A scanning electron microscopic study of debris and smear layer remaining following use of AET instruments and K-floexofiles

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SUMMARY

Aim To compare in vitro the cleanliness of root canal walls following automated or manual instrumentation.

Methodology Thirty extracted human maxillary central incisors, maxillary and mandibular canines and premolars with single root canals were used in this study. The teeth were divided into two groups. In group 1 (20 teeth) automated canal preparation was performed using Anatomic Endodontic Technology (AET). In group 2 (10 teeth) manual instrumentation was performed with K-Flexofiles. Irrigation was performed using alternately 3.00% NaOCl and 18% EDTA, followed by rinsing with saline. The roots were split longitudinally into halves and the canals examined using a scanning electron microscope. The presence of debris and smear layer was recorded at coronal, middle and apical thirds of root canals using a four-step scoring scale. Mean scores for debris and smear layer were calculated and statistically analysed for significance ($P < 0.05$) between and within groups, using the Mann-Whitney-Wilcoxon and Friedman nonparametric tests.

Results At coronal and middle thirds the root canals prepared with manual instrumentation had significantly less surface debris on the canal walls compared with canals prepared with AET ($p<0.05$). At apical third root canals prepared with manual instrumentation had significantly more debris compared with AET group. The amount of smear layer was greater in the apical than in the coronal and middle thirds of the root and significantly less amount was in the AET group ($P<0.05$).

Conclusions Complete cleanliness was not achieved by any of the techniques and instruments investigated. It may be inferred that the choice between AET and hand instrumentation should be based on factors other than the amount of root canal debridement, which does not vary high significantly according to the instruments used.

Key words: Anatomic Endodontic Technology, cleaning efficacy, EDTA, endodontics, K-Flexofiles, root canals, root canal instrumentation, scanning electron microscopy, sodium hypochlorite.

INTRODUCTION

The removal of debris and smear layer from the root canal system prior to obturation is one of the primary aims of endodontic treatment [1]. Smear layer differs from the „dusty” pattern of superficial debris in that it is a layer of „muddy” material, composed of an amorphous layer of organic and inorganic debris, and sometimes bacteria [2], which is compacted against the dentine walls as a result of the rasping action of endodontic instruments [3,4].

It has been suggested that the presence of a smear layer may prevent bacterial penetration into the underlying dentinal tubules [5]. On the contrary, the presence of an infected smear layer may prevent antimicrobial agents from gaining access to the infected dentinal tubules [6]. Furthermore, the removal of the smear layer may enhance the penetration of sealers into dentinal tubules and adaptation of obturation materials to the root canal walls [7,8,9].

Recently, an innovative concept of mechanical root canal preparation, the Anatomic Endodontic Technology (AET) has been introduced [10]. AET was specifically designed to maintain the natural shape of the root canal during preparation. The manufacturer claims that this system is intended to minimize the number of steps and instruments required for effective preparation of root canals.

Numerous studies have been reported on the relative effectiveness of different instrumentation techniques, based on a variety of ways of evaluating canal debridement. Outcomes of instrumentation differ according to the method of canal preparation and evaluation, each method showing advantages and disadvantages [11]. Introduction of the scanning electron microscope (SEM) has proved to be a valuable method for assessment of the ability of the endodontic procedures to remove debris from root canals, thus enabling comparison of instruments and techniques. Therefore, a number of stud-
ies about the debridement of the root canal wall have been carried out by using SEM [12,13,14,15]. However, as far as is known, only few studies for specifically testing the AET instrumentation for canal debridement have been carried out [10].

The aim of this study was to compare by means of scanning electron microscopy, the presence of a smear layer and remnants of debris on the walls of root canals after preparation with AET instruments and manual instrumentation.

MATERIALS AND METHODS

Thirty freshly extracted single-rooted maxillary central incisors, maxillary and mandibular canines and premolars with closed apices were used. None of the teeth had received restorative or endodontic therapy. Following extraction, the teeth were stored in isotonic saline solution to avoid any effect that fixative might have on the dissolution of organic tissue. Conventional endodontic access cavities were prepared (Endo Access Bur, Dentsply Maillefer, Ballaigues, Switzerland) in a high-speed handpiece. To determine working length a size 10 K-file was inserted until it reached the apical foramen and one millimetre subtracted from this length. A small amount of wax was placed on the tip of each root to prevent irrigating solutions from passing through the apical foramen.

