Ti:Sapphire femtosecond laser action on the intraradicular dentine: analysis in electronic scan microscopy

Ação do laser Ti:Sa Femtosegundo sobre a dentina intrarradicular: Análise em Microscopia Eletrônica de Varredura

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ABSTRACT
Dental elements with structural loss greater than 50% require the use of root anchorage, characterized by the use of pins. The major obstacles to clinical success of fiber pins are related to adhesion difficulties within the root canal. This work aimed to morphologically evaluate the treatment of the in-root dentinal substrate with Ti:Sapphire Femtosecond laser as a conditioning agent. Human premolars submitted to endodontic treatment were used. Teeth crowns were sectioned and the roots were cleaved and longitudinally separated with diamond discs. The in-root dentin of each specimen was submitted to different surface treatments according to the following groups: G1: only root canal desobturation, G2: root canal desobturation and surface conditioning with 37% phosphoric acid, G3: root canal desobturation and surface conditioning with 37% phosphoric acid and deproteinization with sodium hypochlorite, G4: root canal desobturation and Ti:Sapphire Femtosecond laser application G5: preparation, acid conditioning and Ti:Sapphire Femtosecond application. After the protocols, the roots were submitted to dehydration through successive alcohol baths in concentrations up to 100%, metallized and analyzed in Scanning Electron Microscopy. For G1, the presence of dentin smear layer was observed, in G2 open dentinal tubules were observed, in G3 it was observed the removal of the collagen layer by the action of the hypochlorite, in G4 and G5 groups it was not possible to observe dentinal tubules opening. The Femtosecond Laser Ti:Sapphire was not capable of surface conditioning the in-root dentinal tissue at neither of used strategies.

Keywords: Dentin, Dental Pins, Tissue Adhesion, Laser.

RESUMO
Elementos dentais com perda estrutural superior a 50% requerem a utilização de ancoragem radicular, caracterizada pelo uso de pinos. Os principais obstáculos para o sucesso clínico dos pinos de fibra estão relacionados às dificuldades de adesão dentro do canal radicular. Este trabalho teve como objetivo avaliar morfologicamente o tratamento do substrato dentinário intrarroxial com o laser Ti: Sapphire Femtosecond como agente condicionador. Foram utilizados pré-molares humanos submetidos a tratamento endodôntico. As coroas dos dentes foram seccionadas e as raízes clivadas e separadas longitudinalmente com discos de diamante. A dentina radicular de cada espécime foi submetida a diferentes tratamentos de superfície de acordo com os grupos: G1: apenas desobturação do canal radicular, G2: desobturação do canal radicular e condicionamento superficial com ácido fosfórico 37%, G3: desobturação do canal radicular e condicionamento superficial com Ácido fosfórico a 37% e desproteinização com hipoclorito de sódio, G4: desobturação do canal radicular e aplicação do laser Ti: Sapphire Femtosecond G5: preparação, condicionamento ácido e aplicação do Ti: Sapphire Femtosecond. Após os protocolos, as raízes foram submetidas à desidratação através de sucessivos banhos de álcool em concentrações de até 100%, metalizadas e analisadas em Microscopia Eletrônica de Varredura. Para o G1 foi observada a presença de esfregaço dentinário, no G2 observou-se túbulos dentinários abertos, no G3 foi

observada a retirada da camada de colágeno pela ação do hipoclorito, nos grupos G4 e G5 não foi possível observar a dentina. abertura dos túbulos. O laser Femtosegundo Ti: Sapphire não foi capaz de condicionar a superfície do tecido dentinário intrarroxial em nenhuma das estratégias utilizadas.

Palavras-chave: Dentin, Pinos dentais, Adesão Tecidual, Laser.

1 INTRODUCTION

Functional rehabilitation of dental elements endodontically treated, with significant structural loss, still represents one of the most complex problems of Restorative Dentistry, as they are considered more fragile and susceptible to fractures, which may be associated with the removal of intraradicular dentin structure and/or decreased humidity, which results in alteration of tooth resilience (Zhu, Rong, Wang, & Gao, 2017).

