Two lasers x ultrasonic on root canal walls treatment: a scanning electron microscopy study

Dois lasers x ultrassom no tratamento das paredes do canal radicular: um estudo de microscopia eletrônica de varredura

Tratamiento de dos láseres x ultrasonidos en las paredes del conducto radicular: un estudio de microscopía electrónica de barrido

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ABSTRACT
The aim of this study was to assess the efficacy of smear layer/debris removal with EDTA, passive ultrasonic irrigation (PUI), Nd:YAG or GaAlAs laser, using surface topography analysis using scanning electron microscopy (SEM). Forty maxillary canines were decoronated, instrumented and randomly assigned (n = 10): G1 - 17% EDTA; G2 - PUI; G3 - Nd:YAG laser; G4 - GaAlAs laser. The roots were prepared for SEM and the cleanliness of each third of the root canal walls was accessed using a predetermined rating system. Kruskal-Wallis test was performed for statistical analysis. A significant difference was observed for G1 and the other three groups in the coronal third (p < 0.01), between G1, G2 and G4 (p < 0.01) and between G1 and G3 (p < 0.05) for the middle third, and between G1 and G4 (p < 0.01) and G1 and G2 (p < 0.05) in the apical third. Not one sample showed a perfectly clean surface of all levels of the root canal.

KEYWORDS: Smear layer. Ultrasonics. Endodontics.
RESUMO
O objetivo deste estudo foi avaliar a eficácia da remoção de smear layer/detritos com EDTA, irrigação ultrassônica passiva (PUI), laser Nd: YAG ou GaAlAs, utilizando análise topográfica de superfície em microscopia eletrônica de varredura (MEV). Quarenta caninos superiores foram decoronados, instrumentados e distribuídos aleatoriamente (n = 10): G1 - 17% EDTA; G2 - PUI; G3 - laser Nd: YAG; laser G4 - GaAlAs. As raízes foram preparadas para MEV e a limpeza de cada terço das paredes do canal radicular foi avaliada usando um sistema de classificação predeterminado. O teste de Kruskal-Wallis foi realizado para análise estatística. Foi observada diferença significativa para G1 e os outros três grupos no terço coronal (p < 0.01), entre G1, G2 e G4 (p < 0.01) e entre G1 e G3 (p < 0.05) para o terço médio, e entre G1 e G4 (p < 0.01) e G1 e G2 (p < 0.05) no terço apical. Nenhuma amostra mostrou uma superfície perfeitamente limpa em todos os níveis do canal radicular.

PALAVRAS-CHAVE: Camada de esfregaço. Ultrassom. Endodontia.

RESUMEN
El objetivo de este estudio fue evaluar la eficacia de la eliminación de la smear layer con EDTA, irrigación ultrasonora pasiva (PUI), láser Nd: YAG o GaAlAs, utilizando análisis de topografía de superficie mediante microscopía electrónica de barrido (SEM). Se decoronaron, instrumentaron y asignaron aleatoriamente cuarenta caninos maxilares (n = 10): G1 - 17% EDTA; G2 - PUI; G3 - láser Nd; láser G4 - GaAlAs. Las raíces se prepararon para SEM y se evaluó la limpieza de cada tercio de las paredes del conducto radicular utilizando un sistema de clasificación predeterminado. Se realizó la prueba de Kruskal-Wallis para análisis estadístico. Se observó una diferencia significativa para G1 y los otros tres grupos en el tercio coronal (p < 0.01), entre G1, G2 y G4 (p < 0.01) y entre G1 y G3 (p < 0.05) para el tercio medio, y entre G1 y G4 (p < 0.01) y G1 y G2 (p < 0.05) en el tercio apical. Ninguna muestra mostró una superficie perfectamente limpia de todos los niveles del conducto radicular.

PALABRAS CLAVE: Capa de barro dentinario. Ultrasonido. Endodoncia.
INTRODUCTION

Teeth root canal cleaning is the goal of endodontic therapy. Removal of smear layer, vital or necrotic pulp tissue and microorganisms maintain the health of the periradicular tissue, allowing for adequate obturation without leakage. The smear layer is composed of organic and inorganic material consisting of reacted coagulated proteins, necrotic or viable pulp tissue, odontoblastic processes, as well as, blood cells and microorganisms and their by-products. It was firstly reported in instrumented root canals in 1975, and since then various forms for its removal have been proposed.

