Evaluation of canal cleanliness and tubular penetration of root canal sealers in extracted primary second molars: a SEM study

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ABSTRACT

Objectives: To evaluate the remaining debris/smear after canal preparation, and the penetration of root canal sealers into dentinal tubules in extracted primary second molars, using scanning electron microscopy (SEM).

Materials and Methods: The widest roots of 120 recently extracted human primary second molars were used. The roots were randomly distributed into four groups, according to instrumentation techniques [conventional stainless-steel hand files / nickel-titanium (Ni-Ti) rotary files] and irrigation solutions [0.5% sodium hypochlorite (NaOCl) and 0.9% saline solution/0.4% chlorhexidine gluconate (CHX)] employed. The debris/smear layer remaining after instrumentation/irrigation, and the tubular penetration of root canal sealers [zinc oxide and eugenol (ZOE)/Apexit Plus (AP)] were evaluated using SEM. All data were analyzed statistically using the Kruskal-Wallis and Mann-Whitney U tests.

Results: No significant difference was found between the debris/smear layer scores based on root canal preparation technique and irrigant solution ($P>0.05$). ZOE cement was unable to enter dentinal tubules, while AP-based calcium hydroxide was able to gain limited entry to the tubules of some roots, but not others.

Conclusions: No differences in canal cleanliness were noted among the instrumentation and irrigant protocols evaluated. AP was found in this *SEM* study to penetrate the dentinal tubules of prepared primary molar root canals more effectively than ZOE; though, penetration with AP was not noted in every root canal.

Keywords: Primary second molar, root canal treatment, debris and smear layer, tubular penetration.

INTRODUCTION

Root canal treatment procedures have been recommended to prevent the premature loss of primary teeth, even those teeth with evidence of severe chronic inflammation or necrosis in the radicular pulp. The success of a root canal treatment depends on the method and the quality of instrumentation, irrigation, disinfection, and three-dimensional obturation of the root canal.

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Recently, several limited in vitro studies related to using the primary teeth of the Ni-Ti rotary files have been prepared.[1-5] These studies explained that Ni-Ti rotary files can be used successfully for root canal treatments in primary teeth.

Irrigants, such as 0.5-1% sodium hypochlorite (NaOCl) or 0.4% chlorhehexidine (CHX) solutions can be used in primary teeth. However, using NaOCl for irrigation in primary teeth can damage peripheral tissues, oral mucosa, and underneath permanent tooth follicles, so it has been suggested that CHX should be used as an alternative to NaOCl.[6]

The penetration of root canal sealers into dentinal tubules is very important for

the success of root canal treatments. Lateral, and apical leakage can be prevented, and penetration can improve the sealing of the root canal system by increasing the interface between the filling material and dentin.[7] Alaçam[8] reported that the penetration of zinc oxide and eugenol (ZOE) was insufficient into dentinal tubules in primary teeth. However, the penetration of Apexit Plus (AP) into dentin tubules in primary teeth have not been studied yet.

The null hypotheses tested were that there would be differences in debris/smear remaining after canal preparation in extracted primary second molars prepared using two different root canal instrumentation methods [conventional stainless-steel hand file and nickel-titanium (Ni-Ti) rotary files] and two different irrigation solutions [0.5% sodium hypochlorite (NaOCl) and 0.9% saline solution combination and 0.4% chlorhexidine gluconate (CHX)]. The second aim of the present study was to compare the penetration of two different root canal sealers [zinc oxide and eugenol (ZOE) and Apexit Plus (AP)] into dentinal tubules in extracted primary second molars using scanning electron microscopy (SEM).

