# In vitro study on the action of MTAD and EDTA for removal of the smear layer in the apical third of the root canal

## Estudo in vitro da ação do MTAD e EDTA na remoção da smear layer no terço apical do canal radicular

**Maria F. M. GESTEIRA[1]; Roberto P. C. ARAÚJO[2]; Silvio J. A. SILVA[3]; Carlos ESTRELA[4]; V. VITA[5]**

1 - Associate Professor IV of Endodontics - School of Dentistry, Federal University of Bahia (FO/UFBA);

2 - Titular Professor of Oral Biochemistry - ICS/UFBA. Coordinator of Postgraduate Program on Interactive Processes of Organs and Systems. Institute of Health Sciences, Federal University of Bahia;

3 - Titular Professor Endodontics - FO/UFBA. School of Dentistry, Federal University of Bahia;

4 - Titular Professor Endodontics - School of Dentistry, Federal University of Goiás (FO/UFG);

5 - Department of Dentistry, Federal University of Bahia, Salvador, Bahia, Brazil.

**ABSTRACT**

Objective: This study aimed to evaluate the *in vitro* effects of BioPure™ MTAD® and 17% EDTA on the removal of the smear layer on instrumented teeth with the aid of 1% NaOCl. Methodology: Human single-rooted teeth (*maxillary* incisors and canines) (n=25) were randomly divided into two Experimental Groups (n=10/group) and a Positive Control Group (n=5/group). After the instrumentation, the final irrigation was performed with 10 mL of 17% EDTA/1% NaOCl solution in Experimental Group 1 for 1 minute and with BioPure™ MTAD® in Experimental Group 2. Next, the teeth were cleaved, and the degree of cleanliness of the apical dentin surface was evaluated using scanning electron microscopy (SEM). Results: This study revealed that the 17% EDTA/1% NaOCl solution was more *effective* in *removing* the *smear layer in the apical third of the root canal* compared to MTAD ($p<0.05$). However, statistical analysis of the results revealed that 17% EDTA was more effective in removing the smear layer 6 mm from the apex in EXPG 1-6, which indicates that the more distant the smear layer is from the apex, the more effective is the chelating solution in removing the smear layer. Conclusion: The two tested substances were not fully effective in the *complete* removal of the smear layer in the *apical third* of the root canal, although 17% EDTA/1% NaOCl solution showed greater efficacy when compared to BioPure™ MTAD®, particularly at 6 mm from the apex.

KEYWORDS: Smear layer; EDTA; Endodontics; Root canal; Scanning electron microscopy.

**INTRODUCTION**

Adequate scientific knowledge and operator technical accuracy are necessary for an excellent chemical-surgical *preparation* of the *root canal*. Knowing the internal dental anatomy and seeking to visualize it through magnification, identifying and treating the main root canals and cleaning and shaping these root canals using endodontic instruments associated with chemical substances that play a role in this process are the goals of all endodontic treatments.

Studies have observed that a layer, called the smear layer, is deposited on the dentin surface after root canal preparation. This layer results from mechanical action of either rotary or hand endodontic *instruments* on the dentin wall, leading to the release of dentin chips and organic residues. When mixed with chemicals, these dentin chips and organic residues form a pasty residue that tends to impregnate the dentin surface and those sediments with greater intensity in the apical region of the root canal. The smear layer is more concentrated in this region due to its smaller diameter, which is considered insufficient for the action of *auxiliary chemicals* on the instrument/root canal wall interface.

The smear layer results from a physicochemical phenomenon that occurs during endodontic instrumentation, given that it is not present on uninstrumented surfaces. This layer is a thin and superficial layer of residues that are deposited into the intertubular dentin and dentinal tubule orifices (1-2 μm thick) and can reach *depths of* up to 40 μm, with an average of 10 μm. The smear layer basically consists of inorganic particles from calcified tissues and organic components, including bacteria, *odontoblastic processes*, blood cells and *necrotic* and *vital* pulp tissue. The smear layer is visualized *only* under a scanning electron microscope and exhibits an amorphous appearance, with an irregular and granular surface[1-7].

