

## The effect of tetracycline hydrochloride on the smear layer formed by various root planing modalities: A scanning electron microscopy study

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هدفت هذه الدراسة الى فحص تشكل طبقة اللطاحة بعد تطبيق أساليب مختلفة لتسوية الجذور ، ومن ثم نزعها بواسطة التتراسيكلين هايدرو كلورايد . شملت الدراسة اثنان وثلاثون سن بشرياً بإصابة حول سنوية ، جرى تقسيمها عشوائياً الى ثمان مجموعات تحتوي كل واحدة على أربعة أسنان . أجريت عملية تسوية الجذور بواسطة الأدوات اليدوية ( غريسي ١ و ٢ ) للمجموعتين الأولى والثانية ، بينما استعملت سنبل ماسية في المجموعتين الثالثة والرابعة . أما في المجموعتين الخامسة والسادسة فجرى استعمال جهاز البيزو الكهربائية ( البيزون ) ، وفي المجموعتين السابعة والثامنة استعمل الجهاز المغناطيسي ( أمذنت ) . وجرى بعد ذلك تحضير سطح الجذر لسنة عشرساً ( اثنان في كل مجموعة ) بواسطة التتراسيكلين هايدرو كلورايد لمدة ثلاث دقائق ، بينما استخدمت الأسنان عشرة الباقية كعينات للمراقبة . أوضحت النتائج على أن عملية تسوية الجذر ، قد أدت الى تشكل طبقة اللطاحة المغطية لسطح الجذر ، بغض النظر عن نوعية المعالجة . وأظهرت النتائج أيضاً ، أن التتراسيكلين هايدرو كلورايد قد أبدى فعالية في إزالة طبقة اللطاحة مع كشف للألياف الكولاجينية بدرجات متفاوتة .

The purpose of the present study was to examine the smear layer formation following different root planing modalities and its removal using tetracycline hydrochloride. Thirty-two periodontitis involved human teeth were randomly divided into 8 groups of 4 each. Groups 1 & 2 were root planed with hand instrument (Gracey 11 /12) while in groups 3 & 4, a flat diamond bur was used. In groups 5 & 6, the roots were scaled with a piezoelectric unit (Piezon) while in groups 7 & 8, a magnetostrictive unit (Amdent) was used. Sixteen teeth (two in each group) were conditioned with tetracycline hydrochloride for 3 minutes while the other sixteen teeth were not conditioned and served as controls. The surfaces were examined by a scanning electron microscope. The results showed that root planing resulted in a smear covering the root surfaces, Irrespective of the treatment modality. Tetracycline hydrochloride efficiently removed the smear layer with collagen fibers exposed to varying degrees.

### Introduction

One of the objectives of periodontal therapy is to convert the periodontitis affected root surface into a substrate which is biologically hospitable for epithelial and connective tissues cell adherence and attachment.<sup>1,2</sup>

Methods to achieve this objective have included scaling and root planing,<sup>3</sup> as well as treatment with various chemicals and antimicrobial agents.<sup>4,5</sup>

Hand or ultrasonic scaling of root surfaces always produces a non-biocompatible smear layer.<sup>6</sup> Conventionally, this smear layer has been removed with citric acid at low pH etchant.<sup>7</sup> However, citric acid has recently been shown to interfere with wound healing because of its low pH.<sup>8</sup>

In vitro studies on the effects of tetracycline hydrochloride on dentin have revealed properties which may be beneficial in periodontal reconstructive therapy. Surface demineralization with tetracycline enhances binding of matrix proteins to dentin and stimulates fibroblast attachment and growth.<sup>9</sup> Furthermore, topical

tetracycline is absorbed to and subsequently desorbed from dentin maintaining antimicrobial activity for at least 14 days.<sup>10</sup> Such substantivity may be beneficial since tetracycline has been shown to inhibit mammalian neutrophil collagenase and in vitro bone resorption.<sup>11</sup> These findings have led to the widespread use of tetracycline treatment of root surfaces in periodontal surgery.

The purpose of this study was to evaluate the smear layer removal and collagen exposure capacity of tetracycline hydrochloride applied to root surfaces treated by different root planing modalities.

### Materials and Methods

Thirty-two periodontally involved human teeth scheduled for extraction were used in this study. All teeth had lost more than two-thirds of their radiographic attachment or had severe through and through furcation defects (Fig. 1). Following extraction, the border between the healthy and diseased root surfaces was marked with a small dental bur. Only the diseased part of the root surface was used in the study. The teeth were randomly divided into 8 groups of 4 each. The diseased root surfaces of the teeth in groups 1 and

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Fig. 1. Radiograph showing extensive bone destruction in the area of the first premolar before its extraction.