Dental elements with a structural compromise greater than 50%, besides being more fragile, require the use of an intraradicular anchorage, characterized by the use of pins, whose main purpose is to promote retention to the restorative material, either through direct or indirect restoration (Doshi, Kanaparthy, Kanaparthy, & Parikh, 2019). The major obstacles to the clinical success of fiber posts are related to the difficulties of adhesion inside the root canal, which may determine the displacement of the restoration (Yagci, Ustun, Zortuk, & Agirnasligil, 2019).

Dentin adhesion is still poor when compared to enamel, mainly due to its composition and physiology. The dentin tissue is composed of a large amount of organic substance, where the presence of collagen fibers can be evidenced. Although they allow the formation of the hybrid layer, which may contribute to increase the bond strength of fiber-reinforced pins, they are quite susceptible to technical failures, becoming the weak bond of tooth adhesion (Erik, Kaya, Maden, & Orhan, 2020).

In this sense, there are some alternatives to the conventional hybridization adhesive technique, including the use of self-etching resin cements and self-adhesive resin cements, which do not require prior acid etching or do not perform dental surface pretreatment, respectively (Barreto et al., 2016), the use of use of matrix metalloproteinase (MMPs) inhibitors, to inhibit the degradation of the hybrid layer after acid conditioning (de Faria et al., 2017) and the deproteinization of the hybrid layer, which is based on the application of sodium hypochlorite, which alters the ultramorphology demineralized dentin surface by dissolving exposed collagen fibers after acid etching (Jitumori, Bittencourt, Reis, Gomes, & Gomes, 2019).

High power pulsed lasers have also been used as a much newer alternative to promote chemical/morphological changes on the tooth surface to improve adhesion. Ultra-short pulse lasers, including femtosecond lasers, have been tested as a potential tool for dental surface conditioning based on the correct focus, intensity and power parameters, allowing ablation of surfaces with extreme precision and reproducibility. As a result, they cause much less collateral damage to the irradiated material than any other thermal, chemical or mechanical method (Le, Bertrand, & Vilar, 2016a; Portillo Munoz et al., 2012).

A femtosecond laser is an ultrashort pulsed laser with a pulse duration of less than 1ps (1 PS = 10-12 seconds), which is a typical value on commercial lasers of 50-150fs (1 femtosecond = 10-15 seconds). enough to plasma ionize the dental structure without time for thermal diffusion (Loganathan, Santhanakrishnan, Bathe, & Arunachalam, 2019). When treated with the femtosecond laser, the dentin surface has an irregular appearance, no smear layer, open dentinal tubules, complete absence of thermal and mechanical damage, no modification of dentin composition by laser, resulting in significant improvement in bonding (Le, Bertrand, & Vilar, 2016b).

However, there are few studies evaluating the action of this type of laser on intraradicular dentin. Thus, the objective of this study was to morphologically evaluate the treatment and action of the Ti:Sa Femtosecond Laser on intraradicular dentin substrate following two strategies: before and after conditioning with 37% phosphoric acid.

2 METHODS

This study was submitted to and approved by the local Human Research Ethics Committee, protocol n. 99775018.5.0000.5208.

In this experimental study were used five uniradicular human premolars with no apical curvature, healthy or with small coronary restorations, and stored for seven days in Chloramine solution 0.5 %, and then in sterile saline, renewed every seven days, refrigerated, until testing.

The crowns were sectioned transversely with a double-sided diamond disc and discarded to standardize the root height at 15 mm and the roots were submitted to endodontic treatment using the manual ProTaper technique, irrigation was performed with Milton solution (1% NaClO and 16.5% NaCl), and he obturation comprised Cement Sealer 26 (Dentsply Sirona), and gutta-percha points, always according to the manufacturer's instructions. After this step the samples were sealed with provisional restorations (Coltosol/Coltene) and stored in sterile saline (Phormula Active) at 37 ° C for 7 days for complete setting of the provisional restorations.