Due to the presence of microorganisms and their products, necrotic debris and fragments from *odontoblastic processes*, there seems to be a greater tendency toward removal of the smear layer to ensure better action of disinfectant solutions *inside the dentinal tubules*, better mechanical interlocking of filling materials to the walls of the dentinal tubules and better antimicrobial activity for intracanal medications[8-10].

Several studies name 5.25% NaOCl as the most effective substance used during endodontic instrumentation, while 17% EDTA (ethylenediaminetetraacetic acid) followed by 5.25% NaOCl are more often indicated for removing the smear layer and debris[11-12].

Simpler and more effective treatment methods that meet the cleaning and disinfection requirements have been evaluated. Torabinejad et al.[4] introduced the BioPure™MTAD® (Dentsply, Tulsa) in endodontic practice because it combines a tetracycline isomer (doxycycline), an acid (citric acid) and a detergent (Tween 80). When

demonstrating the efficacy of this product in removing the smear layer, the authors also state that this product, in contrast to EDTA, does not cause dentin erosion. MTAD has been described as effective in removing the smear layer, able to eliminate microorganisms resistant to conventional *irrigating solutions* and intracanal medication and capable of effective antimicrobial activity due to the *affinity* of *doxycycline* in *binding* to *dental hard tissues*[13,14].

This study aimed to evaluate in vitro the effects of MTAD and 17% EDTA/1% NaOCl on smear layer removal in teeth instrumented with 1% NaOCl.

## MATERIALS AND METHODS

This study was submitted and approved by the Research Ethics Committee of the School of Dentistry, Federal University of Bahia (Universidade Federal da Bahia - UFBA). The reasons for extraction were not related to this study, and the ethics committee of the Faculty of Dentistry, UFBA, Bahia, Brazil approved the research protocol (process number 102.370). A total of 25 human single-rooted teeth (maxillary incisors and canines), freshly extracted and stored in a container with 0.1% thymol solution, were used. After washing in running water, the teeth were dried and kept in saline solution for rehydration in a bacteriological incubator at 37°C for seven days.

To standardize the sizes of the specimens, a distance of 19 mm was measured from the apex toward the crown using an endodontic ruler. Horizontal sections were obtained using a double-faced diamond disc. Next, longitudinal grooves were prepared on the buccal and lingual surfaces to facilitate the subsequent cleavage of the roots after endodontic treatment.

A single operator with expertise in endodontics performed the entire experimental protocol. After individual clamping of the specimens in a vise, the *shaping of the entrance orifice* of the root *canal* and the removal of secondary dentin deposits were performed, allowing free access to the interior of the root canal.

Once the endodontic access was completed, the working length was standardized by introducing a K-Flexofile #15 instrument (Dentsply- Maillefer) into the root canal until its tip was visualized at the apical foramen, minus 1 mm. All root canals were manually instrumented with 10 mL of 1% NaOCl using the crown-down technique. The first instrument of the apical dentin matrix was defined from the adjustment of the instrument that reached the working length after enlargement in the crown-apex direction. Then, three more instruments were used in thepre-established working length, and new scaling was conducted in the apex-crown direction.

The samples were randomly divided into 3Groups, with 2 Experimental Groups (EXPG1 and EXPG 2) and 1 Positive Control Group (PCG). Upon completion of the chemical-surgical preparation of the root canal of EXPG 1 (n=10),a final irrigation with 10 mL of 17% EDTA and 10 mL of 1% NaOCl was performed, while in EXPG 2 (n=10),5 mL of BioPure™ MTAD® was used for the irrigation according to the manufacturer's instructions.For the PCG (n=5), the procedure was limited to root canal preparation with 1% NaOCl.

Four Experimental Subgroups (EXPG 1.3, EXPG 1.6, EXPG 2.3 and EXPG 2.6) and two Positive Control Subgroups (PCG 1.3 and PCG 1.6) were formed from the main Experimental and Positive Control Groups according to the reading area of the dentin surface where the *photomicrographs were taken*, i.e., 3 mm and 6 mm from the apex.

