Optical properties of human radicular dentin: ATR-FTIR characterization and dentine tubule direction influence on radicular post adhesion

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ABSTRACT

Knowledge of dental structures is essential for understanding of laser interaction and its consequences during adhesion processes. Tubule density in dentin ranges from 4.900 to 90.000 per mm^2, for diameters from 1 to 3 µm. Light propagation inside the tubules is associated with tubules orientation. To the best of our knowledge, there is no previous work in literature characterizing physical-chemical alterations in dentin. The dentin samples were irradiated with a Er,Cr:YSGG Laser at wavelength 2.78 µm, with an energy density of 9.46 J/cm^2 , above the ablation threshold. ATR-FTIR at wavenumbers 2000 to 700 cm^{-1} was used to evaluate the differences among third root region and tubules orientation.

Keywords: ATR-FTIR, human dentin, dentinal tubules, laser, Er:Cr,YSGG, Optical properties, post adhesion

1. INTRODUCTION

Extensive knowledge of dental structures is helpful to understand the various interaction behaviors of dental materials. For example, adhesion plays a very important role for dentistry. The dentinal tubules are the basic unit of human dentin; their density range from 4.900 to 90.000 tubules per mm^2, and diameter from 1 to 3 µm. Light propagation inside the tubules depends on tubules orientation, that have been found to amplify light. Laser is routinely used in dentistry and it may affect dentin, but as far as it is known, there is no publication yet demonstrating the physical-chemical alterations in dentin. Thus, we irradiated tooth samples with a Er,Cr:YSGG laser with an energy density 9.46 J/cm^2, above the ablation threshold. An ATR-FTIR system was used to evaluate the influence of dentinal tubules orientation in respect to the laser beam and look for a correlation effect when the tubules are in the same orientation or perpendicular. Another goal is to analyze the changes that occur in different areas of the root canal, since there are differences in tubule density inside each root location and optical density variations in the intertubule region (n=1.45), peritubule (n=1.65) and intertube solution (n=1,33).

2. MATERIAL AND METHODS

FTIR spectroscopy

FTIR spectroscopy is a non-destructive testing for the evaluation chemical changes on the surface of dental tissues. The advantage of this technique is that the reflectance of the analyzed tissue is influenced only by the beam incidence on the studied surface. Superficial changes of the order of 10 µm can be analyzed by this technique. In a previous work studying the effect of Er,Cr:YSGG laser on human dentin at different energy parameters, we analyzed the

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Biophotonics South America, edited by Cristina Kurachi, Katarina Svanberg, Bruce J. Tromberg, Vanderlei Salvador Bagnato, Proc. of SPIE Vol. 9531, 95311V · © 2015 SPIE
CCC code: 1605-7422/15/$18 · doi: 10.1117/12.2181162

chemical changes in five different chemical elements (Mg, P, Ca, K and Na) by the process of "inductively coupled plasma - atomic emission spectrometry" (ICP-AES) 45. We demonstrated differences between the proportions of chemical elements, with the exception of K, which did no change significantly 46. Thermal, physical and chemical changes in the target tissue can also influence the ablation quality during the removal process, leading to a decrease of its removal efficiency with consequent increase in temperature of the adjacent tissue and even loss of ability to remove more tissue with subsequent laser pulses 44.

Tooth preparation

Sixty recently extracted single rooted human teeth were used after approval from ethical committee (CEP FOUSP 177/2010) and in accordance with technical specification ISO TS 11405. The coronal portion was removed at proximal face at the CEJ and minimum root canal working length was 14 mm.

All root canals were endodontically instrumented with mechanical Protaper Universal (Dentsply, Bellaigues, Switzerland) rotary system files, in crown down technique, irrigated with 2 ml of 2.5% sodium hypochlorite. A manual K file #35 (Dentsply, Bellaigues, Switzerland) was used to finish the root canal instrumentation. All root canals were then irrigated with distilled water, filled with standardized gutta-percha points (Dentsply, Petropolis, Brazil) and root canal sealer AH Plus (Dentsply, Konstans, Germany). All roots were stored at 37 ˚C during 24 hours after sealing on a closed canister with humidity of 100%. The roots were then randomly divided in six groups (n = 10).

