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The Effect of EDTA on the Attachment and Growth of Cultured Human Gingival Fibroblasts on Periodontitis-Affected Root Surface

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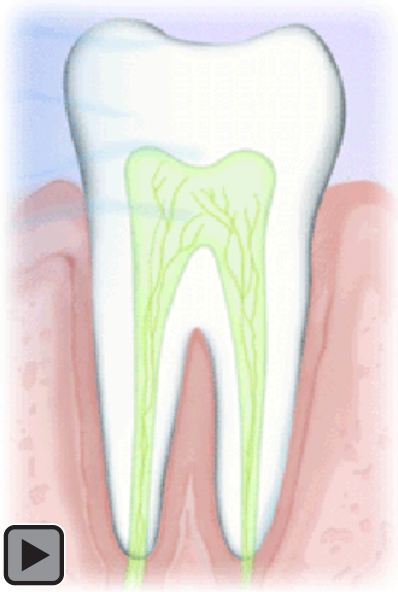
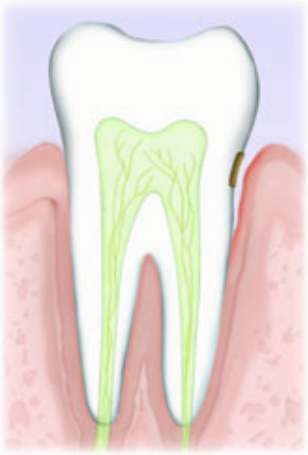


Abstract

The purpose of the present study was to analyze the ef fects of 5% and 24% EDTA on the attachment of gingival fibroblasts to periodontally diseased root surfaces. A flat root surface was created on human teeth that were extracted due to severe periodontitis. The teeth were etched with the following concentrations of etylediaminetetraacetic acid (EDTA) for two minutes: 5% (group I) and 24% (group II). Group III was soaked in saline and served as a control. The specimens and fibroblasts were incubated in a culture medium for 24 hours each day for one and two weeks and photographed using scanning electron microscopy. Each specimen was examined for the migration of cells into the etched and non-etched root surface. No fibroblasts could be detected on the saline groups. More fibroblasts could attach to the surface treated with 24% EDTA than with 5% EDTA. It was concluded that supersaturated EDTA at 24% enhances the attachment of gingival fibroblasts to the root surface.

Keywords: EDTA, periodontitis, gingival fibroblasts

Introduction



Periodontitis-affected root surfaces are hypermineralized[1,2] and contaminated with cytotoxic and other biologically active substances.[3] Such surfaces are not biocompatible with adjacent periodontal cells, the proliferation of which is pivotal for periodontal wound healing.[3] It is not possible to decontaminate a periodontitis-affected root surface completely by mechanical means alone.[4] Hand or ultrasonic scaling of root surface always produces a non-biocompatible smear layer.[5] Conventionally this smear layer has been removed with citric acid, a low pH etchant.[6] Recently, a supersaturated solution of etylediaminetetraacetic acid (EDTA) has been found to be as effective as low pH etchings with respect to smear layer removal.[7] EDTA has also been used in relatively low concentrations (1.5% and 3%) to remove the smear layer in cavity and root canal preparation.[8] Furthermore, cell attachment and periodontal healing has been shown to be promoted by EDTA etchings compared to low pH agents.[9]

A recent investigation suggested that conditioning the dentin with acids will stimulate the attachment of fibroblasts.[10] Another study contrasted with that finding in that root conditioning had little effects on the attachment of fibroblasts.[11] Consequently, the purpose of the present study was to explore the effects of two different concentrations of EDTA on the attachment of gingival fibroblasts to periodontally diseased root surfaces.

