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Original Article

A comparative analysis of root surface biomodification with ethylene diamine tetra acetic acid and tetracycline hydrochloride: An in vitro scanning electron microscopic study

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ABSTRACT

Background: The outcome of periodontal regenerative therapy depends upon the of the biocompatibility root surfaces to the regenerating periodontal tissues. This in vitro scanning electron microscopic (SEM) study was designed to evaluate and compare the demineralizing efficacy of ethylene diamine tetra acetic acid (EDTA), with that of tetracycline hydrochloride applied on to the mechanically treated root surfaces of periodontally involved tooth.

Methods: Forty specimens were prepared from teeth extracted due to advanced periodontal disease and divided into two groups. The study group was treated with an EDTA solution (pH 7.4) and the control group was treated with a tetracycline hydrochloride solution (pH 1.8). The photomicrographs obtained were assessed for presence of smear layer, number of exposed dentinal tubules, area occupied by tubule orifices along with intertubular surface appearance. The results thus obtained were analyzed statistically.

Results: Both EDTA and tetracycline were effective in removing the smear layer and the exposure of the number of dentinal tubules. The diameters of the tubules and thereby the surface area occupied by the tubule orifices in the EDTA treated group were significantly greater than the tetracycline HCL treated group ($p < 0.05$).

Conclusion: The EDTA produced better effects than tetracycline by providing more demineralized area and collagen exposure at a neutral pH.

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Introduction

The final desired outcome of periodontal therapy is the arrest of progressive periodontal disease followed by the predictable regeneration of damaged periodontal tissues.

Periodontal diseases expose the root surfaces of teeth to the contents of the periodontal pocket which are contaminated with periodontal pathogens and endotoxins thereby triggering inflammatory reactions. Consequently, the root surfaces undergo changes in their physical, chemical and immunochemical properties as well as in their histologic appearances. All these changes alter the root surfaces, which become toxic and non-biocompatible to cells associated with periodontal healing and regeneration.[1]

Root biomodification comprises of the alterations produced on the root surface by means of mechanical, chemical or combination of the two procedures to promote favorable root surface characteristics, as the decontamination of a periodontitis affected root surface by mechanotherapy alone is not possible.[2] A smear layer will cover the mechanically treated root surfaces, which contain remnants of calculus, contaminated cementum, subgingival plaque and the associated microorganisms. This smear layer impairs cell reattachment and can serve as a reservoir for microbial growth and thereby will inhibit new attachment.[3]

Demineralization treatment on affected root surface induces cementogenesis and enhances connective tissue attachment by an improved fibrin linkage and the periodontal ligament fibroblast migration. Demineralized dentin surfaces have a greater capacity to bind fibronectin than the surfaces which are not demineralized due to increased number of fibronectin binding sites on the exposed collagen matrix.[4] The tufting of dentin or cementum matrix following demineralization will lead to an increase in the total surface area of the collagen and thereby the total number of binding sites for various proteins or growth factors which play an important role in wound healing and regeneration.[5]

The root treatment protocol earlier centered mainly on application of acids like citric acid and tetracycline hydrochloride.[6] The use of low pH acting agents has been challenged, as their use might be detrimental to the surrounding vital periodontal tissues and may interfere with healing after the regenerative procedures.[7] Studies have shown that ethylene diamine tetra acetic acid (EDTA), a chelating agent which can act at neutral pH is preferable over the other acid solutions because it preserves the integrity of surrounding periodontal tissues along with root surface demineralization and smear layer removal.[8]

With this background, a scanning electron microscopic (SEM) study was designed to evaluate and morphometrically analyze the demineralizing efficacy of an EDTA solution at lower concentration (15% and pH 7.4) with that of a tetracycline hydrochloride solution (pH 1.8), on root surfaces of periodontally involved tooth.

Material and methods

Twenty single rooted human teeth from patients with advanced periodontal disease were selected for use to simulate the clinical use of root demineralizing agents and to account for the possible hypermineralization of diseased root surface.

The criteria for inclusion were unsaveable, single rooted teeth which were periodontally compromised with clinical attachment loss of more than 5 mm. The general exclusion criteria were;

1. Carious teeth with periapical lesions
2. Root caries or erosions
3. Cervical restorations
4. Presence of enamel pearls or any cemental abnormalities
5. Recent periodontal therapy

The root surfaces were thoroughly planed and flattened using a fine diamond finishing bur in high speed hand piece under continuous water coolant to remove the diseased cementum and were re-examined under magnifying lenses to ensure complete calculus removal.

To obtain the experimental surface, the roots were then sectioned longitudinally into two equal halves which yielded a total number of forty specimens. Each of the specimens from the same root were assigned to different treatment groups and numbered. The specimens were cut into slabs of about $6 \times 6 \times 2$ mm in size. The slabs were then preserved in a mixture of anhydrous glycerol/absolute alcohol 1:1 by volume so as to preserve the tissues at the cellular organelle level and without denaturing the dentinal matrix, until the time of treatment as recommended by Wen et al.[9]

The prepared solutions were then applied with the help of applicator tips manually by a single operator to maintain the uniformity in the application pressure. Group I was treated with 15% EDTA solution in phosphate buffer solution (pH 7.4) and Group II were treated with 100 mg/ml tetracycline hydrochloride (pH 1.8) for 3 min.

The samples were prepared for SEM by first rinsing them in buffer solution and then washing with distilled water. They were dehydrated with a graded series of aqueous ethanol solution ranging from 70% to 90% and by air drying using a blower and then by keeping in desiccator jars containing silica gel overnight.

The specimens were then mounted on conductive carbon tape pasted on the standard specimen mounting SEM stubs. As the biological specimens have very low density and will lead to the absorption of electron beam resulting in low resolution, sputter coating was done with a thin layer of gold powder for 1 min using a Polaron SC7610 sputter coating device.

A Scanning Electron Microscope, Philips XL-20 manufactured by Philips Co. Netherlands with a standard computerized control system was used for the study. The electron beam parameters were kept constant while analyzing the samples and the SEM beam was maintained at a 0° tilt angle for achieving the standardization. The microscope accelerating voltage was kept between 15 kV and 20 kV for all the specimens at high vacuum and the photomicrographs were obtained at 1000× and 2000× magnifications. The specimens were then mounted under scanning electron microscope for surface topographical analysis. The specimens under the scanning electron microscope were examined for: -