MATERIAL AND METHODS

Sample Preparation:

The widest roots of 120 recently extracted human primary second molars with a score of Res_i (resorption of the root had not yet begun) or $Res_{1/4}$ (resorption of the root was just beginning), according to the root resorption degree scale of Fanning[9], and teeth extracted because of pulpal abscesses or for orthodontic reason were used in this study. After cleaning, the teeth were immersed in 10% formalin at $+4^0$C. The crowns of the teeth were removed at the cementoenamel junction using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL) under water. The pulp tissues were removed with barbed broaches and the working length was determined using periapical radiograph for all the samples. Roots were then randomly distributed into four groups according to instrumentation method and irrigation solutions (N=30/group):

Group 1: Conventional stainless-steel hand file (FKG Dentaire, La Chaux-de-Fonds, Switzerland) + 5 ml 0.5% NaOCl (Sultan Chemists, Inc, Englewood, NJ) and 5 ml 0.9% saline solution (İ.E. Ulagay, Istanbul, Turkey) combination, irrigation for five min.

Group 2: Conventional stainless-steel hand file + 5 ml 0.4% CHX (Drogsan, Ankara, Turkey), irrigation for five min.

Group 3: Ni-Ti rotary files (Protaper Universal, Dentsplay Maillefer, Switzerland) + 5 ml 0.5% NaOCl and 5 ml 0.9% saline solution combination, irrigation for five min.

Group 4: Ni-Ti rotary files + 5 ml 0.4% CHX, irrigation for five min.

Step-back technique was used in conventional stainless-steel hand file for preparation of root canal. Root canal preparation was performed crown down with Ni-Ti rotary files in strict accordance with the manufacturer's recommendations. All roots were prepared for SEM investigation.

SEM Investigation:

Assessment of Debris/Smear Layer

In every group, 10 roots were randomly separated for the evaluation of debris/smear layer scores, according to root canal preparation technique and irrigant solution. One half of each tooth was prepared for scanning electron microscopy (SEM) evaluation. Specimens were mounted separately on aluminum stubs, coated with gold/palladium, examined using an SEM (JSM-6400; Jeol, Tokyo, Japan) operating at 20 kV, and micrographs were obtained.

A trained operator (C.G.), blinded to the treatment group, evaluated the SEM images using the scoring scale of Hülsmann et al.[10] The amount of

debris/smear layer was graded between one and five at 200X and 1000X magnification, respectively.

Assessment of Tubular Penetration

The remaining 20 roots in every group were separated for evaluating the tubular penetration of root channel sealers; half of them were filled with ZOE (Sultan Chemists, Englewood, USA), and the other half were filled with AP (Ivoclar Vivadent AG, Liechtenstein). Radiographs were taken to determine whether the root canals were completely filled. The roots were wrapped in moist gauze and stored in a 37ºC incubator for one week to ensure the proper setting of the sealers. Thereafter, the roots were split longitudinally into two parts. The roots were mounted separately on aluminum stubs, coated with gold/palladium, examined using an SEM, and micrographs were obtained. The tubular penetration of root canal sealers was investigated at 500X magnification.

Statistical Analysis:

All the statistical analyses were done by using a computerized statistical program (SPSS 15.0, SPSS Inc., Chicago IL, USA). The level of significance was set at 5%.

The scores of the SEM evaluation were compared by using the Kruskal-Wallis and Mann-Whitney U tests.

RESULTS

SEM Investigation:

Assessment of Debris/Smear Layer

The results for mean debris and smear layer scores are shown in Table 1. No significant difference was found between the debris/smear layer scores based on root canal preparation technique and irrigant solution (P>0.05).

Irrigation with NaOCl/ Saline Solution Combination

The NaOCl/saline solution combination failed to remove debris. Dentinal tubules were covered with smear plugs (Figures 1 and 2). Although the debris score was found to be higher in Ni-Ti rotary files than a conventional stainless-steel hand files, significant difference was not found (Figure 2).

