

## Smear layer removal from root dentin using tetracycline hydrochloride concentrations. An SEM study

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استعمل المجهر الالكتروني الماسح لتقييم قابلية نزع طبقة المظاحة من الأسنان ذات الإصابة حول السنية بعد تعرضها للتترايسكلين بتركيز ١٠ ملغ/ملي و ٢٥ ملغ/ملي ولمدة دقيقة وثلاثة دقائق. شملت الدراسة ٣٦ عينة جرى تقسيمها إلى ثلاثة مجموعات متكافئة. المجموعة الأولى صقلت بالماء المقطر كمجموعة مقارنة. أما المجموعتين الثانية والثالثة فصقلت بالتترايسكلين لمدة دقيقة وثلاثة دقائق بتركيز ١٠ ملغ/ملي و ٢٥ ملغ/ملي. أبدت مجموعة المقارنة طبقة غير منتظمة من المظاحة. أما بالنسبة للتترايسكلين وبغض النظر عن التركيز ومدة التطبيق، فبدأ فعلا في نزع طبقة المظاحة عند تطبيق التترايسكلين لمدة دقيقة وثلاثة دقائق بتركيز ٢٥ ملغ/ملي، ظهرت أقنية عاجية عريضة بشكل واضح. لم يلاحظ أي اختلاف بين المجموعات فيما يتعلق بزمن التطبيق.

The scanning electron microscope was used to evaluate the smear layer removal capacity of periodontally diseased teeth subjected to concentrations of 10mg/ml and 25mg/ml of tetracycline hydrochloride for one and three minutes, respectively. Thirty- six teeth were divided into 3 equal groups. Groups 1 and 2 were burnished with tetracycline hydrochloride for 1 and 3 minutes with the following concentrations 10mg/ml and 25 mg/ml, respectively. Group 3 was burnished with cotton swab saturated in distilled water and served as the control. Results showed that the control group exhibited a smear layer. Tetracycline hydrochloride irrespective of the concentration and timing was effective in removing the smear layer. The 1 and 3 minutes application of tetracycline hydrochloride with a concentration of 25mg/ml revealed widely exposed dentinal tubules. No significant difference ( $p>0.05$ ) was found between the experimental groups in relation to timing was noted.

### Introduction

An objective of periodontal treatment is the predictable regeneration of the periodontium in areas previously affected by periodontal disease.<sup>1</sup> For regeneration to occur, it is necessary to eliminate calculus, bacterial plaque, and other cytotoxic substances on or within the diseased root surface.<sup>2</sup>

Human root surfaces have been treated with many substances in an attempt to make the root physiologically acceptable for the regeneration of a new connective tissue attachment.<sup>3,4</sup> For over ninety years, acids of various types had been placed on root surfaces in attempts to modify the diseased tooth structures.<sup>5</sup> Such treatment was to enlarge dentinal tubules into which healing connective tissue could enter.<sup>6</sup>

Surface demineralization with tetracycline hydrochloride or citric acid enhances the binding of the extracellular matrix glycoprotein fibronectin to dentin.<sup>7</sup> This was thought to enhance attachment and growth of gingival fibroblasts on the dentin surface.<sup>8,9</sup> *In vitro* effect of tetracycline hydrochloride on dentin has revealed several properties of tetracycline which indicated its potential usefulness in regenerative periodontal procedures.<sup>10</sup> Tetracycline has a low pH in concentrated solution, acts as a calcium chelator

and its application results in enamel and root surface demineralization.<sup>11,12</sup> Tetracycline hydrochloride concentrations used ranged from 0.5% to 200% and application period varied between 0.5 to 10 minutes.<sup>7,13,14</sup>

Wikesjo *et al.*<sup>15</sup> demonstrated that 10 or 100 mg/ml solutions of tetracycline hydrochloride were sufficiently concentrated to remove the smear layer and expose a regular pattern of open dentinal tubules. Optimal concentration and the application interval of tetracycline to remove the smear layer and achieve root surface characteristics which may support periodontal reconstructive therapy have yet to be established. Since long etching time of 3 minutes and above had been shown to impair periodontal healing,<sup>16</sup> it seems suitable to use short time etching to remove the smear layer and promote new connective tissue attachment to the root surface.

Consequently, the purpose of this scanning electron microscope study was to evaluate the ability of two different tetracycline concentrations to remove the smear layer on root dentin when applied over short times.

### Materials and Methods

#### Dentin Block Preparation

Twelve periodontally involved extracted human teeth were used. After extraction, the teeth

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were immediately cleaned and rinsed in distilled water and then stored for 48 hours at 5°C in distilled water until ready to be used.