Once the endodontic procedures and the final irrigation and aspiration were completed, the specimens were dried with an absorbent paper cone and then *dehydrated in* a graded *ethanol series* (80%, 90% and 100%) for approximately one *hour* each grade. The samples were dried in a bacteriological incubator at 50ºC for one hour and then cleaved into two hemi-sections using a chisel and a surgical hammer. On each sample, distances of 3 mm and 6 mm from the apex were recorded with graphite, with the two points being delimited in the apical third to obtain the photomicrographs.

Standardized photomicrographs of the hemi-sections of each specimen were taken using a LEO 1430 scanning electron microscope (Carl Zeiss) under 1000X magnification. For each specimen, the micrograph with better physical integrity was selected. Thus, 25 photomicrographs were taken 3 mm from the apex, and 25 photomicrographs were taken 6 mm from the apex in EXPGs1 and 2 and the PCG.

The images obtained were interpreted according to the following scores: 0 - absence of a smear layer and presence of dentinal tubules free of debris;1 - presence of a smear layer in the dentinal tubules or smear plugs; 2 - presence of a smear layer and dentinal tubules free of debris in the dentin surface areas; 3- presence of a smear layer and dentinal tubules without clear delimitation in the dentin surface areas; 4- marked smear layer on the dentin surface, according to the scale established by Rome, Doran and Walker[15] and adapted by Malvar[16], as shown in Figures 1A, 1B, 1C, 1D and 1E.

