The root surfaces were cleaned of debris with a Gracey curette. Then, using a dissection microscope, the attachment level was identified and a 1 mm wide horizontal groove was cut with a diamond bur. Another groove was cut vertical to the first groove, dividing the diseased portion into the diseased cementum site and the dentin site. The third site, the nondiseased cementum site was the remaining area where the remnants of periodontal ligaments were carefully removed using a Gracey curette. The roots were then sectioned along the sagittal axis. Experiments were performed on one half and the other half was used as control (Fig. 1).

After total removal of cementum from the dentin site with diamond fissure burs, the diseased cementum site and dentin site were planed by 5 overlapping strokes. The pulpal root surfaces were thinned to 1 mm.

Experimental specimens of the test group were immersed in a saturated solution of citric acid, pH 1, for 3 minutes and rinsed with 0.9% saline solution (3 times, total of 6 min).

2. Cell culture and cell attachment

Human periodontal ligament fibroblast-like cells were obtained from orthodontically extracted teeth and used at subculture 5.

The fragments from each group were placed in separate culture wells (6-well Linbro plate) and incubated with $2.8 \times 10^5$ cells in 1 ml of Dulbecco's modified Eagles medium containing 100 μg/ml Pen-
icillin G., 50 μg/ml Gentamicin and 0.3 μg/ml Fungizone in a humidified atmosphere of 95% air and 5% CO₂ for 1 hour at 37°C. Unattached cells were resuspended every 15 minutes during the incubation period. After 1 hour, the root segments were transferred to fresh wells, washed twice with fresh culture medium and stained with methylene blue.

Cell attachment on the 3 sites of each root fragment were analyzed at magnification ×100 by light microscope and photographed at magnification ×25. The number of cells attached per unit area were determined by the use of an ocular grid (10×10 mm) system at magnification ×100. Quantitative results for each root surface of the fragment were obtained from the counts of 4 different randomly chosen areas.

Four fragments, 2 from the test and 2 from the control groups were processed for scanning electron microscopic observations. They were washed in 0.2-M, 300-mOs sucrose-containing cacodylate buffer pH 7.4 at 37°C and then fixed overnight in 2% gluteraldehyde in 0.2 M cacodylate buffer, post-fixed in 1% OSO₄ for 1 hour, dehydrated in a series of ethanol, critical point dried and coated with platinum. The specimens were viewed using a Leitz AMR 1000 Scanning Electron Microscope (Leitz Co, Germany) and were photographed at magnification ×5000.

3. Statistical Analysis

Statistical analysis were made by paired comparison t-test. A level of P ≤ 0.01 was accepted for statistical significance.

Table 1 Comparison of the mean number of cells on diseased cementum (DC), Dentin (D) and nondiseased cementum (HC) sites of test and control groups at 1 hour.

<table>
<thead>
<tr>
<th></th>
<th>(test group)</th>
<th>(control group)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>X+SX</td>
<td>X+SX</td>
</tr>
<tr>
<td>〈DC〉</td>
<td>145.3+17.8 NS</td>
<td>124.4+17.9 NS</td>
</tr>
<tr>
<td>〈D〉</td>
<td>260.4+33.9 NS</td>
<td>228.6+28.9 NS</td>
</tr>
<tr>
<td>〈HC〉</td>
<td>255.4+33.8 NS</td>
<td>239.6+28.4 NS</td>
</tr>
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X...mean
SX...standard error
*...p ≤ 0.01
Fig. 2 Electron micrographs of fibroblast-like cells on diseased cementum (a), dentin (b) and nondiseased cementum (c) in control group (×5000). The cells which adhered to the 3 types of surfaces showed variations in shape and size. Finger-like projections were observed on the cell surfaces and these organelles seemed to mediate the adhesion of the cells on the root surfaces.

**Results**

1. **Light microscopic observations**

When roots were examined at 1 hour, cells were found to be attached to all 3 surfaces in test and control groups. Cells were easily visible and found not to be only attached, but in the process of spreading.