Following extraction, the border between healthy and diseased root surfaces was marked with a tapered fissure bur. A small diamond disc mounted on mandrel in a slow speed hand piece under continuous irrigation was used to remove the crowns at the cemento-enamel junction and the non diseased part of each root in the apical area as well as sectioning longitudinally the remaining mid-root through the root canal.

Using a high speed hand piece with copious water coolant, the pulpal tissue was removed and a horizontal shallow groove for identification purpose was placed on the pulpal surface of each specimen. To remove the cementum and expose the underlying dentin, each diseased root specimen was thoroughly planed and flattened with a 15µm grit diamond bur\* under continuous water coolant at 20000 to 30000 rpm as described by Trombelli *et al.*<sup>10</sup> The dentin surfaces were scrubbed with a soft brush and distilled water to remove debris. Each root specimen was then sectioned longitudinally into 3 equal parts with a small diamond disk under continuous irrigation with a slow speed hand piece. The specimens were rectangular in shape, approximately 3mm long, 3mm wide and 1mm thick. A total of 36 dentin specimens were randomly divided into 3 groups for conditioning at tetracycline hydrochloride (weight volume) of 10 and 25mg/ml and distilled water for the control group with 2 application periods of 1 and 3 minutes (i.e., 3 samples per group).

#### Preparation of Tetracycline HCL Solutions

A freshly made tetracycline solution was used. The tetracycline solution<sup>†</sup> was prepared by slowly adding pure tetracycline into distilled water and dissolving it in 20 to 35 ml of distilled water using a magnetic stirrer to mix the solution to a final volume of 50ml. The concentrations (weight per volume) of the tetracycline hydrochloride test solutions were 10 and 25mg/ml. The pH of each solution was tested with a hand-held, battery operated pH meter<sup>‡</sup> and they measured 3.4 and 1.8 for the groups with 10 and 25 mg/ml, respectively. These groups served as experimental groups, while group 3 received no tetracycline

hydrochloride and served as control. The specimens of the control group were burnished with a cotton swab saturated in distilled water and the pH was 6.8. The specimens in each experimental group were conditioned with one of the two concentrations and the dentin surface was burnished for 1 and 3 minutes with a cotton pellet soaked in a fresh solution of tetracycline hydrochloride and which was changed every 30 seconds.

#### SEM Study Preparations

All specimens were prepared for scanning electron microscopy in the following manner. After fixation, dehydration was performed in a graded series of ethanol with 100% acetone as a final step. Each of the sectioned pieces was mounted on aluminum stubs, coated in gold with a sputter technique, and examined in the scanning electron microscope<sup>‡</sup>, operated at 15, 20 and 25 kV and with a tilt angle of between 0 and 30 degrees.

The surface characteristics of the dentinal specimens were evaluated descriptively for the presence of dentinal tubules and smear layer. The evaluations were made from the 4 X 5 inch SEM print (made under x 1000, 2000 magnification). All thirty six specimens were examined and photographed at these different magnifications.

Each dentin surface was scanned in its entirety to obtain an overview of the general surface topography. Representative areas that were characteristic of the general surface topography were selected for each specimen and photographed. The dentinal surfaces in the experimental and control groups were assessed for the presence of dentinal tubules and smear layer by a visual method. Assessments were made on photographs using the scale of zero to four as described by Madison *et al.*<sup>13</sup>

**0:** no removal of the smear layer; **1:** greater than no effect but less than one half removal; **2:** approximately one half removal of the smear layer; **3:** greater than half but less than complete removal; **4:** complete removal of the smear layer.

A Kruskal-Wallis test was performed on these evaluations. Tukey's nonparametric multiple range test was used to determine which groups were significantly different. The test was used to compare the three groups separately for the presence of dentinal tubules and smear layer.

#### Results

Scanning electron microscopy examination of control specimens showed an irregular coating

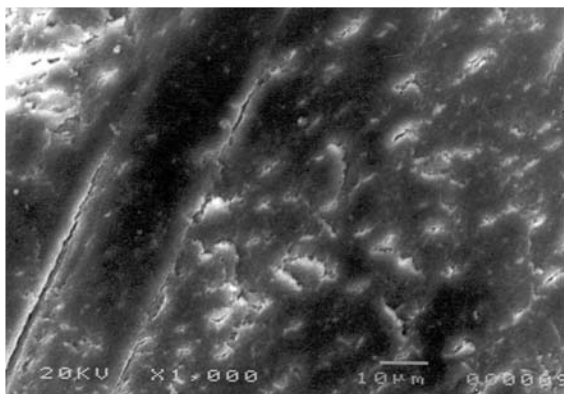
\*Perioset, Intensive Company, Philadelphia, USA

