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Effect of Passive Ultrassonic Instrumentation as a Final Irrigation Protocol on Debris and Smear Layer Removal—A SEM Analysis

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ABSTRACT This study sought to evaluate the efficacy of passive ultrasonic irrigation (PUI) on removing the smear layer and debris from root dentin using scanning electron microscopy (SEM). Twenty-five bovine incisors were manually prepared and divided into three groups according to the final irrigation protocol: EDTA, final irrigation with 12 mL of 17% EDTA for 3 minutes followed by 5 mL of 2.5% NaOCl; EDTA/PUI, final flush with 4 mL of 17% EDTA and PUI for 30 seconds. These procedures were repeated three times to standardize the volume of the irrigant. Control group, after preparation, the specimens were irrigated only with 17 mL of 2.5% NaOCl. The roots were fractured and analyzed using SEM. The intragroup analysis revealed that the EDTA/PUI protocol removed a higher amount of debris at the cervical third ($P = 0.03$). The intergroup analysis revealed that EDTA/PUI presented the lowest amount of debris at the cervical third ($P = 0.007$). Smear layer scores were higher in the control group compared with the EDTA and EDTA/PUI groups, but only at the cervical third ($P = 0.02$). None of the final irrigant protocols completely removed the smear layer and debris. EDTA/PUI only improved the removal of debris at the cervical third. *Microsc. Res. Tech. 76:496–502, 2013*.

INTRODUCTION

Endodontic biomechanical preparation aims to eliminate microorganisms, pulp tissue, and degenerated and infected dentin to promote healing conditions and tissue repair (Barbizam et al., 2002). This process is accomplished via endodontic instruments, irrigating solutions (Barbizam et al., 2002), and the physical process of irrigation-aspiration (Siqueira et al., 1997).

The morphological features of root canals are complex (De Deus, 1975). The presence of isthmi and their ramifications may harbor infected soft and hard tissues after mechanical instrumentation. This organic content may cause endodontic treatments to fail (Barbizam et al., 2002). Debris consists of dentin chips and pulp tissue attached to the root canal walls after root canal preparation (Hülsmann et al., 1997).

The smear layer was described for the first time in instrumented root canals using scanning electron microscopy (SEM) in the 1970s (McComb and Smith, 1975). The smear layer is an agglomeration of dentin, irrigant solutions, and organic tissues poorly adhered to the root canal walls (Teixeira et al., 2005). This surface film presents two distinct zones: The first is superficial and composed of organic matter and dentin particles; the second is primarily formed of dentin chips that extend ~40 μm into the dentinal tubules (Mader et al., 1984). The smear layer is forced into the root canal walls, especially in the apical third where the mechanical action of endodontic instruments has occurred (Teixeira et al., 2005).

The efficacy of root canal cleaning can be evaluated using the presence of debris and the smear layer after chemo-mechanical preparation (Ribeiro et al., 2012). In this sense, not only is the mechanical action of the endodontic instruments responsible for the root canal cleaning, but also chemical irrigants play an important role in removing debris and the smear layer. The irrigation of the root canal is an essential cleaning procedure in endodontic treatments. Currently, a final irrigation with chemicals such as ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite (NaOCl) is recommended to remove the inorganic and organic components of debris and the smear layer (Carvalho et al., 2008). EDTA is used as a chelating agent to remove and reduce debris and the smear layer (Garberoglio and Becce, 1994), thereby creating better access for disinfectants such chlorhexidine and NaOCl and permitting the diffusion of hydroxyl ions to the calcium hydroxide dressings within the dentinal tubules (Pashley et al., 1986).

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Despite the recent advances in rotary instruments, equipment and techniques used in endodontics, studies have shown that mechanical preparation is not completely effective in removing organic and inorganic debris from the root canals system (Barbizam et al., 2002). In particular, rotary instrumentation may have limited action and be unable to effectively clean the areas of flattened root canals that are most likely infected (Barbizam et al., 2002). Thus, the use of ultrasonic equipment enhances the flushing action of the irrigants (Adcock et al., 2011; Blank-Gonçalves et al., 2011).