Canal instrumentation

The teeth were divided into two groups: group 1 (20 teeth) was instrumented with AET instruments (Ultradent Products Inc., South Jordan, UT, USA) and group 2 (10 teeth) was instrumented with K-flexofile instruments (Dentsply Maillefer, Ballaigues, Switzerland). The procedures used for each instrumentation group were standardized.

In group 1, the canals were prepared using the AET (Ultradent Products Inc., South Jordan, UT, USA) according to the manufacturer’s instructions. The operative procedures were as follows. The coronal two-thirds were enlarged with Shaping files 1, 2 and 3. Initially, a size 1 shaping file (2.5% taper) was inserted by hand to approximately 4 mm short of the established working length. The file was then used in a reciprocating 4:1 low-speed handpiece. In teeth in which the mesial and distal aspects provided no resistance, the file was lightly wiped against these walls for a few seconds. For final preparation of the canals, the Apical files 1, 2 and 3, which only cut in the apical area and have a 2.5% taper, were then used by hand to the working length with a step-back technique. Files were changed to the next size when no resistance was felt. Preparation of the apical third of the canals was judged complete when the size 3 Apical file (equivalent to a size 30 K-file at the tip) could be inserted to the working length without force.

In group 2, the canals were instrumented using a step-back technique. The coronal and middle thirds were flared with Gates-Glidden instruments and the apical third was prepared subsequently with sizes 15, 20, 25 and 30 K-files (Dentsply Maillefer) to the full working length. Files were used with in-and-out movements in a circumferential manner. Preparation of the apical third was considered complete when a size 30 file could be inserted without force to the working length. Then, K-files from sizes 35–60, each size 1 mm short of the preceding instrument, were used for final preparation of the coronal and middle third.

In all groups, individual instruments were discarded after use in each root canal and irrigation was performed after each change of instrument using 2.0 mL of a 3.0% NaOCl solution (Chlorcid, Ultradent Products, Inc., South Jordan, Utah, USA) followed by 2.0 mL of a 18% EDTA solution (Ultradent Products, Inc., South Jordan, Utah, USA) and a final rinse with 2.0 mL saline. During instrumentation, the canals were flushed with the irrigation solutions using disposable syringes and 30-gauge needles, which were placed to approximately 3–4 mm from the working length without binding. Upon completion of instrumentation the needles could be placed to approximately 2–3 mm from the working length and the root was finally flushed for 1 min with 2.0 mL of 18% EDTA solution, which was washed with 2.0 mL of 3.0% NaOCl solution followed by copious rinsing with 4.0 mL saline. Finally the canals were dried with paper points. After preparation, the specimens were stored in 100% relative humidity at 37°C until further use.

SEM examination

The crowns were removed at the amelo-cemental junction using a fissure bur in a highspeed handpiece. To facilitate fracture into two halves, all roots were grooved longitudinally on the buccal and lingual surfaces with a small round diamond bur, avoiding penetration into the cavity. Finally, the roots were split with a small chisel into two halves. The two halves were dehydrated in a graded series of ethanol solutions, critical point dried, attached to coded stubs, sputter-coated with 10% gold-palladium, and observed with a scanning electron microscope (Stereoscan 100, Cambridge, England, UK). Photomicrographs at x200 (for debris score) and x1000 (for the smear layer) were taken in the apical, middle and coronal thirds of the canals.

Specimen grading

Separate blind evaluations were undertaken by two trained observers for debris and smear layer using reference photographs.

Superficial debris and smear layer were independently subjected to a standardized semiquantitative evaluation in four grades, according to the classification of Gutmann et al. (1994) [17]. Criteria for the scoring were the following:

- Score of the superficial debris (Fig. 1): (A) score 1, little or no superficial debris covering up to 25% of the specimen; (B) score 2, little to moderate debris covering between 25 and 50% of the specimen; (C) score 3, moderate to heavy debris covering between 50 and 75% of the specimen; (D) score 4, heavy debris covering more than 75% of the specimen.