Materials and Methods

Eighteen human teeth, extracted due to severe periodontitis, were used in the present study. Following extraction, the border between the healthy and diseased root was marked with a small dental bur. Only the diseased part of the root was used in this study. Each root surface was thoroughly scaled and root planed with an 11/12 Gracey curette until a smooth, glass-like surface was obtained. The roots were rinsed with distilled water and cut horizontally into two equal pieces under constant irrigation. Thus a total of thirty-six specimens were used in this study. The specimens were randomly divided into three groups, each containing twelve specimens. The specimens in groups I and II were respectively etched for two minutes with 5% EDTA and 24% EDTA. The specimens in group III served as a control and were treated with saline for two minutes.

Cultured fibroblasts

To test whether the root surfaces were suitable for connective tissue reattachment or in vivo regeneration, gingival fibroblasts derived from human primary tissue of patients suffering from periodontal disease were used. Biopsies from the surrounding gingiva were collected, minced, and transplanted to 25 cm^2 tissue culture flasks (Falcon, No 3012, USA) on plasma clots (10 pieces in each flask). After 30 minutes, 5ml of Modified Eagle's Medium (MEM) [Flow, Cat 14-100-54 UK], supplemented with 100/ul of penicillin and 100/ul of streptomycin (SIGMA Ct n° P 3539), 50/ul L-glutamine (Flow, Cat n° 16-801-49,UK), and 10% fetal calf serum (FCS) was added and the transplants were left for outgrowth.

Cell culture

Primary cell cultures and cell lines were cultivated in Modified Eagle's Medium (MEM) supplemented with Penicillin/Streptomycin, L-glutamine, and 10% fetal calf serum (FCS) at 37° C in 5% CO_2 with 0.2% EDTA (Kebob-Lab, Sweden) in phosphate-buffered saline (PBS) buffer and for 1 min with 0.1% crude trypsin (Flow, UK) in phosphate buffer saline (PBS). The contents of each flask were suspended in fresh growth medium and transferred to three new flasks. Primary cell cultures used for the test were in the third to eight

passage.

Cell adhesion test

Each specimen scaled and treated with each of the etching solutions was soaked in PBS (pH 7.3) twice after which 200/µl of the cultures were added (equal to 200,000 cells/well and specimens) and left for 24 hours. Two point five percent (2.5%) glutaraldehyde was added after presoaked with 200/µl X 2. The fixation solution remained for a week.

Cell adherence and migration

The above mentioned fibroblasts were left in solution for 7 days in order to attain confluence when two pieces from the same root surface with dif ferent treatment were placed radially along the periphery of each petri dish (Flacon Denmark 25 cm in diameter). In total six pieces were placed in each petri dish. Pieces in sequence of two were harvested after one and after two weeks of culture together. The harvested pieces were fixed in 2.5% glutaraldehyde in PBS for one week before preparation for scanning electron microscopy. All specimens were photographed after 24 hours, one and two weeks respectively in culture before fixation.

Scanning Electron Microscopy

All specimens were prepared for scanning electron microscopy in the following manner: Dehydration was performed in a graded series of ethanol with 100% acetone as a final step. The root sections were mounted on aluminum stubs sputter coated[12] and examined in a scanning electron microscope[12] operated at 15kv and a tilt angle

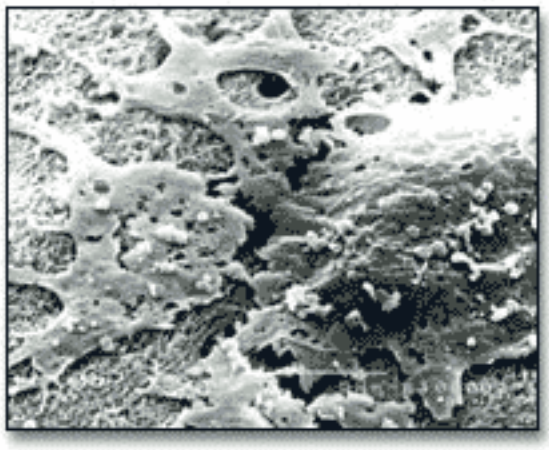


Figure 2.
24-hour seeded fibroblasts grown on treated root with 5% EDTA. Presence of cells with holes in cytoplasm (dead appearance). (Original magnification X 5000)

Results

Controls

The untreated diseased root surfaces did not show any cells attached to the root cementum at 24 hours, one and two weeks (Figure 1). A surface smear layer covered the root surface.