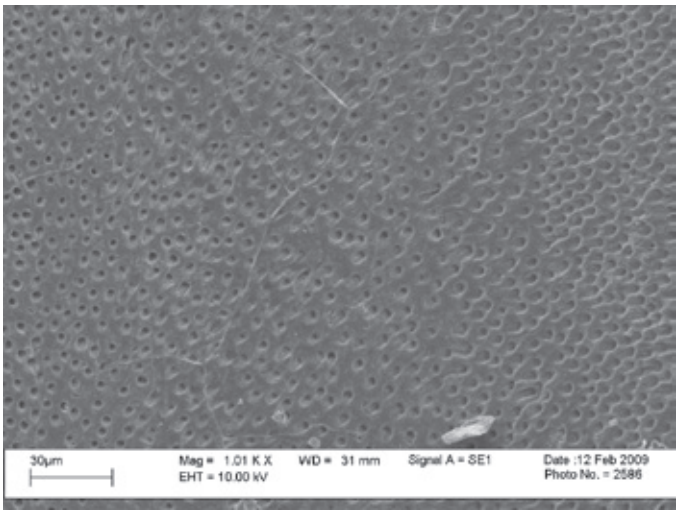


**Figure 1A** – score zero

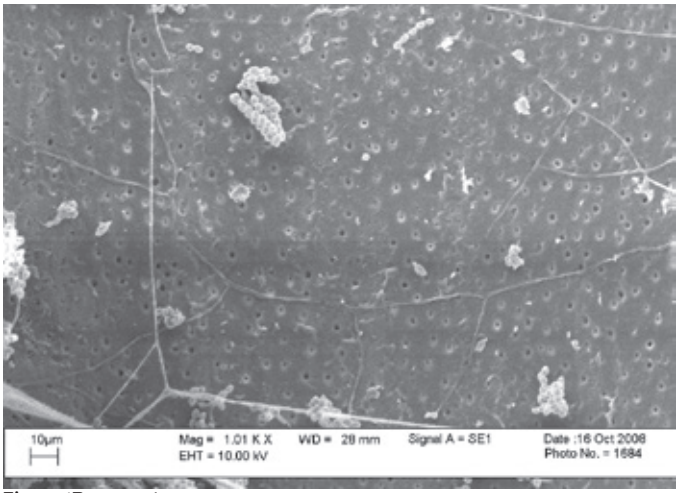


**Figure 1B** – score 1

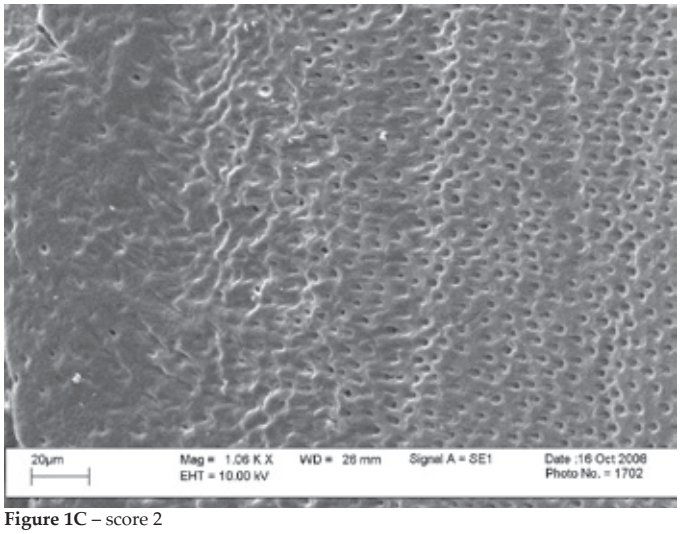


**Figure 1C** – score 2

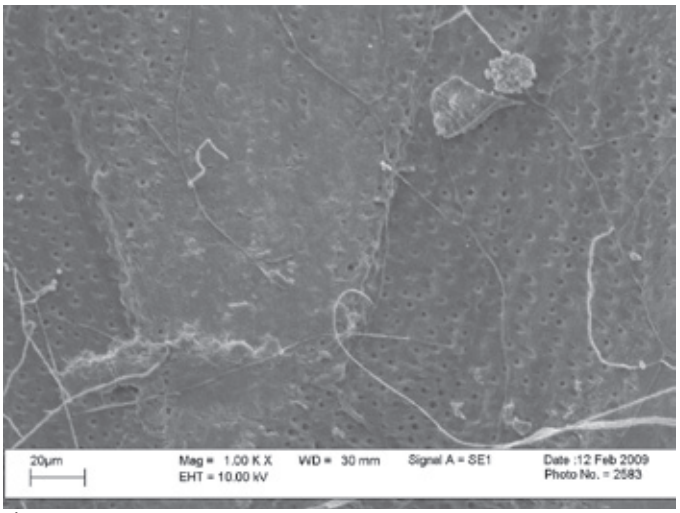


**Figure 1D** – score 3

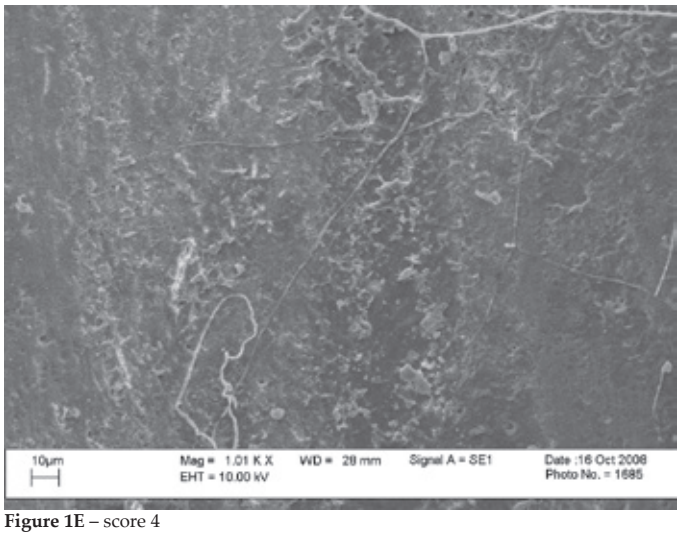


**Figure 1E** – score 4

The results were analyzed using the R statistical package (Development Core Team)[17]. The PCG was compared to the EXPGs, and then the EXPGs were compared among themselves based on descriptive statistics, given that the measurement level of the scale was ordinal qualitative, and multiple comparisons were adjusted using the Benjamini-Hochberg method[18], establishing a level of significance of 5%. The medians of the score reading values were used for the comparisons.

