

## Smear layer removal with 8% EDTA root conditioning: An SEM study of the effect of different application times

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هدفت الدراسة لمراقبة قدرة محلول EDTA بتركيز 8% على نزع طبقة اللطاخة من العاج المشمول بالإصابة حول السنية. كما جرى تحديد زمن التطبيق. جرى تحضير عينات عاجية مسطحة من أسنان بشرية تم خلعها بسبب الإصابة حول السنية. وتم بعد ذلك تحريش العينات بمحلول EDTA بتركيز 8% لمدة دقيقة واحدة (المجموعة الأولى)، وثلاث دقائق (المجموعة الثانية) وخمسة دقائق (المجموعة الثالثة)، أما المجموعة الرابعة فكانت مجموعة المقارنة حيث جرى غمر العينات في السائل الملحي. وجرى بسعد ذلك فحص طبقة اللطاخة بواسطة المجهر الإلكتروني الماسح. دلت النتائج على أن التحريش بمحلول EDTA لمدة دقيقة واحدة ليس كافياً لحل طبقة اللطاخة. أما التحريش لمدة خمس دقائق فبدأ أكثر فعالية من التحريش لمدة ثلاث دقائق. ووفقاً لهذه النتائج، فإن تطبيق محلول EDTA بتركيز 8% على عاج الجذر لمدة خمسة دقائق يزيل طبقة اللطاخة، بشكل كامل، من الألفية العاجية.

The purpose of this SEM study was to evaluate and compare in vitro effects of different timing applications of 8% EDTA on dentin. The surface characteristic was also evaluated. A flat dentin surface was created on human teeth extracted due to severe periodontitis. The teeth were etched with 8% EDTA (pH 7.3) for 1 min (Group I), 3 min (Group II), 5 min (Group III). Group IV served as control and the teeth were soaked in saline (pH 5.1). The teeth were evaluated with scanning electron microscope with respect to smear layer removal. Results showed that 1 min etching with 8% EDTA was not sufficient to dissolve the smear layer. Five min etching dissolved the smear layer more effectively than the 3 min group. Based on these findings, the 5min application of 8% EDTA on root dentin completely removed the smear layer from the tubule opening.

### Introduction

Root surfaces affected by periodontitis become hypermineralized<sup>1,2</sup> and contaminated with endotoxins<sup>3</sup> as well as other toxic bacterial products.<sup>4</sup> Such surface does not encourage cell attachment or migration which are necessary events for optimal spontaneous periodontal healing.<sup>5</sup> It is not possible to decontaminate the periodontitis affected root mechanically using bur or hand instruments,<sup>6</sup> since these methods always produce a smear layer covering the healed root surface.<sup>7</sup> Lately however, a supersaturated pH neutral etching solution of EDTA has been found to be as effective as low pH etchants with respect to smear layer removal<sup>8</sup> and superior in exposing root surface associated collagen<sup>9</sup> in both experimental in vitro and in vivo studies.

Cell attachment<sup>8</sup> and periodontal healing<sup>10</sup> have been shown to be promoted by EDTA etching compared to etching with low pH agents. Of etchants in clinical use, EDTA is the only one which exclusively exerts its demineralizing effect through chelating divalent cations at neutral pH, while phosphoric acid acts through its low pH and dissolves or erodes a mineralized surface.<sup>8</sup> Citric acid (pH 1) functions by a combination of these two mechanisms.<sup>11</sup>

Furthermore, the use of phosphoric acid has

proved harmful to the vitality of periodontal tissues while citric acid does not exerts its devitalizing effect to the same penetration depth.<sup>11</sup> This is in contrast to a supersaturated solution of EDTA (pH 6.1) which does not impair vitality when applied to periodontal tissues.<sup>11</sup> EDTA working at neutral pH appeared preferable with respect to preserving the integrity of exposed collagen fibers, smear layer removal and periodontal healing.<sup>8,10</sup> However most experimental studies on the use of EDTA as an adjunct to conventional periodontal surgery have not attempted to optimize its application time by using different timing.<sup>10,12</sup> Consequently, the purpose of the present SEM study was to evaluate and compare in vitro effects of different timing applications of 8% EDTA on dentin surface. The surface characteristic was also evaluated.