Passive ultrasonic irrigation (PUI) activates an endodontic file with an ultrasonic device inside the root canal to mechanically agitate the irrigant without contacting the root canal wall (Van der Sluis et al., 2007). During PUI, the energy of a freely oscillating file is transmitted to the irrigant within the root canal, which results in acoustic streaming (Roy et al., 1994). PUI is characterized by the noncutting action of the ultrasonic file during irrigation to avoid changes in root canal anatomy (van der Sluis et al., 2007). PUI removes debris and agitates the irrigant. This process alters the hydrostatic pressure. This agitation forms bubbles that burst as well as increases temperature and pressure, which results in a wave impact against the walls of the root canal to remove debris. The process of debris removal is also enhanced by the use of a continuous irrigating solution that leads to adequate root canal cleaning (Vansan et al., 1990).

PUI combined with EDTA or NaOCl is more effective than conventional irrigation at removing debris from the root canal (Goodman et al., 1985; Lee et al., 2004). The amount of irrigant and the use of a chelate are more important than the design of the file used to activate the irrigant (Chopra et al., 2008). The effect of PUI is self-limiting with high-volume flushes of irrigant (Chopra et al., 2008). However, dentin debris is easily removed from simulated canal irregularities when PUI is performed rather than conventional irrigation (Lee et al., 2004).

Several authors have proposed protocols for root canal cleaning using ultrasonic devices (Chopra et al., 2008; Kuah et al., 2009; Rodig et al., 2010). Because of the limitation of instrumentation methods, the study of different protocols that appropriately clean the root canal is recommended. The aims of this *in vitro* study were: (1) to evaluate the efficacy of PUI on the removal of the smear layer and debris from the root dentin using SEM; (2) to compare the scores for debris and smear layer between cervical and apical thirds. The tested hypotheses were that: (1) EDTA/PUI would promote better removal of the debris than the other protocols, (2) the smear layer removal would not be influenced by EDTA/PUI; and (3) the cervical third would present lower scores for debris and smear layer than the apical third after EDTA/PUI.

MATERIALS AND METHODS

The present study was submitted to and approved by the Ethical Committee of the Federal University of Rio Grande do Sul. Twenty-five bovine mandibular lateral incisors were used. Initially, the teeth were immersed in 5% NaOCl for 2 h and then stored in saline solution at 5°C. Afterwards, the crown and the cervical portion of the roots were transversely sectioned using a water-cooled diamond disc (KG Sorensen, Barueri, SP, Brazil) to obtain a standardized root length of 16 mm.

A size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was passively introduced into each canal until its tip was visible at the apical foramen. The working length (WL) was established by subtracting 1 mm from this length. Only straight roots with apical diameters equal to the K-file size 20 or size 25 were included in this study. To standardize apical preparation, root canals were prepared using a step-back technique up to a size 60 K-file at the working length. Root canals were irrigated with 2 mL of 2.5% NaOCl after each file change. The NaOCl was delivered using disposable 5 mL syringes (Ultradent Products, South Jordan, UT) and a 30-gauge needle (Endo Eze Tip, Ultradent Products). The needle was advanced into the canal 1 mm from the WL to allow back flow for the irrigant. Next, the teeth were randomly divided into two test groups ($n = 10$) and one control group ($n = 5$) to evaluate the final irrigation protocols.

- EDTA: After preparation, the root canals were continuously irrigated with 12 mL of 17% EDTA for 3 min followed by 5 mL of 2.5% NaOCl.
- EDTA/PUI: Root canals were irrigated with 4 ml of 17% EDTA. Then, an ultrasonic tip ($D_0 = 0.35$ mm and $D_{16} = 0.75$ mm) coupled to the file-holding adapter of the handpiece of a conventional dental ultrasonic device (NAC Plus, Adiel LTDA, São Paulo, SP, Brazil) was used to oscillate inside the root canals at 40 kH for 30 s. After 30 s intervals, the root canals were reflushed with 4 mL of 17% EDTA, and PUI was performed as previously described. Finally, a third irrigation with 4 mL of 17% EDTA and ultrasonic activation was conducted. Finally, a final flushed with 5 mL flush of 2.5% NaOCl was performed.
- Control group: Neither EDTA nor PUI were used. After root canal preparation, the specimens were continuously irrigated with 17 mL of 2.5% NaOCl. Irrigation was performed using syringes (Ultradent Products) and a 30-gauge needle (Endo Eze Tip, Ultradent Products).