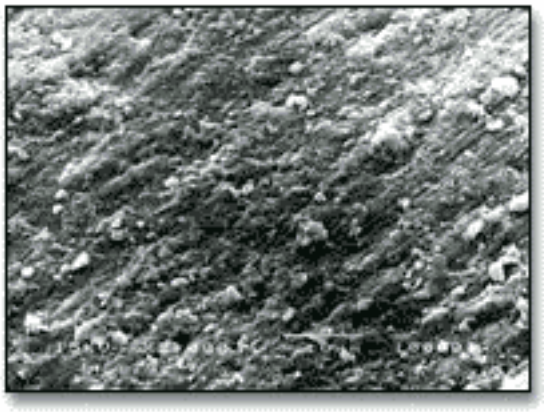


Figure 1.
Untreated root surface (control). The surface has an amorphous appearance with no presence of fibroblasts. (Original magnification X 2000)

Figure 3.
24-hour seeded fibroblasts grown on treated root surface with 25% EDTA. The root surface is seen with a confluent monolayer of cells well attached to it. (Original magnification X 3500)

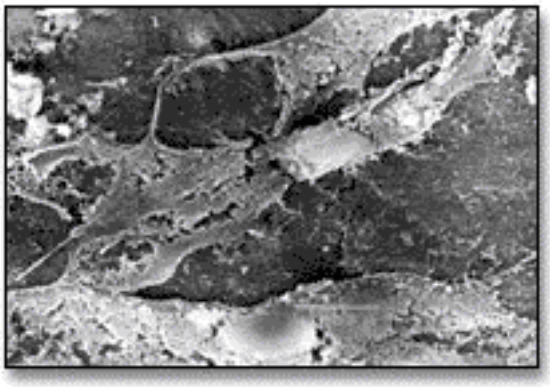


Figure 4.
One week seeded fibroblasts grown on treated root with 5% EDTA. Presence of cells appearing dry. (Original magnification X 3500)

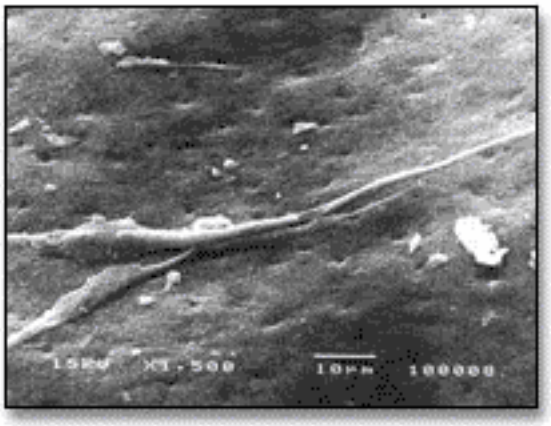


Figure 5.
One week seeded fibroblasts grown on treated root surface with 25% EDTA. Presence of well spread spindle shaped fibroblasts. (Original magnification X 1500)

Figure 6.
Two weeks seeded fibroblasts grown on treated root with 5% EDTA. A single fibroblast is present with philopodia indicating cell shrinkage. (Original magnification X 5500)

