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

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Abstract: This in vitro study was undertaken to evaluate the attachment of human periodontal ligament fibroblast-like cells to root surfaces treated with tetracycline HCl (100 mg/ml, pH 1.9, 5 min, 37°C). 24 root fragments were obtained from 12 patients. These roots were prepared so that a comparison could be made between the initial attachments to instrumented diseased cementum (scaled and root planed), dentin and nondiseased cementum of tetracycline HCl treated (test) and nontreated (control) root surfaces. Both test and control root fragments were incubated with human periodontal ligament fibroblast-like cells for 1 hour. The adherence of the fibroblast-like cells was determined by Light Microscope using an ocular grid system and orientation was evaluated by Scanning Electron Microscope. Results indicated that initial cell attachment was significantly enhanced on dentin surfaces of tetracycline treated root fragments (p<0.05).

Key words: fibroblast-like cell, initial attachment, tetracycline, root surface

A number of studies suggest that endotoxin from periodontal pathogens bound to the root and damage both cellular functions and attachment apparatus1–3). Removal of cytotoxic factors by various detoxification techniques will help attain the goal of a biologically acceptable root surface other than by mechanical means. An approach to detoxifying diseased root surfaces other than by mechanical removal involves use of chemicals and biological agents such as acids and attachment factors3,4).

Studies have shown that tetracycline HCl not only effectively demineralizes root surfaces, but acts favorably toward fibroblastic attachment by inhibiting collagenase activities and bone resorption. Its slow release may also act effectively in maintaining antibacterial activities5–7).

From literature, it could be seen that the interactions between root surfaces and connective tissues are not completely understood and contradictory knowledges on initial attachment of fibroblastic-cells on conditioned root surfaces co-exist8–13).

Consequently, the purpose of this in vitro study was to evaluate the initial attachment of human periodontal ligament fibroblast-like cells to tetracycline HCl treated (test group) and non-acid-treated (control) 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 single rooted periodontally involved carious free teeth with attachment loss of 6 mm and more, were obtained from patients aged 43 to 50 years old, having no systematic diseases and no history of antibiotic treatments 6
months prior to tooth extraction. Experimental teeth had not received any type of root preparations including prophylaxis within 6 months prior to tooth extraction. Following an atraumatic extraction, the crown was excised slightly below the cemento-enamel junction. The root surfaces were cleaned of debris and the attachment level was identified by a 1 mm wide horizontal groove. 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. The roots were then sectioned along the sagittal axis. Experiments were performed on one half and the other half was used as control.

After total removal of cementum from the dentin site, the diseased cementum site and the dentin site were planed by 5 overlapping strokes. The pulpal root surfaces were thinned to 1 mm. Root segments (diseased cementum site, dentin site and nondiseased cementum site) were immersed in an aqueous solution of tetracycline HCl of 100 mg/ml. pH 1.9, for 5 minutes and rinsed with 0.9% saline solution (3 times, total of 6 minutes).

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 of each group were placed in separate culture wells (6-well Linbo plate) and incubated with $2.8 \times 10^5$ cells in 1 ml of Dulbecco’s modified Eagles medium in a humidified atmosphere of 95% air and 5% CO$_2$ 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 with an Olympus Vanox light microscope and photographed at magnification $\times 25$. The number of cells attached per unit area was determined by an ocular grid ($10 \times 10$) system at magnification $\times 100$. Quantitative results for each root surface were obtained from the counts of 4 randomly chosen areas.

Four fragments, 2 from the test and 2 from the control groups were processed for scanning electron microscopic observations. The cultures were rinsed in sucrose containing cacodylate buffer, fixed in 0.1 M cacodylate buffer containing 2% gluteraldehyde, post-fixed in 1% Osmium tetroxide for 1 hour, dehydrated in a series of ethanol, critical point dried and coated with platinum. The specimens were examined using a Leitz AMR 1000 Scanning Electron Microscope (Leitz Co., Germany) and were photographed at magnification $\times 5000$.

3. Statistical analysis

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

Results

1. Light microscopic observations

When roots were examined at 1 hour, cells were observed to be in very close adherence with the root surface of both test and control group.

Fig. 1 Schematic drawing demonstrating the preparation of a root fragment.
2. Quantitative results of light microscopic observations (Table 1)

Statistical comparisons of initially attached cells to root specimens of test and control groups are shown in Table 1. In control group, the number of cells adhered to dentin and nondiseased cementum sites were statistically greater than the number of cells adhered to instrumented diseased cementum sites ($p<0.01$). In test group, cells adhered in significantly greater number of dentin sites as compared to diseased and nondiseased sites ($p<0.01$). Cell adherence to diseased cementum sites was significantly less as compared to dentin and nondiseased cementum sites ($p<0.01$). In the inter-group comparison of initial cell adherence, cells were adhered to dentin sites of test group in significantly greater number ($p<0.05$).