SEM Preparation and Analysis

Two longitudinal and symmetrical grooves were performed in the external root surface to facilitate the separation of the samples into halves. Subsequently, a chisel was used to fracture the roots. The halves were randomly chosen for SEM preparation and analysis. The specimens were dehydrated in ascending ethanol concentrations up to 100% and mounted on aluminum stubs. Next, they were coated with gold palladium and examined in a scanning electron microscope (JEOL 6060, JEOL, Tokyo, Japan) operated at 10 kV. The cervical and apical thirds of the roots were used to evaluate the removal of debris and the smear layer after the final irrigation protocols magnified by 2,000×.

The electron micrographs were obtained from the cervical (3 mm from the cervical limit) and apical thirds (3 mm from the apical constriction) of each root canal. They were then transferred and recorded on a CD and evaluated by a trained examiner blind to the

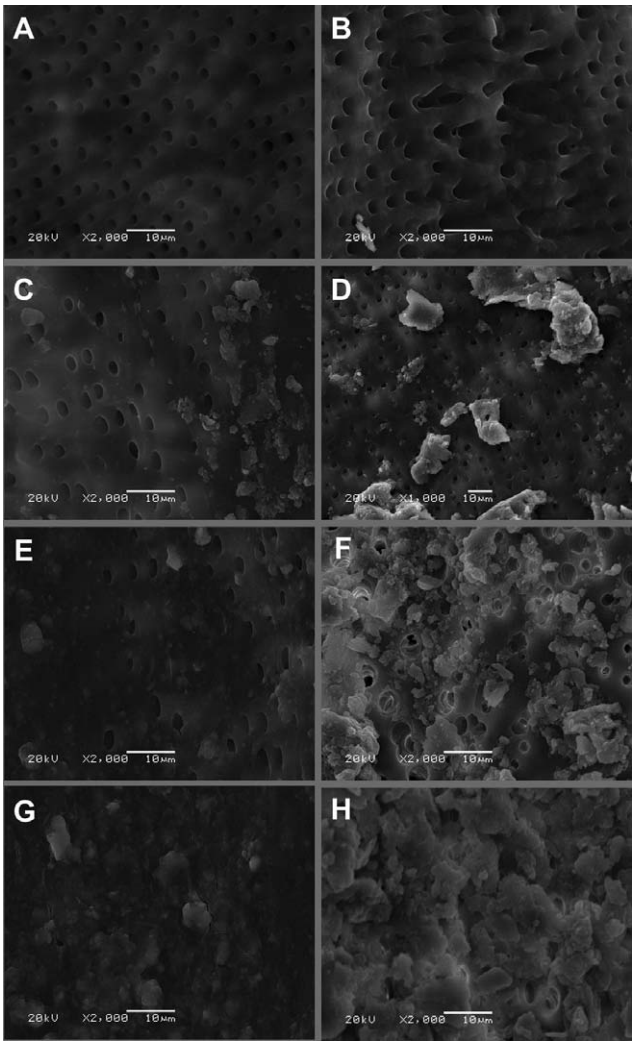

Fig. 1. Representative images of the scores assigned to the smear layer and debris: **A** and **B** = Score 0, **C** and **D** = Score 1, **E** and **F** = Score 2, **G** and **H** = Score 3.

final irrigation protocols. Thus, each micrograph was scored using a semiquantitative analysis with a four-step scale (Chopra et al., 2008) as follows: (1) all dentinal tubules visible; (2) more than 50% of dentinal tubules visible; (3) less than 50% of dentinal tubules visible; and (4) no dentinal tubules visible (Fig. 1).

Cohen's kappa coefficient was used to indicate the reproducibility between the examiner's readings across 7 days. The data were analyzed using the Wilcoxon test for intragroup comparisons as well as the Kruskal–Wallis test and Dunn's multiple comparison analysis for intergroup comparisons (Starview; SPSS, Cary, NC). The significance threshold was set at 5%.

RESULTS

Cohen's kappa coefficient was 0.91. In the intragroup analysis, the final irrigation protocol that used EDTA/PUI removed the greatest amount of debris in the cervical third of the root canal ($P = 0.03$). However, no differences were observed between the control and EDTA groups with regard to the root thirds ($P > 0.05$).