2 were root planed with hand instrument (Gracey 11/12, Hu-Friedy, Chicago, USA) while the root surfaces in groups 3 and 4 were root planed with a flat diamond bur (Periotest, Philadelphia, USA). The root surface in groups 5 and 6 were ultrasonically scaled with a Piezon (Piezon, EMS Switzerland) while the root surfaces in group 7 and 8 were root planed with another ultrasonic (Amdent 830, Sweden).

Piezon is a piezoelectric unit where the pattern of vibration of the tip is linear meaning that only the two sides of the tip are active. Amdent is a magnetostrictive unit where the pattern of vibration of the tip is elliptical.<sup>12</sup> The specimens were prepared in the following manner. Calculus covering the specimen was removed. Attention was paid to avoid over-instrumenting or gouging the underlying root surface.

Following root planing, the teeth in groups 2,4,6 and 8 were immersed in a solution of tetracycline hydrochloride (pH 1.8) for 3 minutes. These groups served as experimental groups, while the teeth in groups 1, 3,5 and 7 received no tetracycline hydrochloride and served as controls (Table 1).

Table 1. Distribution of teeth between groups and treatments.

|   | Group   | Root Planing Method  |          |        |         |              |
|---|---------|----------------------|----------|--------|---------|--------------|
|   |         | Hand Instrumentation | Flat Bur | Piezon | Am-Dent | Tetracycline |
| 1 | Control |                      |          | +      |         |              |
| 2 | Test    |                      |          | +      |         | +            |
| 3 | Control |                      |          |        | +       |              |
| 4 | Test    |                      |          |        | *       | +            |
| 5 | Control |                      | +        |        |         |              |
| 6 | Test    |                      | +        |        |         | +            |
| 7 | Control | +                    |          |        |         |              |
| 8 | Test    | +                    |          |        |         | +            |

Freshly made tetracycline solution was used. The tetracycline Hcl was prepared by slowly adding pure tetracycline (Spimaco, Qassim, Saudi Arabia) into distilled water until a saturated solution of 0.5%g/ml was obtained with constant stirring at 37°C for 10 minutes. This gave a pH 1.8 solution when checked with a pH meter. A magnetic stirrer was used to mix the solution.

After acid conditioning, specimens were rinsed for 3 minutes with distilled water. All specimens were prepared for scanning electron microscopy (SEM) in the following way. After fixation, dehydration was performed in a graded series of ethanol with 100 % acetone as a final step. The teeth were mounted on aluminium stubs and sputter coated<sup>13</sup> with a gold palladium alloy to a thickness of 10nm. All teeth were examined in the scanning electron microscope (Jeol, Japan), operated between 20 and 25 kv and with a tilt-angle of between 0 and 30 degrees.

The roots were examined with respect to the presence or absence of the smear layer and to exposed collagen fibers. Open dentinal tubuli on the root surface were a criteria for the efficacy of the smear-layer removing capacity of the treatment as advocated by Poison et al.<sup>7</sup>

## Results

### *Smear Layer*

SEM examination of control, root planed, non-conditioned specimens showed an irregular, amorphous surface which correspond to a smear layer. No differences in root surface texture following the four treatment modalities were observed. No open dentinal tubuli could be seen. Following ultrasonic scaling with either Piezon or Amdent, the root surface revealed a wave like appearance with presence of debris (Fig. 2), while the root surfaces treated manually or with a bur

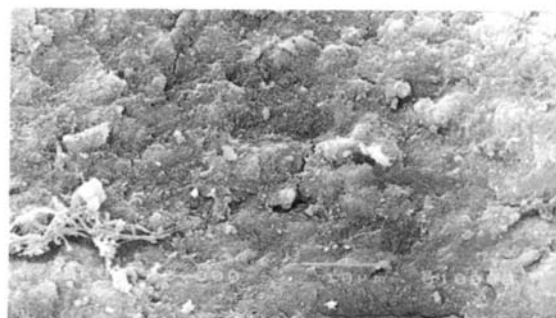


Fig. 2. SEM photomicrograph of root surface following ultrasonic scaling with Piezon. The amorphous smear layer covers the underlying dentinal tubuks. (Original magnification x 2000)

showed a smoother surface interrupted by longitudinal grooves (Fig. 3).