### Material and Methods

#### Dentin block preparation

Dentin blocks were prepared from 12 extracted periodontally involved human teeth. After extraction, the teeth were immediately cleaned and rinsed in distilled water and then stored for 36h at 4°C in distilled water until ready to be used. The dentin specimens were prepared as described by Demirel et al.<sup>13</sup> The crown and root coronal to the mid root region were sectioned through the

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root canal in a plane parallel to the root surface using a diamond disc under water irrigation. The pulpal tissue was removed using a round carbide bur, and the pulpal dentin was disked until smooth and hard. An identification notch was placed on the pulpal root surface. The cementum was removed by water-cooled diamond burs to a depth of at least 0.25 mm, a depth which exceeds the average thickness of human cementum at the midportion of the root.<sup>14</sup> Mesiodistal vertical separation cuts, 1 mm apart, and a horizontal cut, released the specimens from the root. Each dentin specimen was about 4x7x2 mm in size. This yielded a total of 48 dentin specimens; 36 experimental and 12 control. 48 blocks were randomly divided into 4 groups and each tooth contributed one block to each treatment group.

The treatment groups were as follows:

- Group I: Immersion in 8% EDTA solution for 1 min
- Group II: Immersion in 8% EDTA solution for 3 min
- Group III: Immersion in 8% EDTA solution for 5 min
- Group IV: Control

The control specimens were obtained from the non-chelated (EDTA) treated part of the root and were soaked in saline.

The following solutions were used for treatment of the root dentin:

1. Saline (pH 5.1) for control group
2. 8% EDTA dissolved in phosphate buffer solution (pH 7.3) for the experimental group

Following conditioning, each specimen was rinsed in distilled water and placed into a well of a 96-well microtiter plate (Honeycombplates, Labsystem) containing glutaraldehyde in cacodylate buffer at pH 7.3.<sup>15</sup>

#### Preparation for SEM

All specimens were prepared for scanning electron microscopy (SEM). After fixation dehydration was done in a graded series of ethanol and with 100% acetone as a final step. Each of the sectioned pieces was mounted on aluminum stub, coated in gold with a sputter technique.<sup>16</sup> The specimens were examined in the scanning electron microscope (Jeol, Japan) operated at 20 to 25 Kv and with a tilt angle of between 0 and 30 degrees.

## Results

### Control teeth

SEM examination of control, untreated specimens exhibited a significant amount of grinding debris. It also revealed a thin, mineralized smear layer. Nowhere were the dentin surfaces or any patent dentinal tubules visible (Fig. 1).

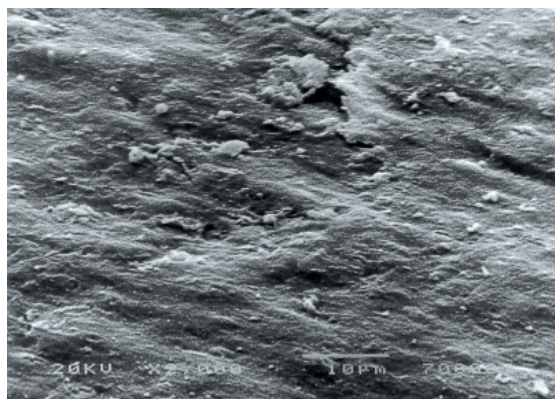


Fig. 1. SEM photomicrograph of saline irrigated dentinal surface. The surface has an amorphous appearance smear layer covering dentin surface. (Original magnification x 2000)

### 8% EDTA for 1 minute

The surfaces of samples soaked with 8% EDTA for 1min showed partial removal of the smear layer. Remnants of debris were seen in most of the dentinal tubule. However, nowhere were patent dentinal tubules visible (Fig. 2).