TABLE 1. The means (±SDs) of the debris and smear layer scores for the final irrigation protocols at the cervical and apical root canal thirds

	Debris				Smear layer			
Groups	Cervical		Apical		Cervical		Apical	
EDTA	2.3^{aA}	0.67	2.0^{aA}	0.81	1.2^{aA}	0.63	1.5^{aA}	0.84
EDTA/PUI	1.3^{aB}	0.48	2.1^{bA}	0.73	1.3^{aA}	0.48	1.5^{aA}	0.66
Control	2.4^{aA}	0.89	2.4^{aA}	0.54	2.4^{aB}	0.89	2.4^{aA}	0.54

The same lowercase letters in the same row denote nonsignificant differences in the intragroup analysis (Wilcoxon test). The same uppercase letters in the same column denote nonsignificant differences in the intergroup analysis (Kruskal–Wallis and Dunn *post hoc* tests). The significance threshold was set at 5%.

No group differences existed between the cervical and apical thirds with regard to the smear layer (Table 1).

When the final irrigation protocols were compared (i.e., the intergroup analysis), the EDTA/PUI group presented the lowest mean score of debris in the cervical third ($P = 0.007$); however, no differences existed among the final irrigation protocols in the apical third. Differences were observed between the control and test groups only at the cervical third with regard to the mean smear layer scores ($P = 0.02$). The EDTA and EDTA/PUI groups presented similar smear layer removals regardless of the root third. Figures 2 and 3 show the distribution of debris and smear layer scores, respectively, according to each irrigation protocol and root third. Figure 4 shows the SEM-obtained images for each test group.

DISCUSSION

Chemo-mechanical preparation is primarily used for root canal cleaning because it eliminates bacterial content and sub-products and removes pulp tissue and degenerated dentin (Baratto-Filho et al., 2004). Chemo-mechanical preparation results in the surgical space to promote the appropriate sealing of root canal system (Baratto-Filho et al., 2004). The mechanical action of the endodontic instruments associated with the chemical action of irrigants and the physical actions of irrigation and aspiration comprise the tools used to eliminate the septic and toxic content of root canals. Although technology is incorporated into endodontics, the existing endodontic techniques, instruments and devices cannot thoroughly clean the root canal system.

Because of the difficulty of obtaining single-rooted human teeth, bovine teeth are often used in dental research and have become an alternative for *in vitro* studies. Previous research presented few microscopic differences between bovine and human teeth (Camargo et al., 2007). Importantly, the volume as well as the internal and external morphologies of these teeth is similar to human canines (Camargo et al., 2007).

SEM (Blank-Gonçalves et al., 2011; Chopra et al., 2008; Ribeiro et al., 2012) and optical microscopy (Baratto-Filho et al., 2004; Barbizam et al., 2002) have been used to evaluate root canal cleaning. The advantage of using SEM over optical microscopy is that SEM enables researchers to view both debris and the smear layer (Ribeiro et al., 2012). The present study used 2,000× magnifications to evaluate the effectiveness of the irrigation protocols tested (Chopra et al., 2008).

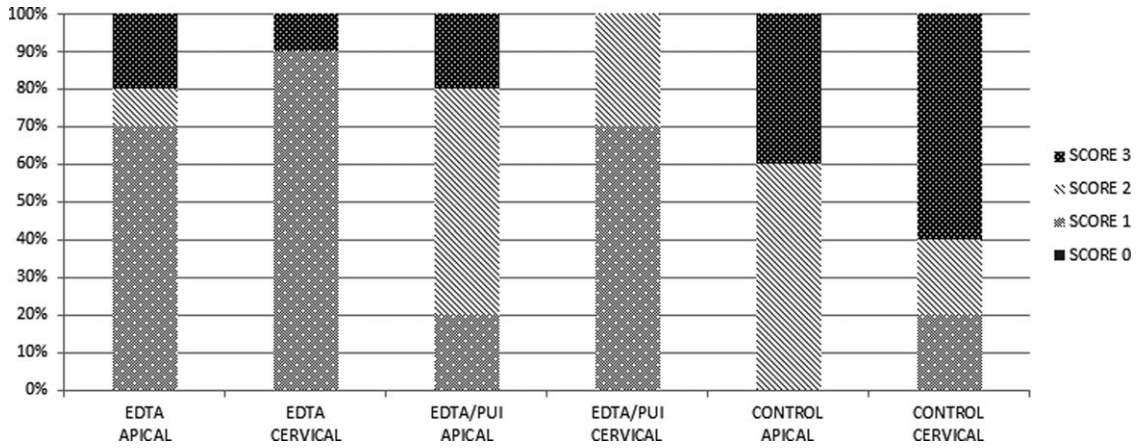

Fig. 2. The distribution of debris scores according to the irrigation protocols.