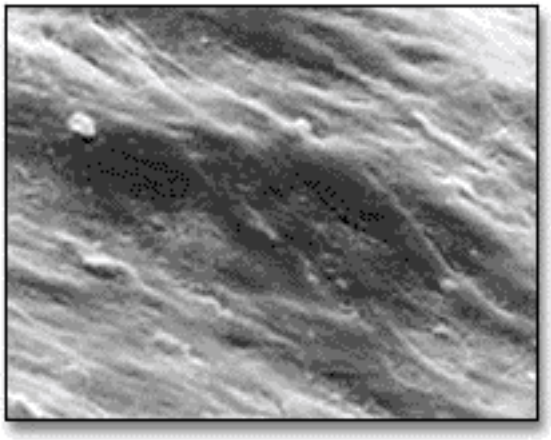


Figure 7.
Two weeks seeded fibroblasts grown on treated root with 25% EDTA. The surface is populated with well spread fibroblasts manifesting surface villous processes. (Original magnification X 1500)

24 hours seeded fibroblasts

Cell attachment and morphology were markedly altered when fibroblasts were grown on root surfaces treated with 5% EDTA concentration (Figure 2). The cells appeared to be retracted from the root surface. The cytoplasm of these cells showed holes giving them a dead appearance. An increase in cellular debris was noted. In contrast, cells grown on a root surface treated with 24% EDTA displayed a smooth surface and appeared to be tightly attached to the root surfaces (Figure 3). between 0 and 40 degrees.

Colonization of specimens after one week

Similar results were observed for cultured fibroblasts as in the 24 hours group. In the 5% EDT A group, cells had a rough appearance (Figure 4); while in the 24% EDTA group, cells have long filopodia and appeared smooth (Figure 5).

Colonization of specimens after two weeks

Concentrations of 5% EDTA and 24% EDTA did not adversely affect the morphology or attachment of human gingival fibroblasts to root surfaces. The 5% EDTA group displayed a typically flattened cell that appeared attached to the surface (Figure 6). However in the 24% EDT A group, the cells were flatter, more in numbers, and appeared to be tightly attached to the surface. (Figure 7)

Discussion

In periodontal therapy citric acid has been the most commonly used etching agent, but recent experimental studies have indicated that 24% EDTA may be the etchant of choice.[7] For several years, EDTA has been used in relatively low concentration to remove the smear layer in cavity and root canal preparation.[8]

The purpose of the present study was to explore the effects of 5% and 24% EDTA on the attachment of gingival fibroblasts in periodontally diseased root surfaces. A root surface treated with 24% EDTA was significantly more attractive to gingival fibroblasts. Root surfaces treated with 5% EDTA showed cells with a necrotic like appearance except in the two week period.

The present results regarding the lower EDTA concentration suggest the higher concentrations would enhance the smear-removing effect, thus, making it more biocompatible and attractive to gingival fibroblast.[3] The presence of adhering cells and tissues on the EDTA treated root surfaces in contrast to a complete absence of cells in the non-etched control teeth implies etching itself produced a root surface which facilitated adherence in vitro. These differences found in cell colonization between the test and control areas are in agreement with earlier findings where adherence and spreading on acid etched root surfaces were compared to non-etched root surfaces.[3,13]

This study also indicated the concentration of EDTA influences the nature of cell attachment to the etched root surface similar to a close response. It indicates the biochemical modifications of the root surface induced by highly saturated EDTA is responsible for an increase in the attachment of human fibroblasts. These modifications could be either a direct consequence of root conditioning by the exposure of some of the extra-cellular matrix constituents acting on the attachment mechanism of gingival epithelial cells or an indirect effect by the increased fixation on the demineralized root surface of biochemical factors.[14]

SEM observations showed major morphological modification of fibroblasts attached to the root surface (Figure 7). This indicates the root surface modifications are recognized by periodontal resident cells and influence their morphological features. It is interesting to observe, contrary to what has been observed in the kinetics of attachment, major differences appeared regarding cell attachment in relation to the type of conditioning. Twenty-four percent (24%) EDTA induced a dense network of filipods. It suggests that 24% EDTA induced demineralization could be somewhat physically different from that of 5% EDTA. In this study, fibroblasts did not attach to periodontally involved root surfaces that were scaled and root planed but treated with saline. This finding is in contrast with Fardal & Lowenberg 1999 study[15] that showed equivalent (citric acid dem-