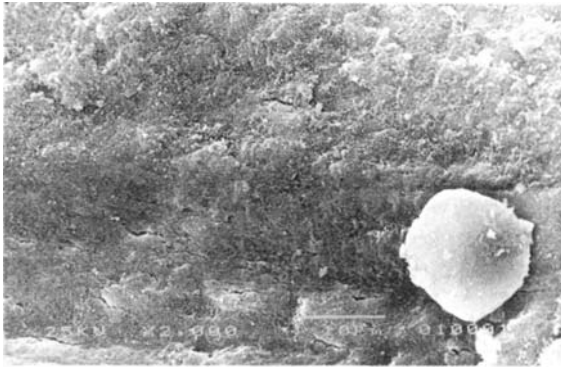


Fig. 3. SEM photomicrograph of root surface following hand instrumentation. Smear-plugged dentinal tubuli with artifactual cracks are visible. (Original magnification x 2000)

The effect of tetracycline hydrochloride treatment on diseased root surface after all four treatment modalities is illustrated in Figs. 4 & 5. Etching with tetracycline for 3 minutes removed most of the smear layer from the root planed surfaces in all four treatment modalities. Little or no variations in the presence or absence of smear layer could be detected following the different root planing and etching modalities. All specimens exhibited distinct areas of patent dentinal tubules.

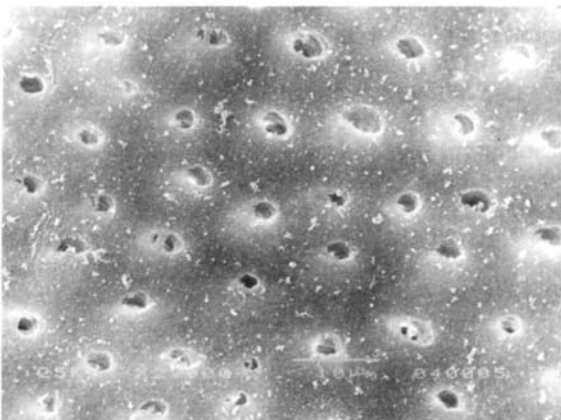


Fig. 4. SEM photomicrograph of ultrasonically scaled root surface (Amdent) conditioned for 3 min with tetracycline. Patent dentinal tubuli with debris are displayed. (Original magnification x 2000)

#### Collagen

Fibrous structures were evident after root conditioning. Surfaces scaled with ultrasonic displayed intertubular surfaces covered by a fibrous meshwork with fibers extending into patent dentinal tubules (Fig. 6). The root surfaces treated manually or with a bur showed fewer and less well defined structures (Fig. 7).

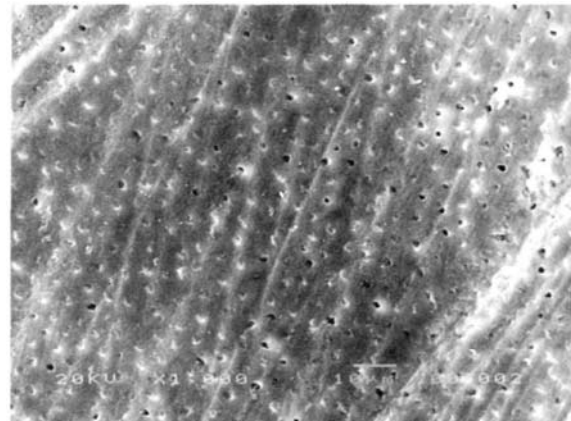


Fig. 5. SEM photomicrograph of root surface scaled with bur and conditioned for 3 min with tetracycline displaying patent dentinal tubules. (Original magnification x 1000)

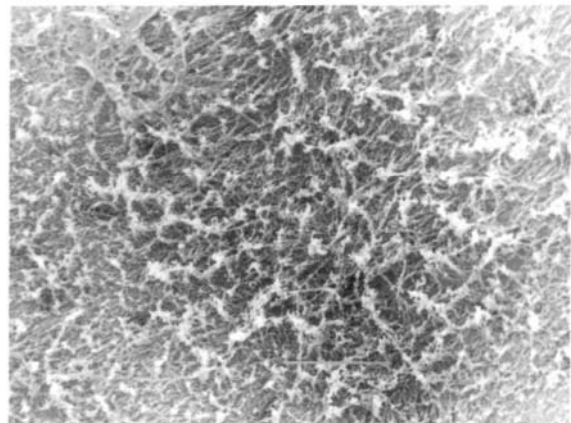


Fig. 6. SEM photomicrograph of root surface ultrasonically scaled (Piezon) and conditioned for 3 min with tetracycline displaying a fibrous meshwork. (Original magnification x 3000)

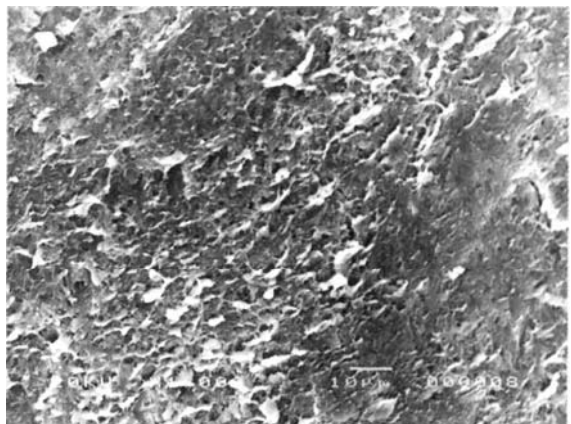


Fig. 7. SEM photomicrograph of root surface following manual root planing and tetracycline hydrochloride conditioning displaying fewer fibrillar structure. (Original magnification x 1000)

