Evaluation of the dentin changes in teeth subjected to endodontic treatment and photodynamic therapy

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Resumo

Introdução: A PDT antimicrobiana é um eficiente método coadjuvante na desinfecção do sistema de canais radiculares, o que torna importante o estudo sobre a possibilidade de alterações morfológicas e permeabilidade provocadas pelo laser de diodo na estrutura dentinária radicular. Objetivo: A proposta deste estudo foi investigar as alterações morfológicas e percentual de infiltração apical radicular após o uso do laser diodo. Material e método: Quarenta dentes monorradiculares foram instrumentados pelo sistema rotatório Protaper e irrigados com NaOCl 5,25% e EDTA 17%. Depois, foram divididos aleatoriamente em: G1: não receberam PDT (controle); G2: receberam pré-irradiação com fotosensibilizador azul de toluidina e irradiação com laser diodo de AsGaAl. Em seguida, 10 dentes de cada grupo foram analisados por MEV quanto as alterações morfológicas. Os outros 10 dentes foram obturados e corados por Rodamina B para avaliar o percentual de infiltração apical. Resultado: Os resultados mostraram diferença significativa entre G1 e G2 (p<0,001 - qui-quadrado), verificando maior presença de debris em G1 (conrole) e maior ocorrência de túbulos dentinários abertos em G2 (PDT). Erosões e cracks foram observadas em ambos os grupos, com diferença estatística significativa (p>0,001). O percentual de infiltração apical foi significativamente maior em G2 que em G1 (p<0,001 – t student). Conclusão: Concluiu-se que a utilização do laser diodo promoveu a redução de smear layer e abertura dos túbulos dentinários, não influenciando na ocorrência de erosão, cracks e carbonização. O laser aumentou a permeabilidade da dentina apical.

Descritores: Fotoquimioterapia; laser; endodontia.

Abstract

Introduction: Antimicrobial photodynamic therapy (PDT) is an efficient adjuvant technique to promote disinfection of the root canal system. Therefore, it is important to investigate changes to dentin morphology and permeability induced by the use of diode laser on the root dentin. Objective: The purpose of this study was to investigate morphological changes and the percentage of apical leakage after the use of laser. Material and method: Forty single-rooted teeth were instrumented using rotary system and irrigated. Teeth were randomly divided in two groups: G1 - not exposed to PDT (control), and G2 - pretreated with toluidine blue photosensitizer and irradiated with AsGaAl laser diode. Ten teeth in each group were evaluated by SEM for morphological changes. The other ten teeth were filled and stained with Rhodamine B to evaluate the apical leakage. Result: The results showed significant difference between G1 and G2 (p<0.001 - chi-square), with greater presence of debris in G1 and higher incidence of open dentinal tubules in G2. Erosions and cracks were observed in both groups, with no statistically significant difference (p>0.001). The apical leakage was significantly higher in G2 than in G1 (p<0.001 – Student’s t-test). Conclusion: It was concluded that the use of low-level laser reduced the smear layer and opened the dentinal tubules. Use of laser increased the permeability of the apical dentin.

Descriptors: Photodynamic therapy; lasers; endodontics.

INTRODUCTION

Persistence of microorganisms in the root canal system (RCS) is the main cause of endodontic therapy failure owing to the presence of metabolic products and to formation of biofilm, which acts as a source of infection. These microorganisms may colonize dentinal tubules, accessory canals, isthmuses, and apical deltas, rendering their eradication difficult solely by instrumentation, irrigation, and use of intracanal medications1.

Additionally, the host defenses or microbial agents are often unable to reach the microorganisms remaining within the RCS or in extraradicular regions2. In these situations, endodontic therapy...
requires complementary techniques to enhance the antimicrobial action and achieve decontamination.

Low-level laser irradiation has been proposed as an adjuvant to chemomechanical preparation (CMP) of the root canal in cases of infection, with the goal of increasing the efficacy of endodontic treatment. Several researchers have studied the bactericidal effect of low-level laser irradiation, in a procedure known as photodynamic therapy (PDT)

In PDT, microorganisms are exposed to a light-sensitive compound and become susceptible to irradiated light, which induces a photochemical reaction that generates free radicals and singlet oxygen. This leads to rupture of bacterial cell walls and destruction of the microorganisms.