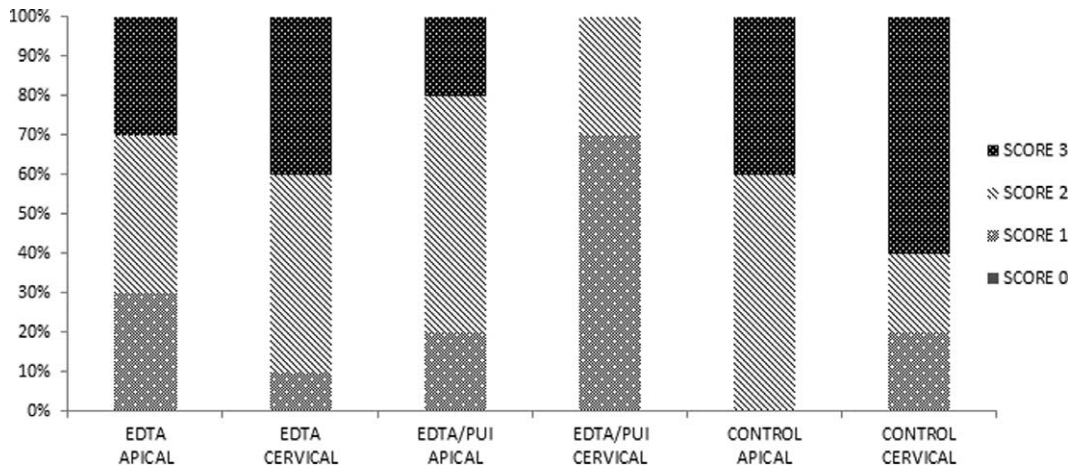

Fig. 3. The distribution of smear layer scores according to the irrigation protocols.

All specimens were irrigated with 2.5% NaOCl solution due to its considerable capacity to dissolve organic tissues. This property cleans root canals by transformation the pulp tissue and necrotic debris into soluble substances such as soap, chloramines, and amino acid salts (Ribeiro et al., 2012). The soaps produced in this reaction maintain fatty bodies in suspension (micelles), thereby facilitating their subsequent aspiration (Spanó et al., 2001). The EDTA used in the test groups was a weak organic acid that has a chelating effect and acts on the inorganic components of the smear layer and root dentin, thereby decalcifying the peri and intertubular dentine matrices (Mello et al., 2010).

In this study, the root canals were irrigated with 12 mL of 2.5% NaOCl, 2 mL after each instrument change during root canal preparation. The irrigant volume simulated a clinical condition, and this protocol matches previous investigations (Rodig et al., 2010; Walters et al. 2002). Likewise, the EDTA volume used in the EDTA and EDTA/PUI groups matches previous studies with similar methodologies (Chopra et al., 2008; Goel and Tewari, 2009). To standardize the volume of the final irrigant, the procedure was conducted for the control group using 17 mL of 2.5% NaOCl.

None of the final irrigation protocols thoroughly cleaned the smear layer and debris from the root canal walls. These results match those of previous studies, including those using SEM (Blank-Gonçalves et al., 2011; Chopra et al., 2008; Goel and Tewari, 2009) and optical microscopy (Barbizam et al., 2002; Baratto-Filho et al., 2004).

The smear layer was not completely removed in the test groups (i.e., EDTA and EDTA/PUI), regardless of the root third. In contrast, the control group showed more smear layer compared with the other groups at the cervical third ($P = 0.002$) (Fig. 2). Thus, the second hypothesis was confirmed, the smear layer removal was not influenced by PUI. On the other hand, the first hypothesis was partially confirmed, once the PUI only promoted better removal of debris at the cervical third.