- Score of the smear layer:
  - (A) score 1, no smear layer;
  - (B) score 2, smear layer 1–25% of the canal wall;
  - (C) score 3, smear layer 26–50% of the canal wall;
  - (D) score 4, smear layer 51–100% of the canal wall.
• **Score of the smear layer** (Fig. 2): (A) score 1, little or no smear layer; covering less than 25% of the specimen; tubules visible and patent; (B) score 2, little to moderate or patchy amounts of smear layer; covering between 25 and 50% of the specimen; many tubules visible and patent; (C) score 3, moderate amounts of scattered or aggregated smear layer; covering between 50% and 75% of the specimen; minimal to no tubule visibility or patency; and (D) score 4, heavy smear layering covering over 75% of the specimen; no tubule orifices visible or patent.

**Evaluation**

Scoring was performed in the coronal, middle and apical third of each longitudinal half of the root. For superficial debris, 9 microscopic fields at x200 were randomly assessed in each third of each half-root and 9 fields at x1000 were, respectively, examined for the smear layer. Each field was graded from 1 to 4 according to the scoring system, and the mean value was calculated for each region of each half of the root.

A preliminary series of four teeth, not included in this study, served for training and calibration of the procedure, both for operator and observers. Four photomicrographs, taken as representative of the four grade scoring system for both superficial debris and smear layer, served as visual reference standards throughout the evaluation. Each examiner assigned his score independently from the other.

The data on the score levels were recorded directly onto coding sheets and transferred to a desktop computer. The statistical analyses were carried out by means of nonparametric tests (Mann-Whitney-Wilcoxon test between the groups and Friedman test within the groups). A probability value equal to or less than 0.05 was considered to indicate significance.

**RESULTS**

At x200 and x1000 magnification the instrumented canal walls from both groups appeared smooth and exhibited varying amounts of remaining debris and smear layer along the entire length of the root canal. The mean scores of debris and smear layer between groups re-
corded at coronal, middle and apical thirds are shown in Tables 1 and 2, respectively. In Tables 3 and 4 are shown the mean scores of debris and smear layer at different thirds within experimental groups. However, completely clean root canals were not observed in any group.

**Superficial debris**

In AET instrumentation group the removal of superficial debris appeared more effective in the middle than in the coronal and apical parts of the root, but this was not statistically significant. In K-Flexofile instrumentation group the removal of superficial debris appeared more effective in the coronal and middle than in the apical part of the root, and this was statistically significant by the Friedman test. A statistically significant difference (P<0.05) was noted between the two instrumentation techniques concerning the amount of superficial debris. The manually instrumented canals had less debris than those using AET technique in coronal and middle thirds of the root canals.

**Smear layer**

Few surfaces showed smear layer to be absent and dentinal tubules completely patent (Fig. 3). Amongst the groups, the Mann-Whitney-Wilcoxon test displayed statistically significant differences at the apical level of the root (P<0.05), the AET-prepared teeth showing the lower score (2.6277 AET vs. 3.2385 manual instrumentation). Smear layer removal at the middle third, although slightly more effective with AET instrumentation (score 2.1602 vs. hand 2.55), did not differ significantly according to the same statistical test. Smear layer removal at the coronal third, slightly more effective with hand instrumentation (score 2.186 vs. AET 2.4145), however, this was not statistically significant (p>0.05).

Statistically significant differences for smear layer debridement between the thirds of the root were evident in both groups. In AET group smear layer removal was more effective in middle third and worst in the apical third. In K-Flexofile group smear layer removal was more effective in coronal third. In apical third the amount of smear layer was greatest (mean score 3.2385).

**DISCUSSION**

Neither of the instrumentation techniques achieved total debridement of the root canal, with both debris and smear layer remaining on the dentinal walls. This finding is supported by earlier reports [12]. One source of bias in studies of this kind is the selection of teeth, i.e. identical shapes of root canals in natural teeth are almost impossible to obtain. However, it is essential to use natural teeth in studies such as this [18].