3. Scanning electron microscopic observations

In the test group, the cleaning and demineralizing action of tetracycline HCl prepared a relatively flat surface with numerous widenings of the dentinal tubules. Fibroblast-like cells appeared to be tightly attached to the conditioned root specimens via their cytoplasmic extensions (Fig. 2). On the contrary, the root surface of the control specimens appeared to be comparatively rough and irregular in texture. The adherence of the fibroblast-like cells to the control specimens appeared to be less intimate as compared to the adherence to the test specimens (Fig. 3).

Concerning the morphology of the fibroblast-like cells, no apparent differences were observed between the cells attached to test and control groups. Adhered cells exhibited variations in size, shape and surface topography (Figs. 2 and 3).

The plasma membrane of the cells was covered with numerous microvilli and filopedia. In some of the attached cells, the cytoplasmic process was prominent, and it appeared that the adherence of the cells to the root specimens were mediated by these cytoplasmic extensions (Fig. 2).

Discussion

This in vitro study was carried out to determine the initial attachment of human periodontal ligament fibroblast-like cells to tetracycline HCl treated (test) and non-acid-treated (control) root surfaces.

The light and scanning electron microscopic observations indicated that the number and orientation of initially attached cells on dentin sites of test and control group emphasizes the importance of root surface characteristics, referring to the exposed dentinal tubules possibly providing a more hospitable environment for cell attachment. This result corroborates with the assumption of Pitaru et al.10, Sato et al.13 and Boyko et al.14

However, sterilized root specimens stored from days to weeks had been utilized in most of the past related in vitro studies1,8,11, and no quantification on number of cell attachment have been made.

In this study, unsterilized root specimens were used immediately after extraction and following 5 overlapping root planing strokes. This experimental schedule together with the short incubation period was designed to keep test conditions as close as possible to clinical situations.

The concentration of tetracycline used in this study was chosen based on the results of past investigations. Authors have demonstrated that maximal absorption of tetracycline enhanced fibroblast attachment and growth is favorably attained when dentin surfaces are conditioned with concentrations of 100 mg/ml tetracycline HCl4–7.

Sato et al.13 in an in vitro study on the characterization of root dentin surface after root treatments reported that tetracycline treatment of
root surfaces proceeding scaling and rootplaning enhances more connective tissue regeneration, as compared to manual scaling and rootplaning.

Wikesjo et al. demonstrated the antimicrobial activity and the substantiality of tetracycline HCl to dentin in their in vitro study and reported that tetracycline HCl inhibits collagenase activity and in vitro bone resorption. Hence, preparation of
root surfaces with tetracycline will promote attachment and growth of fibroblast with its antimicrobial and other anti-enzymatic properties. This provides a key to regeneration of connective tissue attachment in periodontal lesions.

The scanning electron microscopic observations obtained, were consistent with past studies which have shown that tetracycline treatment of root surfaces removes substances which inhibit or prevent fibroblastic attachment and synthesis activities4-7). The cells appeared to attach firmly to tetracycline treated root specimens with their cytoplasmic extensions. Furthermore, these projections are most likely a functional adaptation of the cells to the cell surfaces. As Wikesjo7) and Lindskog15) have mentioned, the vesicles may represent secretory vesicles containing hydrolytic enzymes, while pit formation of the cell may be the result of endocytic activity.

On the other hand, our findings were inconsistent with the results of our previous study121 and the study by Fardal et al.11) on citric acid, and the study by Lowenberg et al.9) on EDTA. No significant difference in gingival fibroblast cell attachment on instrumented, non instrumented or chemically treated root surface was observed. Furthermore, in an experimental study in dogs, Nyman et al.16) have reported that polishing of root surfaces is effective in eliminating cementum endotoxins. These observations may be supported by the findings of Ohtake17), Suda18), Mitsuzaki et al.19) and Nakib et al.20), indicating that the bacterial endotoxins adhere to the surface of the roots rather than penetrating into it.

Clinically, Harris21) has demineralized root surfaces with tetracycline HCl in his partial thickness double pedicle graft technique, probably advocating the merits of root surface conditioning. Similarly, Miller et al.22) and Corn et al.23,24) advocates that graft procedure be proceeded by root demineralization. On the other hand, Ibott et al.25) does not conclude root demineralization a justifiable procedure.

In view of these contradictory discussion, it may be suggested that biologic preparations of periodontally diseased root surfaces should be further investigated.

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References

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