2. **Quantitative results of light microscopic observations (Table 1)**

In the comparison of the mean value of initially adhered cells in both test and control groups, the number of cells attached to dentin and nondiseased cementum sites were significantly greater than the number of cells attached to instrumented diseased cementum sites (P ≤ 0.01).

In the comparison of the number of fibroblast-like cells attached to instrumented diseased cementum, dentin and nondiseased cementum sites of test and control groups, there were no significant differences in the number of cells adhered to the 3 types of root surfaces at 1 hour.

3. **Scanning electron microscopic observations (Fig. 2, 3)**

There were no apparent differences in the morphology of cells attached to instrumented diseased, dentin and nondiseased cementum sites in test and control groups. The cells which adhered to the 3 types of surfaces showed variations in shape and size, from slightly rounded to a slightly flattened appearance.

Furthermore, in both groups, finger-like projections and filopedia were prominent on most of the cell surfaces, and it appeared that the adhesion of the cells to the root surfaces was mediated by these organelles. The attachment of the cells thus seemed to be in very close contact with the root surface.
Fig. 3  Electron micrographs of fibroblast-like cells on diseased cementum (a), dentin (b) and nondiseased cementum (c) in test group (×5000). The cells which adhered to the 3 types of surfaces showed variations in shape and size from slightly rounded to a slightly flattened appearance. Finger-like projections were observed on cell surfaces and adhesion of cells seemed to be in very close contact.

Discussion

This study was designed to evaluate the attachment of human periodontal ligament fibroblast-like cells to different root surfaces treated and nontreated with citric acid.

Results of the study showed that citric acid treatment of cementum and dentin surfaces promoted cell attachment to a certain degree, although this was not statistically significant. Consequently, the present observations could not statistically support the in vitro findings that fibroblasts attach significantly better to demineralized root surfaces\(^{10-12}\). On the other hand, a number of studies have demonstrated that citric acid treated root surfaces do not promote cell attachment and growth\(^{2-9}\). The results of our study are consistent with the above mentioned findings.

Histological in vivo studies have implied that citric acid, due to its cytotoxic effects, impairs wound healing by damaging the adjacent tissues\(^{17,18}\). Due to the differences in applying citric acid to root surfaces in in vivo situations, direct contact of the soft tissues with citric acid may occur. In addition, when evaluating the clinical trials presented in literature, no clinical advantages were found from the use of citric acid which happen to support our findings\(^{19-21}\). It must be emphasized that it was from this background that unlike some of the experiments quoted in the literature\(^ {3,12,22}\), we have tried to keep conditions of root surfaces as close to the clinical conditions as an in vitro study would permit. Therefore, in the present study, fragments were not sterilized and cells were added as quickly as possible. Also, the root planing which we performed was similar to the root planing one could make during an operation.

Recently, a number of investigators have tried to correlate the degree of fibroblast attachment to the
partial or total removal of cementum. Some of these authors maintain that fibroblast attachment will only occur following root planing of diseased surfaces\textsuperscript{[1–14]}, whereas others have stated that fibroblast attachment occurs independently of any removal of diseased cementum\textsuperscript{[22,23]}.

Finally, results have shown that the number of fibroblast attachments to dentin and nondiseased cementum were equal in both test and control groups. This finding also indicated that total removal of cementum may be unnecessary. However, the lesser number of fibroblasts on planed but diseased cementum reminds us of the importance of careful root planing, or by other means converting a diseased cementum into a healthy cementum. However, further indications are necessary before conclusions can be drawn regarding the pathology of the cementum.

Conclusions

1. Periodontal ligament fibroblast–like cells initially attached to all diseased and healthy root surfaces regardless of citric acid treatments.

2. Cell attachment to citric acid demineralized root surfaces was greater in number than non –demineralized surfaces, but this was not significant.

3. Cell attachment to dentin and non–diseased cementum was significantly greater compared to diseased cementum in both citric acid treated and non–treated groups.

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References