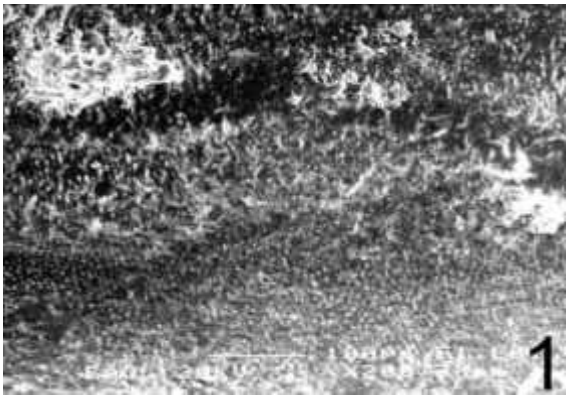

Figure 1. More than 50% of the root canal wall covered by debris in a G1 sample.

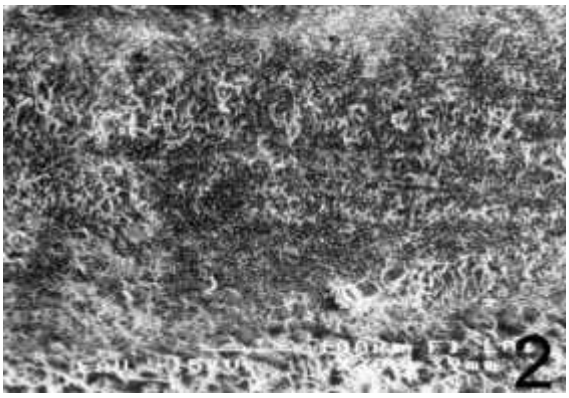

Figure 2. Complete or nearly complete root canal wall covered by debris in a G3 sample.

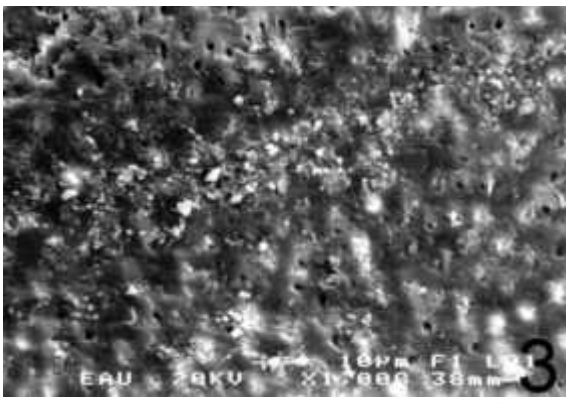

Figure 3. Homogenous smear layer covering the root canal wall, and only few dentinal tubules open in a G1 sample.

The NaOCl/saline solution combination failed to remove smear layer. Dentinal tubules were covered with debris and smear plugs. Only some dentinal tubules were opened (Figures 3 and 4). The smear layer score was found to be similar to Ni-Ti rotary files and a conventional stainless-steel hand file (Figures 3 and 4).

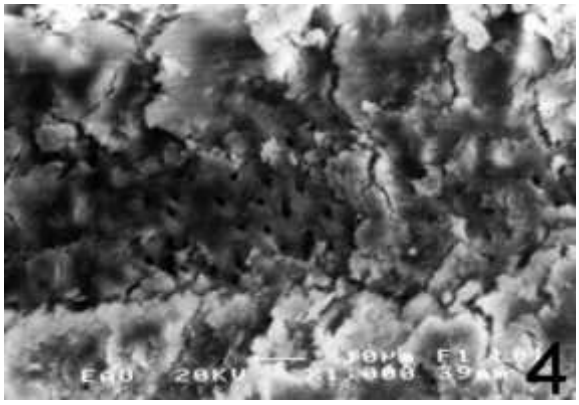

Figure 4. Homogenous smear layer covering the root canal wall, and only few dentinal tubules open in a G3 sample.

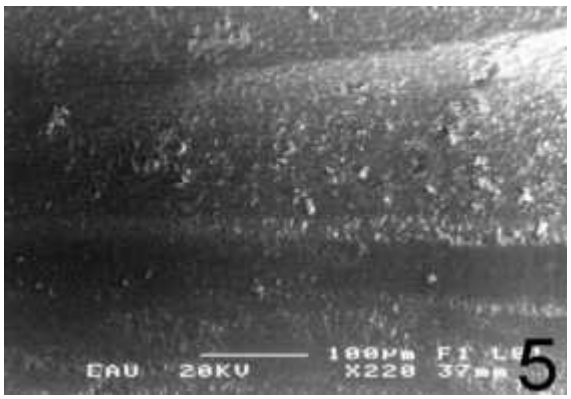

Figure 5. Clean root canal wall, only few small debris particles, cracks, no smear layer in a G2 sample.