The main advantage of SEM is that it allows evaluation of both halves of the canal wall along their entire length. However, only the surface can be examined, and the depth of debris cannot be determined precisely. Preparation of the specimen may also induce artefacts [12]. Moreover, there are practical limitations for grading the root canal surface when a scoring system is used. In fact, magnification is a compromise between the need to observe large areas of the root internal surface, yet still maintaining the possibility of identifying specific structures. This considered, it is estimated that a sufficiently representative view of the debridement of the root canal was achieved in the present study. One weakness of the evaluation of the micrograph was that the measurements of debris and smear layer were arbitrary and at best ordi-

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**Table 1. Mean differences in the superficial debris score between groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Third of the root</th>
<th>Mean score</th>
<th>SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AET</td>
<td>coronal</td>
<td>1.8200</td>
<td>0.4999</td>
<td></td>
</tr>
<tr>
<td></td>
<td>middle</td>
<td>1.3925</td>
<td>0.2063</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>K-Flexofile</td>
<td>coronal</td>
<td>1.5780</td>
<td>0.2388</td>
<td></td>
</tr>
<tr>
<td></td>
<td>middle</td>
<td>1.36</td>
<td>0.1906</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>AET</td>
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<td>1.7475</td>
<td>0.3494</td>
<td></td>
</tr>
<tr>
<td>K-Flexofile</td>
<td>apical</td>
<td>2.148</td>
<td>0.2969</td>
<td>p&lt;0.05</td>
</tr>
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</table>

**Table 2. Mean differences in the smear layer score between groups**

<table>
<thead>
<tr>
<th>Group</th>
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<th>SD</th>
<th>P value</th>
</tr>
</thead>
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<tr>
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<td></td>
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<td>2.186</td>
<td>0.3877</td>
<td>p&gt;0.05</td>
</tr>
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<td>0.3431</td>
<td></td>
</tr>
<tr>
<td></td>
<td>middle</td>
<td>2.55</td>
<td>0.7421</td>
<td>p&gt;0.05</td>
</tr>
<tr>
<td>AET</td>
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<td>2.6277</td>
<td>0.3240</td>
<td></td>
</tr>
<tr>
<td>K-Flexofile</td>
<td>apical</td>
<td>3.2385</td>
<td>0.5120</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

**Table 3. Scores of superficial debris within groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Third of the root</th>
<th>Mean score</th>
<th>SD</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>AET</td>
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<td>1.8200</td>
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<td></td>
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<td></td>
<td>middle</td>
<td>1.36</td>
<td>0.1906</td>
<td>0.0011</td>
</tr>
<tr>
<td></td>
<td>apical</td>
<td>2.148</td>
<td>0.2969</td>
<td></td>
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</tbody>
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**Table 4. Scores of smear layer within groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Third of the root</th>
<th>Mean score</th>
<th>SD</th>
<th>P value</th>
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<td>0.4404</td>
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</tr>
<tr>
<td></td>
<td>middle</td>
<td>2.1602</td>
<td>0.3431</td>
<td></td>
</tr>
<tr>
<td></td>
<td>apical</td>
<td>2.6277</td>
<td>0.3240</td>
<td>0.00063</td>
</tr>
<tr>
<td>K-Flexofile</td>
<td>coronal</td>
<td>2.186</td>
<td>0.3877</td>
<td></td>
</tr>
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<td></td>
<td>middle</td>
<td>2.55</td>
<td>0.7421</td>
<td>0.00123</td>
</tr>
<tr>
<td></td>
<td>apical</td>
<td>3.2385</td>
<td>0.5120</td>
<td></td>
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</table>
nal in nature. However, there is currently no consensus in the standardization of measurements of debris and smear layer.

It should be emphasized, as with most in vitro studies, that a degree of caution should be exercised in the interpretation of the findings and their extrapolation clinically [19]. Many variables were encountered in the clinical and experimental techniques used in the literature, i.e. freshly extracted or saline- or formalin-stored teeth, instrumentation following decoronation or through a clinical access cavity, different irrigating solutions and/or procedures. This makes every comparison impossible, and could account for the apparent conflict in results [14].

