Analysis of smear layer removal by different irrigants

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ABSTRACT

Objective
This study assessed in vitro the ability of different irrigating solutions to remove the smear layer after root canal instrumentation.

Methods
Twenty upper human canines were instrumented and irrigated with different irrigating solutions: Group I (positive control) - saline; Group II (negative control) - 17% EDTA in water; Group III - 24% EDTA gel; and Group IV - BioPure MTAD.

Results
Micrographs of the middle and apical thirds were taken for determination of the area percentage covered by smear layer. Analysis of variance and the Tukey test showed that BioPure MTAD and 17% EDTA in water had similar efficacy (p>0.05), producing clean canal walls with open dentinal tubules. Saline and 24% EDTA gel also had similar efficacy, but in this case much of the root canal walls were still covered by the smear layer. The results from Groups I and III were significantly different from those of Groups II and IV (p<0.01). There were no differences between the root thirds of each group (p>0.05).

Conclusion
None of the irrigating solutions were able to completely remove the smear layer from the root canal walls. Biopure MTAD and 17% EDTA in water proved to be more effective for removing the smear layer than 24% EDTA gel or saline.


RESUMO

Objetivo
Avaliar in vitro a ação de diferentes soluções auxiliares quanto à capacidade de remoção da smear layer após o preparo biomecânico.

Métodos
Vinte caninos superiores humanos foram preparados com instrumentos rotatórios ProTaper® (Dentsply-Maillefer, Ballaigues, Suíça), recebendo diferentes soluções auxiliares de acordo com o grupo experimental. No Grupo I (controle positivo), foi utilizado soro fisiológico; no Grupo II (controle negativo), solução aquosa de EDTA a 17%; o Grupo III recebeu gel de EDTA a 24% e o Grupo IV, Biopure® MTAD (Tulsa-Dentsply, Tulsa, OK, EUA).

Resultados
Fotomicrografias dos terços médio e apical foram obtidas e analisadas quantitativamente, obtendo-se a porcentagem de áreas cobertas por smear layer. Os resultados obtidos foram submetidos à análise estatística (teste ANOVA), que indicou o BioPure® MTAD (Tulsa-Dentsply, Tulsa, OK, EUA) e a solução de EDTA a 17% como tendo capacidade semelhante de remoção da smear layer no terço médio (p>0.05). O gel de EDTA a 24% apresentou capacidade de remoção de smear layer semelhante ao soro fisiológico, sendo inferior ao Biopure® MTAD (Tulsa-Dentsply, Tulsa, OK, EUA) ou à solução aquosa de EDTA. Não houve diferenças entre os terços radiculares estudados.

Conclusão
Nenhuma solução irrigadora removeu totalmente a lama dentínaria nos terços estudados. O Biopure® MTAD (Tulsa-Dentsply, Tulsa, OK, EUA) mostrou resultados semelhantes à solução aquosa de EDTA a 17%, e o gel de EDTA a 24% não foi capaz de eliminar de forma satisfatória a smear layer das paredes dentinárias.


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INTRODUCTION

In addition to endodontic instrumentation, the root canal system is also cleaned and disinfected by supplementary solutions that will perform the critical sanitation of the root canal.

The smear layer, a dentinal mud or magma can be defined as an amorphous, irregular and granulose substrate composed of two distinct layers: a 1 to 2 µm superficial layer consisting of organic matter and a second, 6 to 40 µm, more mineralized and deeper layer consisting mainly of dentinal debris. It is produced by the cutting action of endodontic instruments on moist dentin and consists of inorganic matter, odontoblast process fragments, microorganisms and necrotic pulp remnants.

The smear layer can function as an infection route and substrate for bacterial growth, and negatively affect the penetration and adhesion of obturation materials on the dentinal tubules.

Ethylendiaminetetraacetic acid (EDTA) has proven to be the best supplementary solution for the removal of the smear layer from root canals. On the other hand, the few published studies on EDTA gel show that its ability to remove the smear layer is different.

Biopure MTAD is marketed as a bactericidal solution for root canal irrigation capable of removing the smear layer. It consists of a tetracycline isomer (doxycycline), 10% citric acid and polysorbate 80. It has been shown to remove the smear layer better than EDTA.

The objective of the present study was to use scanning electron microscopy (SEM) to determine the ability of different supplementary solutions to remove the smear layer from the walls of the middle and apical thirds of root canals.

METHODS

Twenty upper human canines extracted for different reasons were obtained at the Federal University of Mato Grosso do Sul School of Dentistry tooth bank. All specimens were stored in a refrigerated, 2% glutaraldehyde aqueous solution until ready for use.

The crowns of the teeth were removed by a double-sided diamond disc #7020 (KG Sorensen, São Paulo, Brazil). Canal length was determined by the manual file Flexofile ISO 10 (Dentsply-Maillefer, Ballaigues, Switzerland) and a dental microscope with a magnification of 8x was used to see when the file crossed the apical foramen (DF Vasconcellos, São Paulo, Brazil). The working length was calculated by subtracting 1mm from the canal length.

The canals were instrumented by rotary nickel-titanium ProTaper® files (Dentsply-Maillefer, Ballaigues, Switzerland) as instructed by the manufacturer. At each change of instrument, the canals were irrigated with 3ml of a 5.25% sodium hypochlorite solution (Pharmacêutica, Campo Grande, Brazil).