Irrigation with CHX

CHX failed to remove debris and smear layer for some samples (Figure 6). However, it was successful in removing debris and smear layer in other samples (Figure 5 and 7). However, no significant difference was found between the irrigation solutions for both debris and smear layer scores ($p>0.05$).

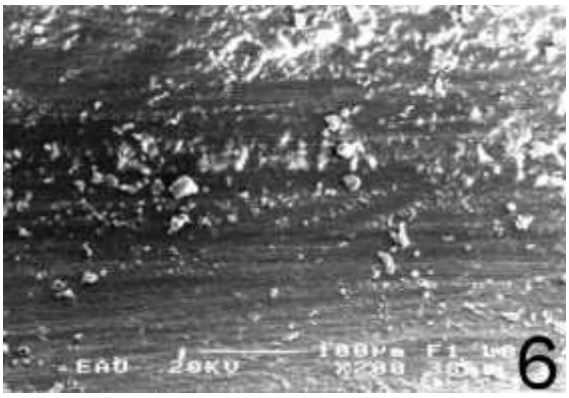

Figure 6. Complete or nearly complete root canal wall covered by debris and heavy smear layer covering the complete root canal wall in a G4 sample.

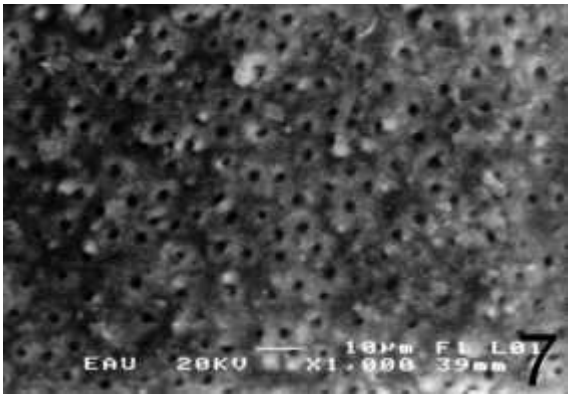

Figure 7. Clean root canal wall, no smear layer, and dentinal tubules open in a G2 sample.

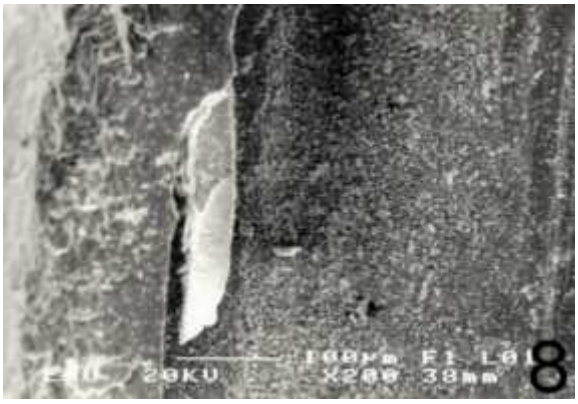

Figure 8. Sample shows that ZOE failed to penetrate dentinal tubules.

Assessment of Tubular Penetration

ZOE cement was unable to enter dentinal tubules (Figure 8), while AP was able to gain limited entry to the tubules of some roots but not others (Figures 9 and 10).