†Vetwic, Egypt

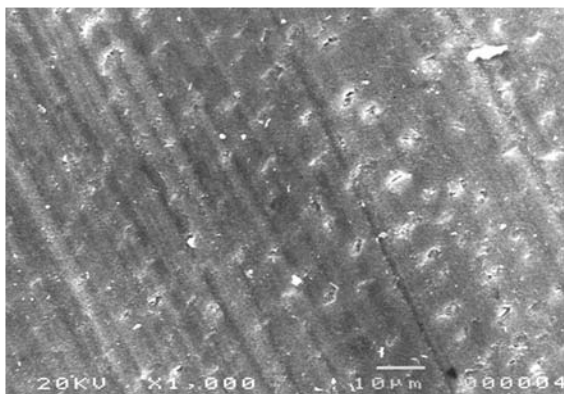
‡Medal 5941.00 Cole Parmer Instrument Company, Chicago, IL, USA

§Jeol, Japan

previously defined as a smear layer. Few dentinal tubule openings were visible and these were partially obscured (Figs. 1&2). When present, tubule orifices were not uniform in diameter. There were no differences in the morphological characteristics between 1 and 3 minutes



**Fig. 1.** Control specimen treated with 0mg/ml Tc-HCl solution. The surface is characterized by irregular, amorphous appearance resembling an encrustation. (Original magnification X1000)



**Fig. 2.** Control specimen treated with 0mg/ml Tc-HCl solution. Dental orifices obscured and occluded. (Original magnification X1000)

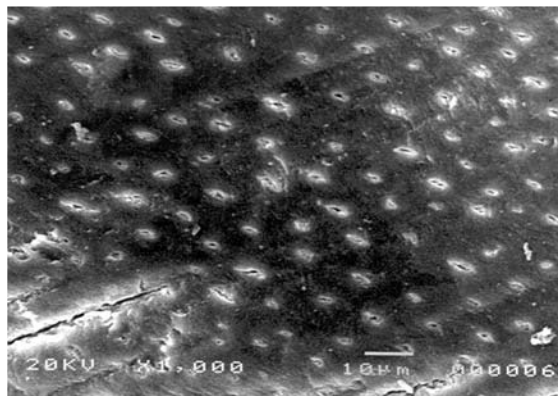
applications.

All specimens exposed to tetracycline hydrochloride solution at different concentrations in two application times demonstrated different degrees of removal of the smear layer and open dentinal tubules.

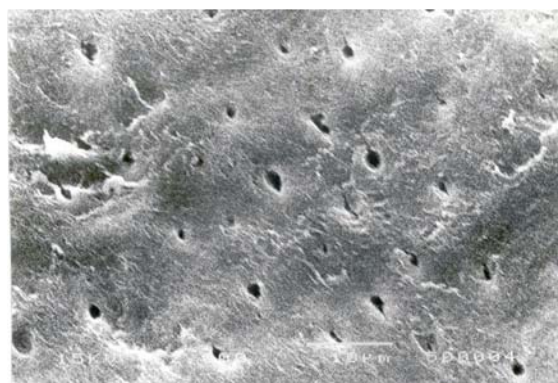
The dentin slabs treated by burnishing with tetracycline 10mg/ml for 1 and 3 minutes demonstrated the removal of smear layer and a homogenous surface with patent tubular openings of variable shapes and sizes. In the 1 minute and 3 minutes groups, a small amount of grinding debris partially occluded the dentinal

tubules (Fig. 3).

Corresponding views of the 25mg/ml tetracycline hydrochloride for 1 and 3 minutes were substantially different in appearance from



**Fig. 3.** Specimen treated with 10mg/ml Tc-HCl solution for 1 minute. Smear layer removed and tubules orifices partially obstructed. (Original magnification X1000)



**Fig. 4.** Specimen treated with 25mg/ml Tc-HCl solution for 1 minute. Open dentinal tubules present. (Original magnification X2000)



**Fig. 5.** Specimen treated with 25mg/ml Tc-HCl solution for 3 minutes. Dental tubules orifices discernible with regular homogenous arrangement than other groups. (Original magnification X1000)

the other surfaces (Figs. 4, 5). The dentinal tubules were found to be strikingly round in shape and very discernable with a more regular homogeneous arrangement than the other tetracycline hydrochloride concentrations.