## RESULTS

The values obtained after statistical analysis are shown in Table 1 and are expressed as medians and values of the first quartile (p 25), second quartile or median (p 50) and third quartile (p 75), with $p<0.05$, which enables comparisons between the PCG and the Experimental Subgroups.

The efficacy of the 17% EDTA /1% NaOCl solution used in the final irrigation was statistically significantly different ($p<0.05$) when the two Experimental Subgroups and the PCG were compared, as illustrated in Table 1, indicating cleaner dentin surfaces after final irrigation with the 17% EDTA/1% NaOCl solution. Regarding the removal of the smear layer, the median values revealed that in EXPG 1.6 (p 50=0.5), the dentin surface was qualitatively better than in the other Experimental Subgroups.

Table 1 also shows that maximal scores (score 4) were observed in all Experimental Subgroups that had their surfaces treated with the17% EDTA/1% NaOCl solution or BioPure™ MTAD®, a result that suggests that none of the substances was able to fully remove the residual layer 3 mm or 6 mm from the apex.

When comparing the p values obtained in the Experimental Subgroups that had their apical dentin surfaces treated with MTAD, it appeared that although EXPG 2.6 (p=0.0373) was statistically significant different from EXPG 2.3 (p=0.0501), the difference in the median value (p 50=3.5) was only 0.5 point, a result that indicates that there was no significant qualitative difference between these two Experimental Subgroups (Table 1).

Table 1 - Positive Control Group and Experimental Subgroups: values of the first, second and third quartiles and p values.

| Study groups | Minimal score | Maximal score | p 25 | p 75 | p 50 median | p value <0.05 |
|---|---|---|---|---|---|---|
| PCG | 4 | 4 | 4 | 4 | 4 | |
| EXPG 1.3 (3 mm) NaOCl 17% EDTA/1% NaOCl | 0 | 4 | 2 | 4 | 2.8 | 0.0227 |
| EXPG 1.6 (6 mm) NaOCl 17% EDTA / 1% NaOCl | 0 | 4 | 0 | 3.75 | 0.5 | 0.0108 |
| EXPG 2.3 (3 mm) NaOCl BioPure™ MTAD® | 3 | 4 | 3 | 4 | 4 | 0.0501 |
| EXPG 2.6 (6 mm) NaOCl BioPure™ MTAD® | 3 | 4 | 3 | 4 | 3.5 | 0.0373 |

Considering the interactions between the Experimental Subgroups analyzed, there were no significant differences (p>0.05), indicating that either 3 mm or 6 mm from the apex, the surface--cleaning efficacy was similar for these groups, although the median values indicate a better efficacy for the 17% EDTA/1% NaOCl solution (Table 2).

Table 2 -Comparison between the Experimental Subgroups using descriptive statistics (quartiles). P values are shown.

| GROUPS | EXPG 2-3 | EXPG 2-6 | EXPG 4-3 | EXPG 4-6 |
|---|---|---|---|---|
| EXPG 13 | _ | _ | _ | _ |
| EXPG 1-6 | 0.2492 | _ | _ | _ |
| EXPG 2-3 | 0.2617 | 0.086 | _ | _ |
| EXPG 2-6 | 0.2981 | 0.0881 | 0.8504 | _ |

## DISCUSSION

The importance of the smear layer has been reported by several authors[1,3,6,7,12] due to the presence of microorganisms and their products, necrotic debris and fragments from odontoblastic processes. Thus, it is reasonable to remove this layer to obtain better efficiency of disinfectant solutions within the dentinal tubules, better antimicrobial activity of intracanal medications and better mechanical interlocking of filling materials to the dentinal tubule walls[9,10,13].

The results of the current study demonstrate that the tested substances did not act equivalently in the different Experimental Groups, indicating the need for finding new substances or more effective methods to carry these substances into the root canal, especially in the apical third, which is a difficult region to reach with irrigating solutions. This fact can compromise both cleaning quality and disinfection efficacy[19, 20].