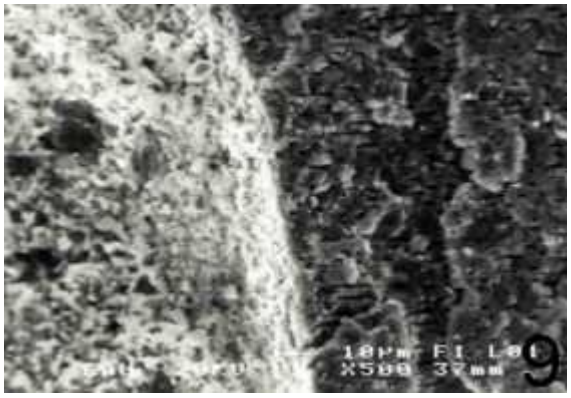

Figure 9. Sample shows that AP limited tubular penetration.

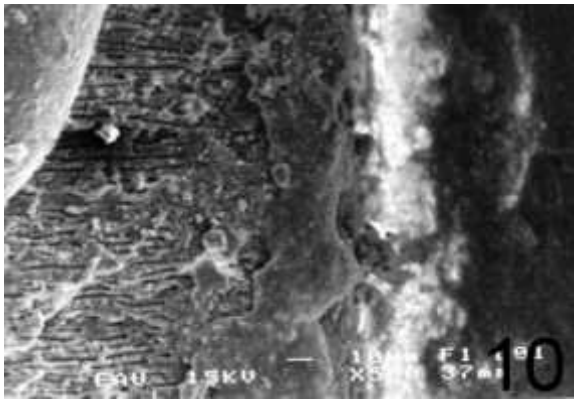

Figure 10. Sample shows that AP failed to penetrate dentinal tubules.

DISCUSSION

Root canal treatment was advocated a method for retaining those primary teeth which would otherwise be lost.[11] An ideal root canal filling material for primary teeth must have several properties, such as resorbing at a rate similar to that of primary root, being harmless to the periaphical tissues and permanent tooth germ, resorbing rapidly if pressed beyond the apex, and being strongly antiseptic. It should easily fill the root canals, adhere to the walls of the canal, not be susceptible to shrinkage, be easily removed if necessary, be radiopaque and nor discolor the tooth. In addition, it should not set to a hard mass which could deflect an erupting succedaneous tooth.[12,13] ZOE paste was the first root canal filling material to be recommended for primary teeth.[11] In addition, as root canal sealer in root canal treatment of primary teeth can be made different materials such as iodoform pate, calcium hydroxide or calcium hydroxide and iodoform paste.[14]

Smear layer can affect to the adaptation into the canal wall of root canal sealers. Organic, bacterial, and necrotic remains in the structure of smear layers can affect the success of endodontic treatment.

McComb and Smith[15] explained that most standard instrumentation techniques produced a canal wall that was smeared and often packed with debris. Besides, researchers explained that both rotary and hand files failed to remove the smear layer.[10,16,17] They reported that irrigation solutions were effective in the removal of the smear layer.[10,16,17]

Cameron[18] explained that the effect of the NaOCl irrigation solution depends on waiting time in a root canal: one min waiting time removed only the surface smear layer, and more waiting time was needed to remove tubular plugs. Thus, five min waiting time was applied in present study.

After the root canal obturation for the setting of root canal sealer, samples waited in a 100% moist environment because test results can be affected before the test.[19] However, samples were not stored completely in water.[19] Thus, samples were wrapped in moist gauze and stored in a 37ºC incubator for one week to ensure the proper setting of the sealers in the present study.

The results of Ferreira et al.[20] showed that distilled water and the 0.2% CHX groups were statistically similar in terms of

a greater amount of debris, whereas 2.5% NaOCl and filtrate obtained after combining 0.2% CHX and 2.5% NaOCl were more efficient in removal of debris. They reported that this can be explained by the lack of tissue dissolving properties of these solutions. Naenni et al.[21] demonstrated that increasing the concentration of CHX to toxic levels did not lead to tissue dissolution.