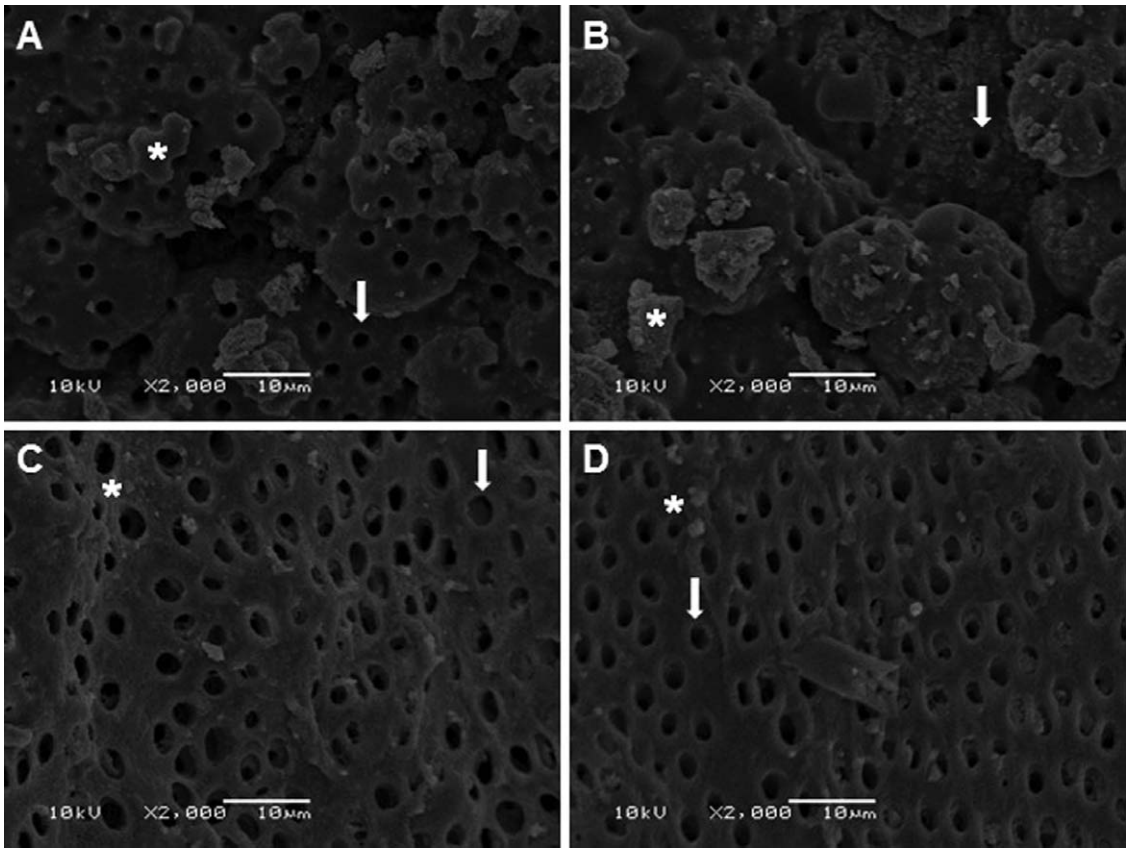

Fig. 4. SEM images after the final irrigant protocols: (**A**) EDTA, cervical third = Score 1 for the smear layer and Score 2 for debris; (**B**) EDTA, apical third = Score 1 for the smear layer and Score 2 for debris; (**C**) EDTA/PUI, cervical third = Score 1 for both the smear layer and debris, (**D**) EDTA/PUI, apical third = Score 1 for the smear layer and Score 2 for debris. Arrows indicate visible dentinal tubules, and asterisks (*) indicate the presence of debris.

The final irrigation protocols showed a similar efficacy in removing the smear layer, with scores ranging between 1.2 and 1.5. These findings confirm the importance of using EDTA to remove the residual layer of the root canal walls. In this study, the chelant remained in contact with the dentin walls for 3 min in both EDTA groups. Previous study has reported satisfactory cleaning levels when EDTA is used in times ranging between 1 and 10 min (Çalt and Serper, 2002). More EDTA time did not improve cleanliness and eventually led to significant erosion of the peri and intertubular root dentin (Çalt and Serper, 2002).