Post space preparation procedure

The glass Fiber Reinforced Composite root canal post (FRC) (Postec plus #3, Ivoclar, Schaan, Liechtenstein) was used, with length of 10 mm measured from cervical. A sequence of increasing diameter of Gates Glidden burs was used to remove the gutta-percha and enlarge the root canal. The post space was shaped with the low-speed preparation bur provided from the post system kit. The root canal was flushed with 2 ml of NaOCl and 5 ml of distillated water to neutralize chemical traces.

Laser irradiation procedure

The Er,Cr: YSGG laser device (Waterlase, Biolase, San Clemente, USA) emits at a wavelength of 2.780 nm with repetition rate of 20 Hz. It was used an exchangeable 400 µm fiber tip model Z4 (Biolase, San Clemente, USA) with settings for water at 37 %, and 34 % for air. Instead of compressed air, pure nitrogen gas 99.999 % (Ni 5.0 White Martins, Brazil) was connected to the laser device in order to avoid any contamination from the air spray. The laser irradiation was performed in helicoidal movements from apical to cervical. Each movement took 6 seconds with 20 seconds interval allowing thermal relaxation and repeated 5 times. The energy per pulse was 45 (± 4) mJ measured by an energy meter (Field Master GS, Coherent, Auburn, CA, USA) connected to a detector for pulsed infrared lasers (LM-P10i Coherent, Auburn, CA, USA) before each irradiation to standardized power outputs.

Post surface preparation

As directed in the manufacturer`s instructions manual the post surface was etched with 37 % phosphoric acid during 1 minute, rinsed with water, dried and coated with a layer of primer Monobond (Ivoclar, Schaan, Liechtenstein) After 1 minute reaction was carefully dried for 15 seconds, with air flow and the surface wasn't touched until its placement.

Post fixation

The FRC posts were immediately cemented after post space preparation (control groups) or right after laser irradiation and the post was kept under manual pressure during 2 minutes to have a mimetic clinical situation. All samples were light cured for 60 seconds (power density of 500 mW/cm^2) and stored in labeled containers with 100 %

humidity at 37 ºC for 48 hours. Three different techniques for dual curing luting adhesive composite were tested, as follows:

1- In the *total etching* technique the dentin was etched with phosphoric acid at 37 % for 15 seconds, water rinsed and dried with tapered endodontic paper points until the dentin surface got a glossy wet appearance. The dual-curing dental adhesive Excite DSC (Ivoclar, Schaan, Liechtenstein) was applied with a second lining after 30 seconds and the excess was removed with paper points. The Variolink II (Ivoclar, Schaan, Liechtenstein) was used, with ratio 1:1 of base and low viscosity catalyst.

2- In the *self-etching* groups, equal amounts of primer A and B (ED primer) were mixed and applied with an endodontic micro brush. The surplus was removed within 30 seconds. The Panavia F (Kuraray Medical, Okayama, Japan) paste A and B were mixed in equal amounts for 20 seconds, applied and Oxigard II (Kuraray Medical, Okayama-Japan) was used after the post placement.

3- In the *self-adhesive* groups, RelyX Unicem Aplicap (3M ESPE AG, Seefeld, Germany) capsules were activated (Aplicap Activator-3M ESPE AG- Seefeld Germany) and mixed in high-frequency mixer during 20 seconds. The cement was applied with elongation tip.

Push out samples preparation

Each root was sectioned in six slices of 1mm, perpendicularly cut to post long axis with a cutting machine (Accutom 5, Struers, Cleveland, OH). Each slice dimension was measured with a digital caliper (Mitutoyo- Japan). The bond surface area of the post in each slice was calculated according to:

$$A = \pi (R + r)\sqrt{(R - r)^2 + H^2}$$

The specimen were loaded into a universal testing machine (Instron 5567, Norwood, MA, USA) placed within a centralizing plate to ensure centered application of the load and tested at a crosshead speed of 0.5 mm/min until obtaining the maximum failure load. The load was applied from apical to cervical, because of post conical shape.