Table 1. Mean Values and Standard Deviations (SD) for Debris and Smear Layer Scores

Groups	**Debris (mean ± SD)**	**Smear Layer (mean ± SD)**
G1 (Conventional stainless-steel hand file + 0.5% NaOCl and 0.9% saline solution combination)	3,6 ± 1,26 a	3,2 ± 1,54 a
G2 (Conventional stainless-steel hand file + 0.4% CHX)	2,8 ± 1,32 a	2,4 ± 0,97 a
G3 (Ni-Ti rotary files + 0.5% NaOCl and 0.9% saline solution combination)	3,9 ± 0,99 a	3,2 ± 1,03 a
G4 (Ni-Ti rotary files + 0.4% CHX)	2,9 ± 1,37 a	2,5 ± 1,27 a

If marked with the same letter, the difference between the groups is statistically insignificant (P>0.05).

Canoglu et al.[5] reported that Ni-Ti rotary files can be a viable alternative to conventional stainless-steel hand files in primary molars. They found that no difference between rotary, ultrasonic and hand files in regards to dentin removal. Silva et al.[1] reported that there was no statistical difference between manual instrumentation with K files and rotary preparation with Profile 0.4 ISO instruments in terms of cleaning in primary root canal. These results are compatible with our study results. No significant difference was found between the debris/smear layer scores according to the root canal preparation technique in the present study (P>0.05). However, mean debris and smear layer scores were found more in Ni-Ti rotary files than conventional stainless-steel hand files. Pinheiro et al.[22] reported that the manual instrumentation resulted in the lowest amount of debris and the highest amount of smear layer when compared with the rotary and hybrid techniques (P<0.05). They found that no difference between rotary and hybrid instrumentation in degree of debris and smear layer. These results are incompatible with our study results. Differences may be due to use of only palatal canal in our study.

Although significant difference was not found, CHX showed better results when compared to NaOCl in this study. These results are in agreement with Gurbuz et al.[23] The ineffectiveness of NaOCl in removing the smear layer agreed with the previous study.[24] Thus, 0.4% CHX can be used for root canal treatment in primary teeth.

The penetration of root canal sealers into dentinal tubules is very important for the success of root canal treatments. Lateral, and apical leakage can be prevented through penetration, which can improve the sealing of the root canal system by increasing the interface between the root canal sealer and dentin.[7] In addition, canals obturated hermetically through the penetration of the root canal sealer into dentinal tubules.[25]

The studies related to the influence of a smear layer on the adhesion of root canal sealers into dentinal tubules were

limited.[26,27] This study explained that the adhesion of root canal sealers into the dentinal tubulus removal of smear layer was changed according to the type of root canal sealer used.[26,27]

Torabinejad et al.[28] determined the smear layer decreased penetration of irrigation solutions, medicament, and root canal sealers into the dentinal tubules. Şen et al.[29] reported that leakage decreased due to an increase in the depth of penetration. Villegas et al.[30] explained that tubular penetration was affected by instrumentation technique, irrigation solution, and obturation technique.

Alaçam[8] investigated the effect of various irrigation solutions on the adaptation of ZOE sealers in primary teeth, and insufficient adaptations were found in all groups. This result is in agreement with our study.

Finally, mean debris and smear layer scores were found in more Ni-Ti rotary files than in conventional stainless-steel hand files, while no significant difference was found between debris/smear layer scores according to root canal instrumentation methods and irrigation solutions in the present study ($P>0.05$). Leakage can be decreased and clinical success can be increased depending on the penetration of root canal sealers into dentinal tubule. In addition, the biocompatibility of using root canal sealer should be considered for a successful root canal treatment. AP solution showed better results when compared with ZOE, and CHX showed better results when compared with NaOCl in this study. However, these results should be supported with further in vivo and in vitro study.

CONCLUSION

Within the experimental conditions of the present study, the following conclusions can be made:

1) Root canal preparation with Ni-Ti rotary files can be a viable alternative to conventional stainless-steel hand files in primary molars.
2) Root canal irrigation with CHX can be a viable alternative to NaOCl in primary molars.
3) AP was found in this SEM study to penetrate the dentinal tubules of prepared primary molar root canals more effectively than ZOE, though penetration with AP was not noted in every case.

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