The specimens were randomly divided into four groups according to the solution used in the last irrigation: Group I – saline (Pharmacêutica, Campo Grande, Brazil) as positive control for the smear layer. Group II – 17% EDTA aqueous solution (Pharmacêutica, Campo Grande, Brazil) as negative control; Group III – 24% EDTA gel (Biodinâmica, Ibiporã, Brazil); and Group IV - Biopure MTAD (Tulsa-Dentsply, Tulsa, OK, USA).

Each supplementary solution stayed inside the canals for 5 minutes. The canals were then rinsed with saline and blotted (Tanari, Manaus, Brazil). The canals in Group IV were irrigated as recommended by the manufacturer, that is, irrigation with MTAD for 5 minutes and no subsequent saline rinsing.

A longitudinal groove was made on the vestibular and lingual surfaces of the roots and a surgical hammer and bieveled chisel were used to cleave the specimens into two parts. One of these parts was randomly selected and prepared for SEM (JEOL JSM-5410, Tokyo, Japan).

The middle and apical thirds of each hemisection were magnified 500 times and representative micrographs were taken from each area. The images were analyzed quantitatively by the software ImageTool version 3.0 (University of San Antonio, Texas, USA), which superposed the images with a grid containing 300 cells. All cells containing smear layer were marked. This cells were analyzed by a previously calibrated doctor in dentistry (emphasis on endodontics), who routinely analyzes micrographs. The results were expressed as percentage of the middle and apical third areas of each group covered by smear layer, calculated by the
software GraphPad Prism v5.0 (GraphPad Software Inc., USA). The groups were then compared by two-way analysis of variance (two-way ANOVA) complemented by the Tukey.

The study was approved by the Research Ethics Committee of the Federal University of Mato Grosso do Sul, protocol number 954.

RESULTS

The results after quantitative analysis of the micrographs are shown in Figure 1.

One of the specimens in Group IV (BioPure MTAD) was lost because of processing error. Figures 2 to 5 show the micrographs of the most representative areas of each experimental group.

The efficacy of the study solutions differed significantly (p<0.001). More smear layer remained in the groups irrigated with saline and EDTA gel, which did not differ from each other (p>0.05). The groups irrigated with 17% EDTA in water or BioPure MTAD had more dentinal tubules exposed, indicating that these substances removed the smear layer more effectively (p>0.05).

The only group whose middle third differed significantly from its apical third with respect to percentage of area covered by smear layer was Group I (saline) (p<0.01). There were no significant differences between the middle and apical thirds of the other groups (p>0.05).

**Figure 1.** Mean and standard deviation of the percentage of smear layer-free area in the middle and apical thirds of the four groups.

**Figure 2.** Micrographs of the apical (A) and middle (B) thirds of the specimens in Group I (saline).

**Figure 3.** Micrographs of the apical (A) and middle (B) thirds of the specimens in Group II (EDTA).
DISCUSSION

Alternating irrigation with sodium hypochlorite and EDTA during instrumentation was proposed by Goldman et al.\textsuperscript{13}, and has since been adopted by endodontists worldwide. Hence, 17% EDTA in water was used by the present study for the last irrigation as negative control, while saline was used lastly as positive control. It is known that this solution, free of chelating agents, is unable to remove the smear layer\textsuperscript{14}.

This study found that Biopure MTAD was not significantly different from 17% EDTA in water in its ability to remove the smear layer (p>0.05), confirming the results of other studies\textsuperscript{15-16}. On the other hand, Torabinejad et al.\textsuperscript{12} reported that MTAD can remove the smear layer more effectively in the apical third than EDTA.

The 24% EDTA gel was not as effective in removing the smear layer as Biopure MTAD or 17% EDTA in water, corroborating the finding. Meanwhile, Dottò et al.\textsuperscript{9}, Sampaio et al.\textsuperscript{10} and Putzer et al.\textsuperscript{11} found that EDTA gel effectively removed the smear layer from dentinal walls.

In the present study, EDTA gel was not capable of removing the smear layer effectively from any of the study thirds. The results obtained by EDTA gel and saline were similar (p>0.05).

None of the specimens irrigated with Biopure MTAD presented regions of dentinal erosion, contrary to what has been reported by Torabinejad et al.\textsuperscript{12}, Tay et al.\textsuperscript{15}, Gonzalez-López et al.\textsuperscript{17} and Grande et al.\textsuperscript{18}.

There were no significant differences between the middle and apical thirds (p>0.05).

CONCLUSION

None of the irrigation solutions tested was capable of fully removing the dentinal mud from the study thirds. The efficacy of Biopure MTAD was similar to that of 17% EDTA in water, both very effective for removing the smear layer. On the other hand, 24% EDTA gel was not capable of removing the smear layer from the dentinal walls effectively, and proved to be similar to saline.
Collaborators

RD GONÇALVES performed the experimental work which included preparing the specimens and taking the micrographs; he also helped to write the article. FHF DOMINGUES helped to write the article and with the literature search. HK ONODA helped to assess the micrographs using the software ImageTool, tabulate the results and write the article. DMZ GUERISOLI supervised the study and was responsible for the study design, statistical analyses and writing of the article. KFS PEREIRA supervised the methodology and writing of the article. GH YOSHINARI conceived the experiment, coordinated the execution team and helped with the experimental tasks.

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