The root canals were prepared with low rotation Largo Peeso drills n° 2, 3, 4 and 5 (Mailefer/Dentsply), with a cursor positioned at 10.0 mm and finished with the drill post # 1.0 White post DC (FGM) provided by the manufacturer. Root canal was irrigated with saline, stored in hypodermic syringe, at each drill change. After removal of obturator material and dilatation of the root canals, a conduit 10.0 mm long and 5.0 mm below the root apex was obtained.

Then, the roots were sectioned to avoid contamination of the intraradicular dentin by debris obtained from rotary cutting instruments. For this purpose, two opposite and longitudinal orientation grooves were made on the outer surface of each root with a double rotating diamond face at low rotation until two slices of each root were obtained.

The roots were divided (n = 1) according to the dentin treatment received in: G1 - Root canal desobturation only; G2 - Desobturation and surface conditioning with 37% Phosphoric Acid for 15 seconds followed by washing for 15 seconds and drying; G3 - Desobturation, surface conditioning with 37% phosphoric acid for 15 seconds, washing for 15 seconds and drying, deproteinization with 5% sodium hypochlorite for 2 min; G4 - Desobturation, Femtosecond Ti:Sapphire Laser application; G5 - Desobturation, Surface Conditioning with 37% Phosphoric Acid for 15 seconds, washing for 15 seconds and drying, application of Femtosecond Ti:Sapphire Laser. For groups G4 and G5, the following parameters were adopted for Femtosecond Ti:Sapphire Laser application: beam intensities of 50 milliWatts (mW) irradiated on dentin substrate at a speed of 0.05 mm per second (mm/s) (Portillo Munoz et al., 2012).

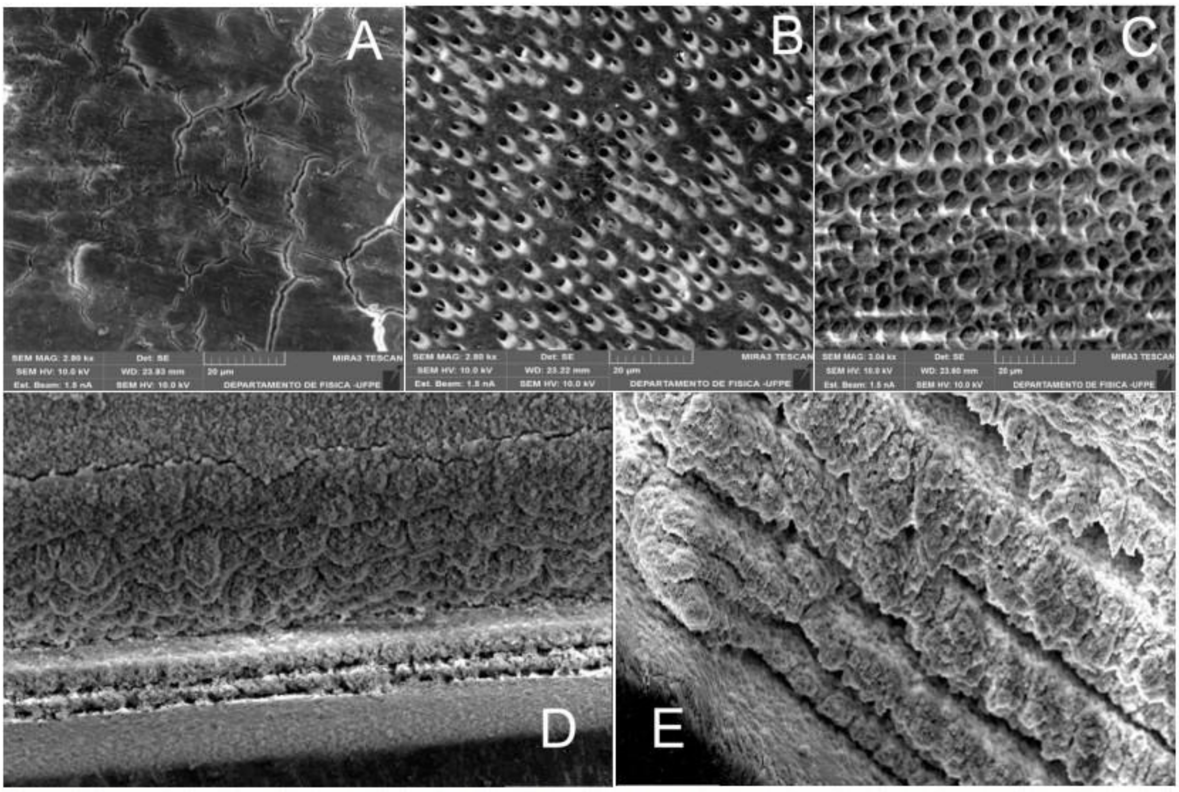
After the treatments already described, the samples were dehydrated by immersion in ascending Ethanol concentrations, following: 50% (20 min), 70% (20 min), 95% (20 min) and 100% (60 min) and dried in silica gel desiccator for 24 hours for solvent evaporation. After drying, the specimens were fixed on glass sheets with double-sided carbon tape and subjected to silver dyeing and metallization (BALTEC SCD 050 Metallizer).