However, the effects of this technology on the dentin structure should be investigated, in view of the possibility that it may induce morphological changes to the dentinal tubules and to dentin permeability, which in turn could hinder the sealing ability of filling cements. If the sealing ability is impaired, reinfection of the RCS may occur, allowing the formation of new sources of infection and culminating in endodontic treatment failure.

With this in mind, the aim of the present study was to evaluate in vitro, by quantitative and qualitative analyses, the changes in permeability of dentinal tubules and of the dentin structure after PDT using a 660nm laser diode as an adjuvant to CMP.

**MATERIAL AND METHOD**

This study protocol was approved by the Ethics Committee of the Federal University of Juiz de Fora. Forty single-rooted extracted human teeth were selected for this study. The presence of a single canal was determined by mesio-distal and buccal-lingual digital radiographs and confirmed after conventional root canal access with diamond and Endo-Z burs (Dentsply/Maillefer, Switzerland). The working length (WL) was established by inserting a size 10 K-file (Dentsply/Maillefer S.A., Petrópolis, Rio de Janeiro, Brazil) into the canal, until its tip was visible at the apical foramen under 10X magnification. The endodontic rubber stop was placed against the edge of the tooth and the file was removed from the canal. The distance from the file tip to the rubber stop was measured on the edge of the tooth and the file was removed from the canal.

Following that, the teeth were subjected to instrumentation, irrigation with sodium hypochlorite at 5.25%, irrigation with 17% EDTA; sodium hypochlorite at 5.25%, irrigation with 17% EDTA; sodium hypochlorite at 5.25%, irrigation with 17% EDTA; and, after that, irradiation with low-level laser (PDT).

**Low-level Laser Irradiation**

Teeth in G2 had their root canals filled with 6 µg/mL toluidine blue stain (TBO) (Fórmula & Ação, São Paulo, Brazil) for 5 minutes, by means of a 0.33 cc insulin syringe. Low-level 660 nm AsGaAl (arsenide, gallium, aluminum) laser was applied for 5 minutes using a continuous wave red laser diode unit (Photon Lase III, DMC Equipamentos Ltda., São Carlos, SP, Brazil). The laser parameters were: 600 nm wavelength, width of approximately 10nm, 100 mW power, and 12 J energy.

A 400 µm optical fiber was attached to the laser unit, inserted into the root canal up to the WL and the laser was activated. Then, the tip was slowly withdrawn while touching the buccal surface of the root canal wall. Afterwards, the same procedure was performed with the tip touching the palatal, mesial, and distal surfaces of the root canal wall.

Following that, the teeth were subjected to morphological analysis by scanning electron microscopy (SEM) and to evaluation of the apical leakage.

**Morphological Analysis of the Root Dentin**

Ten teeth from each group were randomly selected, cleaved in two fragments and examined under an SEM microscope (model JEOL JSM-T220 A) operating at 15 kV. Photomicrographs were taken under 500X and 3000X magnification. A screen with a central square measuring 20 x 20 cm was placed on the computer monitor, in order to outline the area to be analyzed. This allowed the apical third of each specimen to be observed in a standardized manner with the aid of the Measure 2.0 Build 158 image software.

Two trained observers analyzed the fragments. They were previously calibrated by individually evaluating images with characteristics of specific morphological changes, on alternate days.
and times (three times a day for three days). After the third day, the observers analyzed the images together, in order to establish a consistent observation pattern of the morphological features to be scored.

Each fragment was analyzed and scores were attributed to each of the following morphological changes:

1. Presence of debris: none (0); low (1); medium (2); high (3);
2. Incidence of open dentinal tubules: none (0); low (1); medium (2); high (3);
3. Dentinal tubule erosion: absent (0); present (1);
4. Dentinal tubule cracks: absent (0); present (1);
5. Carbonized dentinal tubules: absent (0); present (1);
6. Other changes: absent (0); present (1).

**Evaluation of Root Dentin Permeability**

Ten teeth in each group had their root canals filled according to Tagger’s hybrid technique, using ProTaper F2 gutta-percha cones (ProTaper – Dentsply/Maillefer S.A. Ballaigues, Switzerland) and endodontic sealer (Endoﬁll). The obturating materials were compacted with a size 50 engine plugger attached to a slow-speed handpiece for 7 seconds. After root canal ﬁlling, the coronal portion of each tooth was made impermeable with clear polish, as previously described.