Scanning electron microscopy (SEM) remains a safe method to obtain information about the morphology of the dentin surface and has been used to evaluate the efficacy of several chemicals in removing the smear layer[4]. In the current study, the analysis of the dentin surface by SEM was restricted to the apical third of the root canal, at 3 mm and 6 mm from the apex, a region where the actions of chelating agents in removing the smear layer are limited[16]. The analysis of the photomicrographs revealed that greater accumulation of the smear layer occurs closer to the apex; thus, a greater effort must be taken to achieve better surface disinfection and cleaning. Median values indicated cleaner dentin surfaces at 6 mm from the apex, confirming previous studies that showed that better surface cleaning can be achieved with the distancing of the apical working limit toward the middle and cervical thirds[12, 21].

When used in the chemical-surgical preparation, 1% NaOCl was not able to remove the smear layer from the root surface. Analysis of the photomicrographs from PCG1 and PCG 2 at distances of 3 mm and 6 mm from the apex show thick surface smear layers with irregular and amorphous appearances. These observations demonstrate the inefficacy of 1% NaOCl in removing this layer and the necessity of using chelating substances to remove it. This result is consistent with studies that confirmed the presence of the smear layer in teeth instrumented only with NaOCl[22, 23].

The efficacy of the EDTA alternated with NaOCl is due to the action of NaOCl on the organic components of the root canal system, while EDTA has a sanitizing effect on the inorganic content of the canal walls. Although some authors state that this combination may result in erosion in the dentinal tubule during the removal of the smear layer[24,25], this possibility depends on the concentration and exposure time of the dentinal areas to these substances[26, 27].

Some studies have demonstrated that MTAD has major advantages over 17% EDTA in removing both the smear layer in the entire length of the root canal and organic and inorganic debris. In addition, MTAD does not cause erosions or physical changes in the dentin when the chemical-surgical preparation of the root canal is performed with 1% NaOCl[4-6,13,28]. The erosive effect of 17% EDTA/1% NaOCl solution and MTAD on the apical dentin surface was not evaluated in this study because these two irrigating solutions were not able to fully remove the smear layer present in most of the analyzed specimens, which prevented the full visualization of the dentin surface, an observation that is in agreement with other findings[29].

In the current study, photomicrograph analysis revealed abundant smear layer in the apical third in all studied groups, despite the fact that the median value obtained had indicated a better cleaning efficacy of the dentin surface for the 17% EDTA/1% NaOCl group at6 mm from the apex (EXPG 1.6). In this group, the median value was 0.5 point, with up to 3.5 points of difference compared to the PCG. The results indicated cleaner dentin surfaces in this group, although with no statistically significant difference when compared with GEXP 1.3.

This result corroborates other experimental studies that demonstrated the greater efficacy of chelating solutions in removing the smear layer with increased distance from the apex. Other researchers either observed poor cleaning of the dentin surface in the apical third compared to the cervical and middle thirds[10, 30, 31] or concluded that the final irrigation with 17% EDTA/NaOCl did not produce surfaces free of residues in the apical third[32,33,34]. Conversely, some studies report that the amount of the smear layer removed from the apical dentin surface is acceptable, regardless of the amount of 17% EDTA/NaOCl used and duration of application[35]; some studies also report the full removal of the smear layer in this region in 75% of the samples that used this combination[36].

Although in the current study, the apical dentin surfaces were cleaner in specimens irrigated with 17% EDTA/1% NaOCl based on the median values and the p values<0.05, it is essential to achieve higher cleaning scores for this region.

The median values of the Experimental Groups irrigated with MTAD after chemical-surgical preparation compared with those of the PCG demonstrate that these groups are clinically similar, which suggests a limited action of MTAD in removing the apical smear layer.

In any case, the results indicate the better efficacy of 17% EDTA/1% NaOCl over MTAD in removing the smear layer from the dentin surface, as indicated by the median values. This finding agrees with results obtained in studies that demonstrated that17% EDTA was more effective than MTAD in removing the

smear layer[7,37]. According to some literature on this topic, one must take into consideration that 17% EDTA and MTAD do not completely clean the dentinal walls in the apical third[29], along with the fact that no significant differences are observed when evaluating the presence of smear layers in the cervical, middle and apical thirds after the final irrigation of the root canal with EDTA and MTAD[38, 39]. Finally, still in agreement with the literature, there are reports that MTAD, 17% EDTA and 24% EDTA gel are not able to fully remove the smear layer in the middle and apical thirds of the root canal[31].