The scanning electron microscope (JSM 5900; Jeol Ltd., Tokyo) readings covered the range corresponding to the middle third of root dentin, which were qualitatively characterized by a blinded specialist in the field of Oral Histology, according to aspects such as: surface cleaning; exposure of dentinal tubules; tubular density, presence of secondary canaliculi.

3 RESULTS

The analysis of the images obtained by scanning electron microscopy revealed, for group G1, a dentin substrate covered by a dense material compatible with smear layer, possibly formed by remnants of endodontic cement and debris resulting from root canal treatment. Due to the presence of this material it was not possible to observe the entrance of the dentinal tubules (Figure 1 - A).

Figure 1. Images obtained from the observation by Scanning Electron Microscope



Surface layer formed by smear layer (A); dentinal tubules exposed after acid conditioning (B); removal of collagen matrix by 5% sodium hypochlorite (C); absence of smear layer removal from dentin tissue after Femtosecond Laser Treatment Ti:Sapphire (D); denaturation of exposed dentinal tissue after acid etching without visualization of tubules (E).

In group G2, the peritubular, intratubular and intertubular dentin layer was observed beyond dentinal tubules entrance. The lumen of the tubules was mostly open, but with the presence of artifacts at the entrance of some tubules (Figure 1 - B) suggesting incomplete removal of dentinal sludge by the action of 37% phosphoric acid.

For group G3, a cleaner surface was observed, absence of smear layer with exposure of dentinal tubules, and a larger amount of secondary tubules not usually observed with only conventional surface treatment (Figure 1 - C).

It was not possible to visualize tubular opening in the treatments performed in G4 (Figure 1 - D), in which only erosion bands in dentin tissue were observed. Similar result was obtained in treatment G5 (Figure 1- E), where bands of tissue with denaturation and superficial destruction are observed, evidenced by irregular areas similar to fractures, without visualization of the tubular entrance.

4 DISCUSSION

Smear layer is an adherent layer of debris on the surface of treated teeth formed by its contact with rotary or manual instruments. The smear layer is revealed by scanning electron microscopy as a granular substructure that completely covers the dentin, being approximately 1-2 µm thick. The holes in dentinal tubules are obliterated by extensions of these debris, called smear plugs, which may extend to a depth of 1-10 µm (Suyama et al., 2013). Substrate analysis after root canal desobturation showed, in this study, a high density aspect of dentin sludge disposed over the entire dentin length, showing obliteration of dentinal tubule entrances, as shown in Group 1 (Figure 1 - A).

The complete removal of smear layer by etching with phosphoric acid followed by washing is a step of conventional adhesive technique. The dentin surface is morphologically altered due to the dissolution of hydroxyapatite crystals, leading to a wide opening of the dentinal tubules, exposing collagen fibers (Cardoso et al., 2011; Suyama et al., 2013), as shown in the images obtained from Group 2 (Figure 1- B), corroborating with the descriptions presented in current literature.