It was not possible to determine whether this incomplete debridement occurred because of the nature of the experimental model. Mastering any new endodontic technique is undoubtedly related to the individual’s learning curve [20], however, our results cannot be explained by operator inexperience, since he had been practising hand instrumentation as well as AET instruments for a significant period prior to this study. Indeed, incomplete debridement appears to be a common problem of SEM investigations [12], which have generally concluded that all hand and mechanical instrumentation and irrigation methods leave debris, both organic and inorganic, within the canal [21]. Present findings are in agreement with these observations, demonstrating that untouched dentinal surfaces are usually left and the aim to provide the optimum cleanliness of the root canal is a theoretical one. Indeed, smear layer removal still remains a controversial issue [16], and, since many other bio-mechanical factors may affect the outcome of root canal treatment, further studies are needed to establish the clinical importance of its absence or presence [2]. In this respect, irrigating solutions and procedures appear more critical than instrumentation techniques. More important factors to be considered are the speed and ease of use, canal shaping ability, reduced apex transportation, and the reliability of instruments under mechanical stress.

Overall at the coronal and middle levels, the canals prepared with AET appeared to have less surface contamination compared with using manual instrumentation. However, some isolated areas of unprepared root canal walls were also present in the AET and manual instrumentation groups. There are several reasons that may explain why AET-shaped root canals have lower debris and smear layer scores than canals shaped by manual instrumentation, especially in apical third. The AET technique was performed with stainless steel instruments used in a 30° reciprocating side-to-side and up-and-down motion. These instruments are stiffer than nickel-titanium rotary instruments and can be easier and with less risk forced towards the root canal walls and the polar recesses during the side-to-side lifting motion. The use of stainless steel instruments in this motion was probably more efficient in following the natural shape of the canals and removing tooth structure [10]. This also yielded a larger preparation with an increased volume of irrigants in direct contact with the root canal walls. Another explanation for the reduced efficiency of the manual instruments in the smear layer removal may be the less taper of K-flexofiles (in compare with AET instruments).

Concerning the efficacy of manual instrumentation, the results suggest that although a step-back technique was used for root canal preparation, the files when used in a circumferential motion were not totally effective in cleaning the root canal walls at the different thirds. This can be explained in that it is possible that the file was not sufficiently forced towards the buccal and lingual recesses, thus leaving areas un-instrumented as well as debris and smear layer behind. Clearly, there is a need to determine the importance of these variables in another study.

Another important fact that needs to be emphasized is that efficient cleaning does not necessarily depend only on the type of instrument or instrumentation technique used. In order to dissolve debris and smear layer, chemical irrigation solutions are recommended along with mechanical instrumentation [22,23,24]. Baumgartner & Mader [25] found that alternating solutions of EDTA with NaOCl was the most effective combination to produce clean root canal walls. Their study demonstrated the importance of using a chelating agent such as EDTA in combination with NaOCl, to effectively remove the inorganic and organic components of the smear layer. Therefore, in this study 2.0 mL of 3.0% NaOCl and 2.0 mL of 17% EDTA was used in an effort to maximize the cleansing of the instrumented canal walls, although perhaps not universally recommended. It can be argued that the use of 2.0 mL saline as a final rinse was not necessary, at least not for this study. However, the authors believe that this was an important step to rid the canal of chemicals that had been previously used. To eliminate variables, equal volumes of irrigants were used for all teeth. A potential variable that may have affected the results for all groups is that the use of irrigants appeared to be less effective in areas that were not or partially instrumented.

Although the time required to prepare the root canals in each group was not recorded, it was our impression that the AET technique was simpler and less time-consuming.

CONCLUSIONS

Complete cleanliness was not achieved by any of the techniques and instruments investigated. Whether this translates into a clinically more successful treatment cannot be determined from this study. It may be inferred that the choice between AET and hand instrumentation should be based on factors other than the amount of root canal debridement. Within the limitations of this study, however, the use of AET is promising and warrants further laboratory experiments and clinical trials.

ACKNOWLEDGEMENTS

We wish to acknowledge Doc. Sigutė Vakrinienė for her assistance with the statistical analysis of the data. The authors wish to thank Ultradent Inc. for their partial support of this project.
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Received: 10 07 2006
Accepted for publishing: 26 09 2006