Through the action of irrigation solutions, tissue debris are removed with the intent to clean the root canal systems. Several studies have shown that a combination of NaOCl and EDTA can be recommended for smear layer removal. Ultrasonics is also being used for cleaning root canals. Additional use of different agitation methods contributes to removal of debris and smear layer. Among the techniques currently used, passive ultrasonic irrigation (PUI) has shown better effectiveness in cleaning the root canal, compared with conventional irrigation. Tissue-dissolving capabilities of solutions with a good wetting ability may be enhanced by ultrasonics if pulp tissue remnants and/or smear layer are wetted completely by the solution and become subject to the ultrasonic agitation.

The use of lasers in endodontics has been reported by several investigators to disinfect the root canal, improvement in sealing and remove the smear layer. Evaluating the efficiency of EDTAC activation using a near-infrared-pulsed 940 nm laser delivered by plain fiber tips into 15% EDTAC or 3% hydrogen peroxide showed that the laser protocol used was more effective for smear layer removal than the ‘gold standard’ protocol using EDTAC with sodium hypochlorite. It has been shown that the effect of Nd:YAG laser irradiation on the dentin of root canal walls caused the fusion of the smear layer, producing a glazed, nonporous surface devoid of organic tissue. However, irradiation with Nd:YAG alone has not been able to sealing dentine surface of apicoectomies, been able to clean root canals when used in combination with hand files showing mostly an absence of smear layer and tissue remnants. GaAlAs lasers have also been used in endodontics. However, only a few studies have reported about dentin treatment or smear layer removal.

The purpose of this study was to evaluate the efficacy of EDTA, PUI, Nd:YAG laser or AsGaAl laser in the removal of the smear layer of prepared root canals. The evaluation was carried out using scanning electron microscopy.

MATERIAL AND METHODS

Specimen Preparation

This study was approved by the Ethics Committee for Research at São Paulo State University, Dental School of Araçatuba, São Paulo State University. Forty recently extracted human maxillary canines with single straight canals were used. They were stored at the Human Teeth Bank in a saline solution, which was renewed once a week until use.

Radiographically it was confirmed that the teeth had one root canal, had no complex root canal anatomy and that no calcification or resorption was present. The crowns were sectioned at the cementoenamel junction with a diamond disc under copious water-cooling (ISOMET 1000 Buehler, Lake Bluff, IL, USA).

Preparation of Root Canals

The working length of the teeth was determined by subtracting 1 mm from the length of a #15 K-file (Dentsply, Milford, USA) when the tip was just visible at the apical foramen. All teeth were cleaned and shaped with Pro Taper Universal (Dentsply Maillefer, Ballaigues, Switzerland) using a crown down technique with apical preparation of a F3 file (Dentsply Maillefer, Ballaigues, Switzerland).

During instrumentation, the canals were irrigated with 5 mL of 1% NaOCl solution, dispensed through a 30-g NavTip needle (Ultradent Products Inc.). The NaOCl solution was followed by a final rinse with 5 mL of distilled water dispensed through a 30-g NavTip needle to prevent NaOCl crystals on the root canal walls. Subsequently, the canals were dried with sterile paper points (Tanari Ltda., Manacapuru, Brazil).

Smear Layer Removal

The teeth were then randomly assigned to 4 experimental groups (n = 10), using specific parameters (Table 1).

Table 1 - EDTA, ultrasonic and laser parameters.

| G1  | EDTA | US | Laser            | Parameter | Penetration | Time | Irrigation/Order |
|-----|------|----|------------------|-----------|-------------|------|------------------|
| G1  | 17%  |     | EDTA             |           | 50 seconds  |      | EDTA             |
| G2  |      |     | Int Septo Front | 30%       | 60 seconds  |      | EDTA + Ultrasonic |
| G3  |      |     | NdYAG            | 50±10 W   | 60 seconds  | 60 µm| NdYAG + Ultrasonic |
| G4  |      |     | GaAs            | 60 µm     | 60 seconds  | 60 µm| GaAs + Ultrasonic |

G1: treatment with 17% Ethylene Diamine Tetraacetic Acid (EDTA) (Herpo Ltda., Rio de Janeiro, Brazil).
solution was dispensed through a 30-g NavTip needle, left in the canal for 3 minutes, followed by a final rinse with distilled water.

G2: treatment with passive ultrasonic irrigation (PUI) (Multisonic Four Plus, Gnatus, Ribeirão Preto, Brazil). IrriSafe tip (Satelec, France) was used at 1mm of the working length using a setting power of 30% with distilled water dispensed through a 30-g NavTip needle and PUI was used at 1mm of the working length for twice 30 seconds activity on the root canal followed by a final rinse with distilled water.