However, debris remains on the root dentin even after 15 seconds of acid etching (Figure 1- B), suggesting some difficulty of this etching method in acting over the entire canal extension. The use of phosphoric acid in gel form is justified to enable control of the application area, which would not happen in presentations with lower viscosities, making them difficult to manipulate. Higher viscosity allows material to not flow promoting greater application control and lower viscosity allows for greater wetting and a lower contact angle, closer to zero. Thus, the use of phosphoric acid in the liquid presentation would have benefits at the root level, since particularly in this case the higher flow allows the contact between the acid and walls that are not normally visible, promoting a better removal of the smear layer in poorly accessible surfaces (Costa Scholz et al., 2020; Salas et al., 2011).

While acid etching only removes hydroxyapatite and exposes the intertubular network of hydrated collagen fibers, subsequent deproteinization of demineralized dentin with sodium hypochlorite removes collagen and exposes several lateral secondary tubules that are not commonly seen on etched dentin surfaces without sodium hypochlorite treatment (Barreto et al., 2016). Thereby, the use of sodium hypochlorite after acid etching creates a more porous dentine structure, as seen in G3 (Figure 1 - C), with multiple irregularities, rich in hydroxyapatite crystals and high wetness, being similar to that of phosphoric acid conditioned enamel, facilitating the access of resin monomers to a more permeable substrate, promoting increased restoration longevity (Hashimoto et al., 2000).

Since its inception in the 1960s, Laser technology has been studied in the dental field due to the wide range of applications of laser light in both soft and hard tissues. High power pulsed lasers have been used as an alternative to promote chemical/morphological changes on the tooth surface in order to improve adhesion (Silva, Melo, Ferreira, Oliveira, & Gutknecht, 2019). An attractive feature of using laser technologies would be to not cause thermal damage to the adjacent tissue, so this technology would be a possible replacement for conventional rotary instruments that generate excessive heat and vibrations due to friction with the dental surface (Le et al., 2016a, 2016b).

The ablation in the ultrashort pulse regimen occurs through the plasma mediated mechanism. The material is heated on a picosecond (ps) time scale, with a direct transition from solid phase to plasma followed by rapid hydrodynamic expansion and ablation. This process occurs in order of magnitude faster than thermal diffusion to adjacent tissue, preserving the structure and properties of the irradiated material (Vogel & Venugopalan, 2003).

In this study, the surface treated with femtosecond Ti:Sapphire laser revealed a superficial denaturation of dentin, where it was not possible to observe the efficiency of the laser in the superficial treatment for the proposed parameters, suggesting the need of use of less radiation power in laser irradiation. Since ablation efficiency of the femtosecond laser on dentin and enamel is closely related to the laser fluence and may reach a maximum when the laser fluence is set to an appropriate value (Chen et al., 2015).

Apparently, when energy is increased, the volume of dentin removed also increases, changing the morphology of the adjacent dentin and a greater sealing of dentinal tubules (Portillo Munoz et al., 2012). Under the average laser fluences of 1.13 to 3.68 J/cm², clean ablated surfaces without debris and microcracks can be obtained. Laser fluence influences the ablated diameter and depth, whereas under a certain fluence, pulse number only affects the depth, without affecting the diameter (Ji et al., 2012).

The limited number of studies that currently exist on ablation of dental tissues with ultrashort lasers, similar to the laser used in this study, suggest that human dentin can be successfully ablated with these devices, in addition, application of subpicosecond pulses almost completely prevents thermal damage and microcracking in dentine tissue (Ji et al., 2012). Therefore, there is a need for further studies on the use of Femtosecond Laser as an alternative to conventional methods, in order to circumvent the failures obtained by them, through greater control and variability of parameters combined with mechanical test results to prove whether this type of treatment is indeed a viable promise for this purpose, aiming to improve the characteristics and increase the adhesion to the substrate. The use of reasonable control for each of these parameters will improve future clinical application, favoring a better retention of the restorative materials.

5 CONCLUSION

The Ti:Sapphire Femtosecond Laser was not able to satisfactorily condition the intraradicular dentin tissue in any of the strategies used.

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