### Discussion

Complete mechanical removal of dental calculus and contaminated cementum is not a realistic objective in periodontal therapy.<sup>14</sup> Consequently, additional methods to detoxify the root surface such as etching has been recommended.<sup>15</sup> By definition, etching involves selective removal of parts or components from a solid surface through the action of etching agents such as solution of acid and other substances like tetracycline hydrochloride. Etching does not, however, imply erosion of the surface or removal of a complete surface layer. Etching performed on a root surface during periodontal surgery thus aims at selectively removing mineral and associated bacterial toxins, leaving an exposed layer of collagen.

The results presented in this study are in accordance with those of earlier studies that have found that root planing with hand instruments or burs always results in the formation of a smear layer.<sup>6,7</sup> When a piezoelectric unit (Piezon) and a magnetostrictive unit were used for root planing, the smear layer in all specimens were found to cover the root planed surfaces (Fig. 2). This is in accord with Hanes et al<sup>6</sup> who reported that periodontitis affected teeth ultrasonically scaled appeared slightly altered showing a faint mat-like texture following a 5-minute tetracycline Hcl or citric acid exposure. The tetracycline Hcl and citric solutions produced comparable morphologic characteristics. However, another study claimed that the smear layer has been removed by the ultrasonic treatment.<sup>17</sup> These contradictory results may be explained by differences in the working principles among ultrasonic scalers, although it seems unlikely that a smear layer with its content of hydrophobic substances can be entirely removed by water irrigation.

The wave-like appearance of the root surfaces following ultrasonic scaling may be explained by the more gentle effect exerted by the scaler to the root surface compared to hand instrument and burs.<sup>18</sup> Following etching with tetracycline hydrochloride for 3 minutes, the smear layer was practically non-existent. In addition to removing the smear layer, tetracycline hydrochloride also enlarged or widened the tubule orifice due to preferential demineralization of the peritubular dentin.<sup>19,20</sup>

Dentin surface conditioning with tetracycline hydrochloride has also been shown to influence in vivo fibroblast attachment and fibroaction binding to tetracycline or minocycline treated dentin

surfaces.<sup>21</sup>

Discrepancies in reported demineralizing effect may relate to the degree of mineralization,<sup>22</sup> nature and concentration of the conditioning agent,<sup>23</sup> application mode and interval<sup>24</sup> or a combination of these factors. The presence of a hypermineralized surface layer or periodontitis affected roots has been assessed by microradiography.<sup>25</sup> Hypermineralization appears limited to the superficial 35 to 50 urn of the root surface. Root surface conditioning has been shown to demineralize the root to a depth of 3 to 12 urn.<sup>26</sup> However, ultrasonic scaling has been reported to result in a loss of root substance varying from 12 to 86 urn.<sup>27</sup> Therefore it is not unrealistic to assume that our cementum specimens exhibited normal mineral content and crystal structure and that observed surface alterations may not necessarily relate to periodontitis affected cementum. These observations may suggest that instrumentation of pathologically altered root cementum, while not extensively removing root structure is necessary to enhance the demineralizing effect of the acid.

In this study, root associated collagen fibers were exposed to varying degrees following etching (Figs. 6 & 7). This was observed in all of the etched groups irrespective of root planing modality. The piezon scaled group displayed more fiber-like structure. This can be explained by the erosive nature of the cavitation activity created by ultrasonic scaling.<sup>28</sup> Root surfaces that had less exposed collagen had probably a higher mineral content and the etching therefore had less effect on the surface in relation to exposure time. However, the biochemical and morphological changes in the root surface produced by the various mechanical techniques and chemical agent used for root surface preparation are poorly understood. A clear understanding of the effects of the methods of root surface preparation and conditioning is essential if one hopes to be able to consistently convert the periodontitis affected root surface into a substrate which is biologically hospitable to epithelial and connective tissue cell adherence and attachment.

### Conclusion

Based on the findings of this in vitro study, the following conclusions can be drawn:

1. Irrespective of the treatment modality, root planing resulted in a smear layer covering the root surface.
2. Tetracycline hydrochloride applied for 3

minutes efficiently removed the smear layer.

3. Collagen fibers were exposed to varying degrees following conditioning with tetracycline hydrochloride.

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