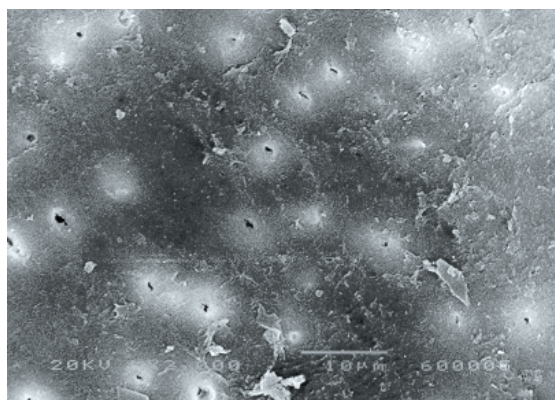
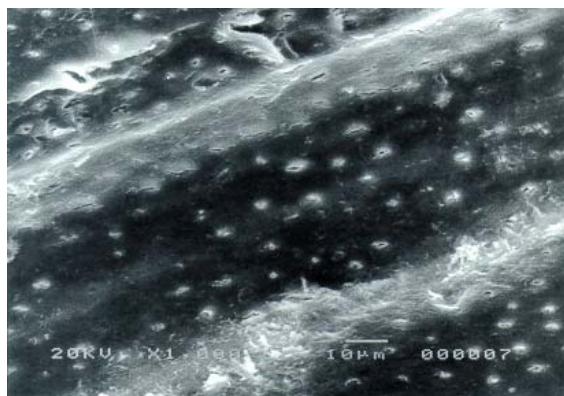


Fig. 2. SEM photomicrograph of dentin surface treated with 8% EDTA for 1 min. Smear layer is partially removed. (Original magnification x 2000)

### 8% EDTA for 3 minutes

There were no traces of smear layer on any of the dentin surfaces in any of the test group. About

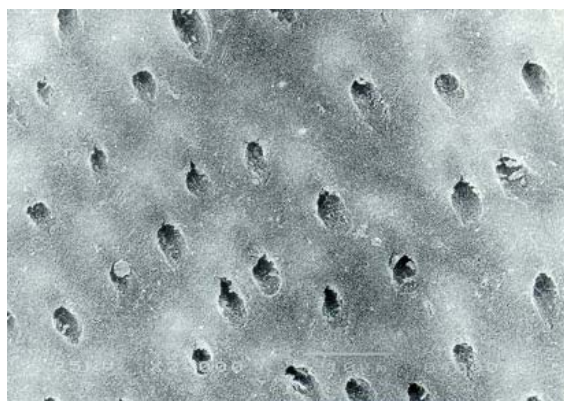
half of the tubules were plugged but the total number of visible tubules opening were higher than in group II (Fig. 3).



**Fig. 3.** SEM photomicrograph of dentin surface treated with 8% EDTA for 3 min. Smear layer is removed. Few dental tubules are partially occluded. (Original magnification x 1000)

#### 8% EDTA for 5 minutes

The dentin surface was readily visible in all root planed "diseased" areas as evidenced by lack of smear layer and an abundance of patent dental tubules. The EDTA treated root surfaces in this group displayed widened dental tubules which appear as funnel shapes due to the removal of the peritubular dentin (Fig. 4).



**Fig. 4.** SEM photomicrograph of dentin surface treated with 8% EDTA for 5min. Dental tubules in the surface of the specimen are widened. The smear layer has been removed and the dental matrix is exposed. (Original magnification x 2000)

### **Discussion**

The smear layer has been described as a layer composed of very small particles of mineralized collagen matrix.<sup>17</sup> Studies have suggested that the presence of this smear layer interposed between the root surface and adjacent connective tissue

may serve as a physical barrier to the development of a connective tissue attachment to the root surface.<sup>18</sup> It has been shown that EDTA treatment of root dentin by the burnishing action results in removal of the smear layer.<sup>9</sup>

In the current study, surfaces that received only root planing presented an amorphous, irregular coating with little evidence of patent dental tubules. This characteristic appearance was probably due to the presence of a surface smear layer. Treatment of dentin specimens with 8% EDTA in three different timings (1, 3 and 5 minutes) resulted in partial or total disappearance of the above mentioned amorphous surface. The dental tubules and the dentin between the tubules were affected differently according to the time used. Application time of 5 minutes showed dental tubule orifices more widely exposed than 3 and 1-minute applications suggesting that the smear layer removal is time dependent.