The literature discusses several effects of ultrasonic activation on smear layer removal (Cameron, 1988; Guerisoli et al., 2002). These variations in the results can be attributed to factors such as (1) the correlation between the moment of ultrasonic activation and the stage of instrumentation and (2) the volume and type of irrigation solution used in the final irrigation. Guerisoli et al. (2002) found that ultrasonic activation increases smear layer removal capacity when performed with EDTAC on the root canal. This finding was attributed to a cationic surfactant with low surface tension (Cetavlon), which was added to EDTA, in addition to ultrasonic activation. These results do not match those observed in the current study because ultrasonic activation did not increase EDTA smear layer removal. Hülsmann et al. (1997) and Chopra et al. (2008) also found no effects of ultrasonic activation in smear layer removal, which reinforce the findings of the present study. Moreover, the use of a chelating is critical for smear layer removal (Gambarini and Laszkiewicz, 2002).

The Figure 3 shows a similar distribution of Score 3 (no visible dentinal tubules) for the smear layer in the apical portion of the test groups. Score 1 (more than 50% of dentinal tubules visible) predominated the EDTA group (70%), and Score 2 predominated the EDTA/PUI group (60%); however, this difference did not influence the mean scores for either group in the analyses ($P > 0.05$). Large EDTA volumes have more potential to remove the smear layer compared with ultrasonic activation (Chopra et al., 2008). Figure 3 shows that the control group did not present any visible dentinal tubules in 40% and 60% of the samples related to the cervical and apical portions, respectively. Furthermore, the similarity of the mean scores matches the values observed by Chopra et al. (2008), regardless of the root portion.

The file must remain loose within the root canal without pressing the walls or intentionally removing dentin during PUI to allow the backflow of the irrigant that is responsible for carrying debris and preventing NaOCl extrusion in the periapical tissues (Sabins et al., 2003). During ultrasonic activation, the

oscillation of an instrument with an irrigant into the root canal transmits energy via ultrasonic waves. This action induces a hydrodynamic turbulence that produces cavitations on the irrigant and bubble bursts. These elements increase temperature and hydrostatic pressure, thereby producing waves that remove debris via continuous irrigation (Ribeiro et al., 2012).

EDTA/PUI removed more debris in the cervical portion than the apical root portion ($P = 0.03$). This aspect partially confirmed the third hypothesis. The cervical third was the only section not to have Score 3 (no visible dentinal tubules); and this third showed more than 50% of dentinal tubules (Score 1) in 70% of its cases. The studies of Goodman et al. (1985) and Jensen et al. (1999) can explain this result. Both studies found improvements in root canal cleaning, especially in the middle and cervical thirds. The oscillation of the ultrasonic tips was reduced at the smaller root canal diameter. Because the oscillation amplitude is greatest at the tip of the instrument, any interference might affect the apical portion significantly (Walmsley and Williams, 1989). The current results confirm these concepts because ultrasonic activation had less influence on the apical portion of the root canals. These concepts might explain why no differences were observed in the mean debris scores of the apical region when the groups with and without ultrasonic activation were compared ($P > 0.005$).

The ultrasonic tips had diameters of $D_0 = 0.35$ mm and $D_{16} = 0.75$. The limited space for the tip to move freely in the apical region may be responsible for the lower efficiency in the debris removal of this region as compared with the cervical third. Furthermore, due to the larger diameters in D_0 and D_{16}, and therefore the greater mass, this tip eventually became less flexible, thereby yielding vibrations of lower intensity. Mayer et al. (2002) found no effect of ultrasonic activation or tip diameter (K file # 15, taper 2% or Ni-Ti # 25, smooth and cylindrical) on the removal of the smear layer or debris. This result may have occurred due to the small difference in the diameters between the two instruments (0.1 mm in D_0). The findings of this study suggest further researches, especially with respect to the diameter and stiffness of the ultrasonic tips and their ability to influence smear layer and debris removal from the apical third of the root canals.

CONCLUSION

Given the experimental conditions of this study, it can be concluded that PUI did not improve smear layer removal by EDTA. Moreover, the smear layer scores were similar regardless of the root canal third after the final irrigating protocols. On the other hand, EDTA/PUI removed significantly more debris at the cervical third as compared with EDTA alone.

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