Teeth were immersed in 0.5% Rhodamine B dye solution (Laboratório Synth, São Paulo, SP, Brazil) in an ultrasonic bath at 37°C for 48 hours. Following that, specimens were irrigated with distilled water and dried with size F2 absorbent paper points. The roots were then sectioned lengthwise and the ﬁlling material was removed with the aid of a cotton plier. Fragments were placed on a sheet of graphing paper and photographed using a Canon T3i camera attached to a stand. The focal distance was set at 40 cm.

Morphometric analysis was carried out using the Image J software. The following areas were measured:

a. total area of root dentin;
b. area of dye-inﬁltrated root dentin.

The percentage of leakage was measured by dividing the dye-inﬁltrated area by the total area.

**Statistical Analysis**

The qualitative data relative to the scores attributed to the morphological changes were evaluated by the chi-square test. Data relative to the percentage of apical leakage were subjected to Student’s t-test for independent samples, by comparing the specimens in the control group with those that were exposed to low-level laser irradiation.

Both tests were conducted using the R statistical software, with the significance level at 5%.

**RESULT**

**Morphological Changes**

The scores for presence of debris were significantly different ($c^2 = 27; p < 0.0001$) between teeth in the control group and those exposed to low-level laser. Specimens in the control group presented higher debris scores. Regarding the presence of open dentinal tubules, significant difference ($c^2 = 60; p < 0.0001$) was also observed: teeth that received laser application showed a greater incidence of open dentinal tubules (Figure 1).

As for the occurrence of erosion and cracks, no significant differences were detected between the groups (Figures 2, 3).

Areas of carbonization or other morphological changes were not observed in any specimen, regardless of the group.

**Root Dentin Permeability**

When the mean ratios of Rhodamine B dye-inﬁltrated areas divided by the total dentin area were compared, it was veriﬁed that the teeth exposed to low-level laser irradiation had signiﬁcant greater dye leakage in comparison with those in the control group ($t = -6.9987, p < 0.001$) (Figure 4).
and a final irrigation with 17% EDTA, pH 7.5, aiming to more closely resemble a clinical situation. This technique corroborates previous studies, which indicate the importance of long exposure periods of NaOCl at high concentrations during irrigation, in order to eliminate resistant bacteria\textsuperscript{14,15}.

Although irrigation using NaOCl followed by EDTA is unable to completely remove the smear layer and debris, this procedure has demonstrated better results than irrigation with each of these solutions separately\textsuperscript{5}. Use of EDTA at 17% and pH 7.5 aims to promote further penetration of the solution and remove the smear layer, which occludes dentinal tubules that may contain microorganisms and their sub-products\textsuperscript{15}.

The results from the present study showed that the smear layer was almost completely removed in the group of teeth subjected to low-level laser irradiation. This group also presented greater incidence of open dentinal tubules. On the other hand, teeth in the control group presented larger amounts of debris on the root canal walls along with occluded dentinal tubules, in spite of the final irrigation with 17% EDTA.

Diffusion of irrigants and intracanal medicaments such as calcium hydroxide and sealers into the dentinal tubules is greater when the smear layer is removed because the presence of debris acts as a physical barrier against these substances by occluding the dentinal tubules\textsuperscript{14,17}.

This study demonstrated that teeth irradiated with low-level laser presented greater percentages of Rhodamine B dye apical leakage compared with the teeth in the control group, which were not exposed to laser.

The results of the present study indicate that PDT is a promising adjuvant therapy to CMP during endodontic treatment. PDT presented adequate results, without inducing morphological changes to the dentin surface or hindering the permeability of the dentinal tubules in the root dentin. Further \textit{in vivo} and \textit{ex vivo} studies are necessary to determine parameters for the use of low-level laser in daily clinical practice.

**CONCLUSION**

Photodynamic therapy with 660 nm laser diode combined with chemomechanical preparation of the root canal system resulted in reduction of the smear layer and opening of the dentinal tubules, without inducing morphological changes such as erosion, cracks, or carbonization. In regards to dentin permeability, it was verified that application of laser increased the permeability of the dentinal tubules.

**REFERENCES**


The authors declare no conflicts of interest.

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