G3: the root canals were dried with paper points and teeth were irradiated with a pulsed Nd:YAG laser (Twin Light Fotona Medical Lasers, Slovenia) sitting on 1.5 W, 100 ml, 10 Hz, t 40s (4 x irradiation for 10s).

G4: the root canals were dried with paper points and then irradiated with a GaAlAs laser (Opto Eletrônica, São Carlos, Brazil). A diode laser (810 ηm, 0.6W, Optical fiber, four applications 10s).

**Scanning Electron Microscopic Preparation and Evaluation**

With a diamond disc at low-speed, a longitudinal vertical groove was made on the buccal and lingual aspect of the root surface, taking care not to perforate the root canal. Then with a chisel, the root was split into two halves. If perforation occurred, the specimen was discarded and replaced. Subsequently, the specimens were dried in ascending series of alcohol, 30, 50, 70, 80, 90, 100% (twice), mounted on aluminum stubs and coated with a layer of gold in a sputter coater (BALTEC-SCD050 Sputter Coater).

The samples were examined at the coronal, middle and apical thirds of the root canal and the presence of a smear layer and overall cleanliness assessed. Four different areas for each third of each root canal were selected. Serial scanning electron photomicrographs at magnifications of 500 x to 1.000 x were made (SEM) (JSM- T330A, JEOL, Tokyo, Japan).

The photomicrographs of this study were randomized and evaluated blindly by three independent calibrated investigators who assigned a score for each representative area of each third root canal. Conflicting scores were discussed until a mutual consensus was reached. Score 0 = no smear layer, dentinal tubules open and free of debris; score 1 = smear layer present only in the openings of the dentinal tubules; score 2 = thin smear layer covers the surface, outline of dentinal tubules indiscernible, tubular openings covered by debris and the location of the tubule indicated by a crack; and score 3 = heavy smear layer, indiscernible tubules openings.

**Statistical Analysis**

The scores were tabulated and subjected to statistical analysis. Kruskal-Wallis non-parametric analysis determined statistical significance between the four groups (p < 0.05 and p < 0.01).

**RESULTS**

The Kruskal-Wallis statistical analyses showed that there were significant differences (p < 0.01) in cleanliness with respect to the smear layer: in the coronal third, between G1 and others groups; in the middle third, between G1 and G2 versus G4 and between G1 and G3; and in the apical third, between G1 and G2 and between G1 and G2. There were no significant differences between G2, G3, and G4 in the cervical, middle and apical thirds (p > 0.01). When the coronal middle and apical thirds of each group were compared, there were no significant differences (p > 0.01), except in G1. There was a significant difference between the apical and coronal third (p<0.01), and between the apical and middle third (p < 0.05) of G1. Table 2 shows ranks average of the thirds. A graphic representation of the SEM evaluation of the effects of various treatments on each third aspect of human teeth is represented in Figure 1.

**Table 2 -** Ranks average of the third coronal, middle and apical.

| Group | Coronal | Middle | Apical |
|-------|---------|--------|--------|
| G1    | 5.65a   | 8.75a  | 13.25a |
| G2    | 24.80b  | 23.70b | 26.60ab|
| G3    | 23.30b  | 19.90ab| 13.90a |
| G4    | 28.25b  | 29.65b | 28.25b |

Different letters show statistical significant difference.

**Figure 1 -** Median of the SEM ratings classified for each group at the third cervical, middle, and apical portion of the tooth.
Representative SEM photomicrographs are shown in Figure 2. SEM findings revealed that the G1 samples had canal walls with open dentinal tubules and an absence of smear layer in the coronal third of most specimens. In the middle third smear plugs were present in the openings of the dentinal tubules, and in the apical third, a thin smear layer covered the surface of the canal walls and the dentinal tubules were covered by debris. In G2, the coronal third was cleaner than the middle third, which had root surfaces and dentinal tubules covered by debris. The canal walls of the apical third had a thick smear layer and the tubular apertures were indiscernible. The G3 in which the Nd:YAG laser was used had canal walls covered with smear layer and debris obscuring the dentinal tubules in most specimens. In the coronal third of some root canal walls, areas were covered with fused smear layer and dentinal tubules. Specimens in G4 presented with a heavy smear layer and tubular openings that were mostly indiscernible.

Figure 2 - Representative SEM photomicrographs of specimens with smear layer and debris score: 0 (A) 1.000x; smear layer and debris score: 1 (B) 1.000x; smear layer and debris score: 2 (C) 1.000x; smear layer and debris score: 3 (D) 1.000x.