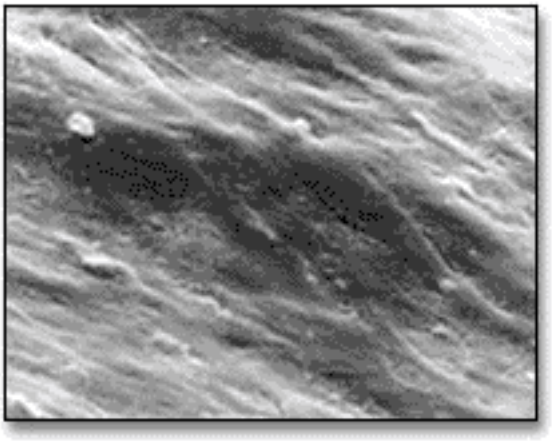


Figure 7.
Two weeks seeded fibroblasts grown on treated root with 25% EDTA. The surface is populated with well spread fibroblasts manifesting surface villous processes. (Original magnification X 1500)

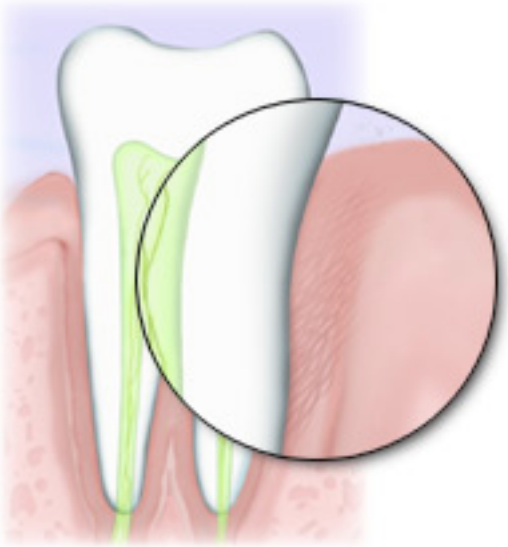
ineralized) fibroblast attachment for both root planed and diseased root surfaces. According to Pitaru et al,[16] removal of endotoxins from diseased root surfaces are required for reattachment. The presence of endotoxins is significant to the potential attachment of the root soft tissue interface and must be eliminated either by chemical means[3] or by meticulous root planing.[4] This study is in agreement with these latter findings. Based on this, it would appear preferable to remove the superficial layers of a "diseased" root prior to any etching since results after etching on root surfaces in the same area were essentially predictable and in accordance with previous studies.[7,10] However, a recent study indicated that demineralization with tetracycline hydrochloride did not statistically enhance the attachment of fibroblasts to the root surface when compared to the non-tetracycline hydrochloride treated groups, but caused changes in the cell morphology.[17]

It is well established nowadays that formation and maintenance of stable bonds between fibrin of the blood clot and the root surface is an essential step for optimal periodontal wound healing.[18]

Based on the findings of this observational study, root conditioning at high concentration (24% EDTA) enhanced periodontal to cellular attachment in vitro. The results suggest that root conditioning at 24% EDTA may transform the root surface to a more biocompatible substrate for new connective tissue attachment as suggested by Hanes et al.[19] Hence, this treatment may attract fibroblasts cells to form new attachment to teeth.

In a clinical setting, this information may aid the therapist in conditioning a periodontal involved root surface, i.e., the removal of the hypermineralized surface and contaminated smear layer without compromising the healing potential of the surrounding tissues. A surface freed from the smear layer may enhance periodontal healing conditions, since attachment and migration of fibroblasts in vitro have been reported to be delayed in the presence of the smear layer.[20]

This study suggests root surface conditioning with 24% EDTA enhances the reattachment of human gingival fibroblasts to root surfaces and favors faster periodontal healing.



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