However, records from some researchers indicate that MTAD is more efficient in removing the smear layer in all three thirds of the root canal compared to 17% EDTA[4, 6, 13, 14], while other authors emphatically report that significantly cleaner dentin surfaces in the apical third can be obtained when the final irrigation is performed with MTAD[28].Finally, other studies that ensure that MTAD is more efficient compared to 17% EDTA in the apical third of the *root canal*, with the caveat that these two substances are not completely effective in removing the smear layer[23].

The lack of standardization in sample sizes; the different irrigation and aspiration techniques used; and the chemical-surgical preparations performed with closed systems (up to the working length), semi-open systems (with patency or cleaning of the foramen) or open systems (with enlargement of the foramen) may influence the results of the experimental studies published up to now. Linear studies are needed to find a consensus regarding the best substance and/or the best method to remove the smear layer. Given the contradictory findings regarding the efficacy of 17% EDTA/1% NaOCl and MTAD on the removal of the smear layer in the apical third of the *root canal*, further studies using different standardized experimental protocols to more clearly elucidate the efficacy of these two products are needed.

The results of this study demonstrate that neither tested substance was fully effective in removing the apical smear layer. It should be stated that the chelating substances are effective in removing the smear layer in the cervical and middle thirds of the root canal, although these results do not imply that all of the smear layer was fully removed in these regions[16]. However, based on the findings of the current study and given the need for removing the maximum amount of the smear layer, the method of choice certainly will lie in the use of 17% EDTA followed by 1% NaOCl.

## CONCLUSION

According to the methodology used in this study, it can be concluded that 17% EDTA/1% NaOCl solution and MTAD, used for the complete removal of the smear layer in the apical third of the *root canal*, are not fully effective chelating agents, although the 17% EDTA/1% NaOCl solution was clinically more effective when compared to MTAD, particularly at 6 mm from the apex.

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**RESUMO**

Objetivo: Este estudo teve como objetivo avaliar os efeitos in vitro do MTAD e EDTA 17%/ NaOCl 1% na remoção da camada de smear layer em dentes instrumentados com o auxílio de 1% NaOCl. Metodologia: dentes unirradiculares humanos (incisivos e caninos) (n = 25) foram divididos aleatoriamente em dois grupos experimentais (n = 10 / grupo) e um grupo controle positivo (n = 5 / grupo). Após a instrumentação, a irrigação final foi realizada com 10 mL de EDTA 17% /NaOCl 1% no Grupo Experimental 1 durante 1 minuto e com MTAD no Grupo Experimental 2. Em seguida, os dentes foram clivados, e o grau de limpeza da superfície dentinária apical foi avaliado a 3 mm e 6 mm do ápice através do MEV. Resultados: Este estudo revelou que a solução EDTA 17% / NaOCl 1% foram mais eficazes na remoção da camada de smear layer no terço apical do canal radicular em comparação com MTAD ($p < 0,05$). A análise estatística dos resultados, revelou que 17% de EDTA foi mais eficaz na remoção da smear layer a 6 mm a partir do ápice no GExp 1-6, o que indica que quanto mais distante do ápice, mais eficaz é a solução quelante na remoção da camada de esfregaço. Conclusão: As duas substâncias testadas não foram totalmente eficazes na remoção da smear layer no terceiro apical do canal radicular, embora a solução de NaOCl 1% / 17% EDTA mostrou maior eficácia quando comparada ao MTAD, particularmente em 6 mm a partir do vértice

PALAVRAS-CHAVE: Camada de esfregaço; EDTA; Endodontia; Canal Radicular; Microscopia eletrônica de varredura

**CORRESPONDING AUTHOR**
Maria de Fátima Malvar Gesteira
Department of Dentistry, Federal University of Bahia,
Salvador, Bahia, Brazil
E-mail: mfmalvar@yahoo.com.br