DISCUSSION

In this investigation, association of 1% NaOCl solution after each file during the instrumentation and 17% EDTA how final rinsed was used to be compared with ultrasonic, Nd:YAG or Diode laser in the removal of the smear layer. According to some authors16-17 the use of EDTA or NaOCl effectively removes the inorganic and organic components of the smear layer, respectively. In the present study, no specimen showed a perfectly clean root canal at the three levels that were evaluated. EDTA produced statistically the best scores of cleanliness than all other groups in the coronal third, than ultrasound (G2) and diode laser (G4) in the medium third and was better than G4 in the apical third. This fact confirms its potential to remove the smear layer, in agreement with other reports1,4,18.

In the G2, the ultrasonic effect was not able to effectively remove the smear layer. This can be explained by the fact that the solution of distilled water had no chemical action aiding in the removal of the smear layer19. The combination of 5 mL of 17% EDTA and 3 mL of 2.5% sodium hypochlorite (NaOCl) with other devices removed the smear layer from the apical area of curved root canals more effectively than other ultrasonic protocols were used4. Just the physical and mechanical actions of ultrasonic treatment were not effective in producing a smear-free surface20. The duration of ultrasonic treatment in this study could be another possible factor that affected the cleaning ability. One minute of ultrasonic removed the superficial smear layer but left the dentinal tubules covered21, which was also observed in this study. Our study agrees22 regarding the power of 30%, however, the total time recommended by the authors is three minutes. The literature reports three minutes of ultrasonics removed the superficial smear layer and most of the dentinal plugs, while five minutes removed all debris in instrumented and non-instrumented areas21. According to the literature, the efficacy of ultrasonic cleaning is controversial. Although some authors have found favorable results using ultrasonic for root canal cleaning2,22-23 while other investigators did not find a difference between ultrasonic and conventional irrigation24. In addition to passive ultrasonic irrigation (PUI) demonstrate better efficacy in root canal cleansing than conventional irrigation6. Research has shown that PUI was superior to other methods like CanalBrush and the Nd: YAG laser in removing the smear layer25. Concerning the last one, our study showed the inverse.

Laser irradiation can have different effects on the same tissue at different settings26. Its effects depend on the power and mode of energy delivery, type and condition of the target tissue. The wavelength for G4 was different from G3 because G4 and G3 are different types of lasers. Furthermore, the size and shape of the optical fiber that transmits the laser beam is a variable27. In this study, the Nd:YAG laser, with settings of 1.5 W, 100 mJ, 10 Hz, t 40s (4x irradiation for 10s), did not remove debris from the walls of the prepared root canals, corroborating with another study27. In G4, was used a GaAlAs diode laser. When this laser was used with 0.6 W (t 40s, 4 x irradiation for 10s), the worst scores of cleanliness were observed. The negative results on the part of both lasers can be explained by the position of the optical fiber parallel to the walls of the root canal, impairing its effectiveness of action. There are reports in the current literature researching GaAlAs on dentin treatment, which demonstrated closed dentinal tubules25. In the present study, this laser was unable to remove the smear layer with the worst results in the apical third. Studies have demonstrated fusion of the smear layer and its recrystallization by scaling the tubules after the application of the Nd: YAG laser, with the fiber positioned perpendicular to the dentinal walls in cases of apicectomy26-28. However, other research in this situation demonstrates equally negative results13.
For all groups, the results indicated that cleanliness was most difficult to achieve in the apical third, which can be confirmed in previous reports. In recent years, nickel-titanium (NiTi) rotary root canal preparation systems, along with several others, have altered the techniques of canal instrumentation. Different types of files and different kinds of movement aim at the removal of the smear layer and debris to promote better penetration of irritants into the periradicular tissues and sealer adaptation to the canal walls. Associated with these advances, an accurate irrigation protocol must be preconized to achieve higher levels of disinfection of root canals.

As shown by this study, no devices or technique showed a suitable clean on all the thirds of the root canal. EDTA produced the best scores of cleanliness. Further research is needed to investigate the efficacy of these devices to remove the smear layer from the root canal walls, especially in the apical third, comparing parameters and defining their respective best form of application for successful endodontic therapy.

CONCLUSION

No device or technique showed adequate cleaning in all thirds of the root canal.

More researches are necessary regarding the parameters of these devices to obtain better cleaning of the dentinal walls, especially in the apical third of the root canal.

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