In clinical dentistry, etching of the teeth is performed for different purposes, consequently different etchants are in use. In surgical periodontal procedures, citric acid has been recommended for removing smear layer from root planed surface in order to delay downgrowth of gingival epithelium<sup>19</sup> and enhance clinical attachment gain.<sup>20</sup> In periodontal therapy, citric acid has been the most commonly used etchant but a recent experimental study has indicated that EDTA may be the etchant of choice.<sup>9</sup> According to these authors, etching at neutral pH (EDTA) for 20 seconds preserved adjacent tissue vitality while etching at low pH (citric acid) necrotized the flap and adjacent periodontium after 20 seconds of exposure in an in vivo animal study.

A recent in vitro study demonstrated that EDTA applied for 3 minutes and citric acid applied for 20 seconds by the rubbing technique were able to remove mineral from the dentin surfaces exposing a matrix structurally resembling collagen fibers of the unmineralized predentin.<sup>8</sup>

The purpose of this study was to explore the possibility of obtaining an acceptable smear removing effect using three different timings of 8% EDTA applied by the soaking technique to the root dentin.

The 5 minutes passive application completely removed the smear layer and exposed the dental tubul while the 3 minutes application was significantly more effective than the 1 minute application with regard to smear layer removing capacity. However, the 3 minutes application partially exposed the dental tubules while the 1 minute application showed occluded dental

tubules.

The present result regarding the EDTA use are in contrast with recently produced results.<sup>8</sup> The authors claimed that 20 seconds of rubbing the root dentin with EDTA was enough to remove the smear layer. The present findings suggest that 5 minutes passive application of 8% EDTA on the root dentin is adequate.

The differences are mainly due to the different modes of application used in this study. As it is difficult to standardize the rubbing technique used in the Blomlöf study, the passive method by soaking the dentin specimen into the 8% EDTA solution was used in this study. Wen et al<sup>21</sup> in an in vitro study, evaluated the effects of citric acid (pH 1.0) application techniques on freshly extracted teeth and concluded that "it may be undesirable to apply citric using excessive pressure by the rubbing technique". Also, they found that with the rubbing technique, the exposed dentin may often be masked creating a "smear" layer with obliterated tubules. These observations are further supported by another study.<sup>22</sup> In this respect, it appears reasonable to assume that the longer exposure times would enhance the smear removing effect. A longer etching may thus be of value only if a more profound effect on the dentin surface is desired, thus assuring the complete removal of smear layer as shown by Trombelli et al.<sup>23</sup> While it has been suggested that EDTA application for 3 minutes may necrotize surrounding cells thus having a negative influence on periodontal wound healing,<sup>24</sup> a recent experimental in vivo study did not show any harmful effects on the vitality or healing capacity of the periodontium.<sup>11</sup>

According to Sterrett,<sup>25</sup> a longer application of a conditioning agent on root dentin will result in a chemical and mechanical action enhancing the removal of chemically loosened organic material and surface debris exposing underlying dentin to the demineralized action of the root conditioning. The present findings are in accord with this concept. The 5 minutes application of 8% EDTA was necessary to completely remove the smear layer. EDTA operating at neutral pH appeared to be able to selectively remove mineral from a dentin surface. This type of dentin surface appeared to produce a more biocompatible surface. EDTA may be needed to achieve smear layer removal within a clinically acceptable time period. However, there may be a risk in etching a root planed surface consisting of dentin. The resultant opening of dentinal tubules between the pulp chamber and the periodontium may cause tooth sensitivity or

interaction between the pulp tissue and the periodontium according to Ehnevid.<sup>26</sup>

This study suggests that a root surface should be etched with 8% EDTA for 5 minutes thus exposing completely the dentinal tubules. This soaking will render the surface chemotactant to fibroblasts, and thereby enhance the possibility of obtaining regeneration in areas of root exposure caused by periodontitis. This hypothesis must however be tested clinically.

### Conclusions

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

1. Saline application on root dentin did not result in the disappearance of the smear layer.
2. 1 minute application of 8% EDTA on root dentin removed the smear layer partially.
3. 3 minutes and 5 minutes applications of 8% EDTA on root dentin dissolved the smear layer with the degree of exposed dentinal tubules greater in the 5min group.

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