The nonparametric one-way analysis of variance (Kruskal-Wallis) indicated a difference, between the three groups ( $P < 0.0001$ ).

The Tukey type nonparametric multiple range tests indicated that group 3 (control) was significantly different from the other two groups ( $P < 0.001$ ). Group 1 was significantly different from group 2 ( $P < 0.001$ ). However, there was no significant difference between the groups in relation to the timing ( $p > 0.05$ ).

### Discussion

In this *in vitro* study, SEM was used to evaluate the morphologic characteristics of diseased dentin treated with tetracycline hydrochloride solutions applied in different concentrations and for different periods of time. The control surfaces presented an amorphous, irregular coating with dentinal tubules obscured.

Tetracycline solutions treatment of root dentin specimens at 10 and 25 mg/ml resulted in the disappearance of the amorphous irregular coating. The results confirmed earlier studies which showed that conditioning with tetracycline hydrochloride removed the amorphous surface layer and exposed dentin with open tubules to produce similar surface characteristics.<sup>12-15</sup> The surface characteristics are represented by the absence of smear layer and the presence of open dentinal tubules.

Root surface conditioning with tetracycline has also been shown to influence fibroblast attachment to tetracycline treated root surface *in vitro*.<sup>17,18</sup> However, a study utilizing dentin blocks and treated root dentin specimens with 0.5% of tetracycline hydrochloride for 5 minutes revealed that tetracycline hydrochloride did not routinely remove the smear layer and did not expose tubules orifices<sup>19</sup> and so the authors concluded that higher concentrations of TC-Hcl might be required to achieve complete demineralization. The findings of this study are in accordance with this observation which demonstrated that tetracycline hydrochloride demineralization of root surface using two different concentrations for 1 and 3 minutes produced different surface characteristics. The concentration of 25mg/ml appeared to be more effective in exposing

dentinal tubules regardless of the application time. The results of this study are limited to the physical root surface changes and the potential of the tested solutions as a root surface conditioner for the removal of the smear layer and exposure of dentinal tubules.

Sterrett *et al.*<sup>20</sup> found that effectiveness of highly concentrated citric acid burnishing was time dependent and acid solution applied for 5 minutes produced a relatively debris free, flat surface. In this study, there was no significant difference among the groups in relation to the timing ( $p > 0.05$ ).

One possible explanation for these varying results could be the frequency of solution application. There are different modes of applying the etching solution. The burnishing technique introduced by Miller is a marked deviation from conventional demineralization techniques including the placement method.<sup>21</sup>

The burnishing effect of the root conditioner on the instrumented tooth surface has been studied<sup>22</sup> resulting in a chemical and mechanical removal of the smear layer and allowing for demineralization of the underlying root surface. In this study, the tetracycline solution was applied using a burnishing technique. It has been theorized that the acid burnishing technique resulted in a chemical/mechanical action that enhanced the removal of chemically loosened inorganic material and surface debris exposing the underlying root surface to the demineralization action of fresh acid solution.<sup>22</sup> This may ultimately achieve an optimal degree of demineralization. In the Sterrett study,<sup>22</sup> a single drop of demineralizing agent was used which may have become saturated, precluding any more demineralization, while repeated applications were used in this study.

This concept contrasts with the findings of the Labahn *et al.*<sup>23</sup> study where the mode of applying the demineralizing solution did not appear to have any consistent effects on the morphologic parameters they studied. One explanation may relate to the mechanical abrading action of the cotton pellet soaked with the demineralizing solution. Its importance could explain why in their study the depth of penetration was less in the burnished group than in the non-burnished group.

According to Register and Burdick,<sup>24</sup> the range of acid penetration depended on the time of application of the acid solution. The present findings suggest that 10mg/ml burnishing with

tetracycline hydrochloride may be enough for smear layer removal, but 25mg/ml burnishing applications of tetracycline was adequate to completely expose the dentinal tubules irrespective of the time used.

There were some relevant differences in the surface morphologic alterations produced in dentin between specimens treated with different concentrations of tetracycline solution. This was possibly due to the differences of pH (3.4 and 1.8.) of all freshly prepared solutions. Further studies are needed to establish whether and to what extent the morphologic surfaces changes produced by tetracycline hydrochloride solution application may provide a biologically favorable substrate for connective tissue cell repopulation of diseased root surfaces.

In summary, the results of this study confirmed that conditioning with tetracycline produced morphological changes to dentin surfaces. Therefore, it was concluded that tetracycline hydrochloride concentration of 25mg/ml will alter the dentin surface by removing the smear layer and opening the dentinal tubules irrespective of the time used.

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