Push-out strength ($\partial = F/A$) was expressed in megaPascals (MPa) by dividing the load at failure (Newton) by the surface area (mm^2).

Statistical analysis

All data were tested regarding its normality and homogeneity of variances using Shapiro-Wilk and Levene´s tests. For groups with homogeny variance, the ANOVA test was performed along with Tukey’s post-hoc and, for non-homogeny variances, the t-test for different variances was performed along with the Ryan-Holm step down Bonferroni procedure (Statistica 7.0 StaSoft-inc, Tulsa, OK, USA). All significance level were p = 0.05.

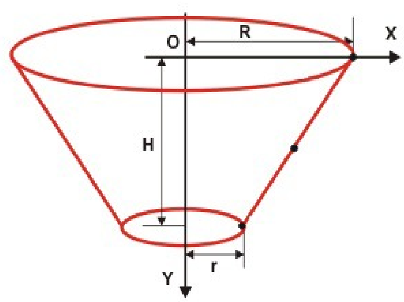

Figure 1 - Arrangement for calculating the area of the samples to determine the bond strength

3. RESULTS

FTIR Results

Figure 2 shows the spectra of different regions of dentin (apical, middle and cervical) to non-irradiated samples, the samples for both cross-sectional and longitudinal. The region is presented 2000-700 cm -1, since in this region the corresponding bands are organic compounds. To make it possible to compare the different regions of the spectra, the spectra were normalized by the intensity of the band phosphate (located at about 970 cm -1).

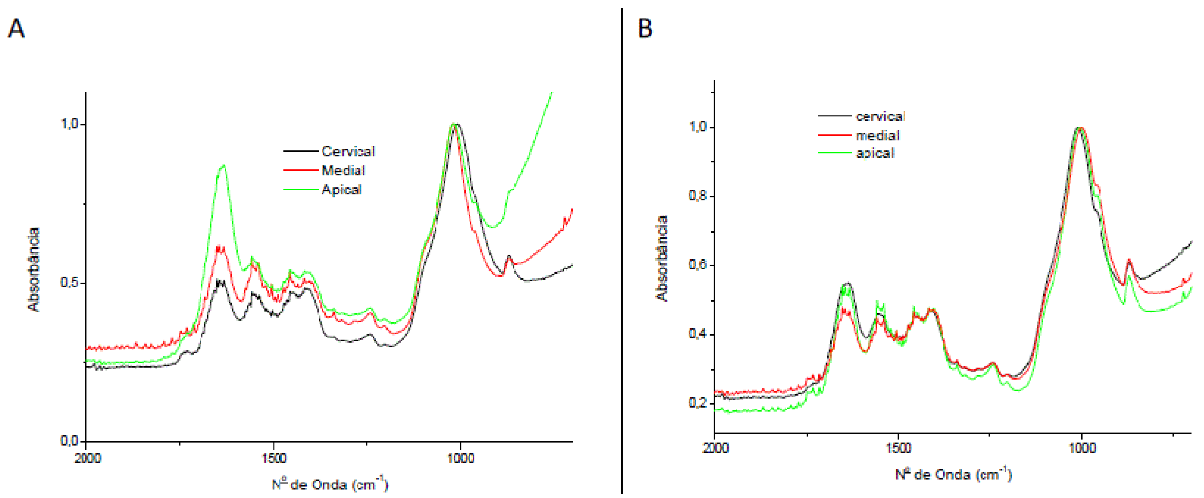

Figure 2: Comparison of the spectra obtained from cervical , medial and apical regions of dentin cut (A) cross and (B) Longitudinal.

Figure 3 shows the spectra obtained from non- irradiated samples of the same region of dentin due to the cutting direction. The region is presented 2000-700 cm -1, which contain bands of interest, since this region are the corresponding bands organic compounds . To allow comparison of the spectra in different regions they were normalized by the intensity of the band phosphate (located at about 970 cm -1).

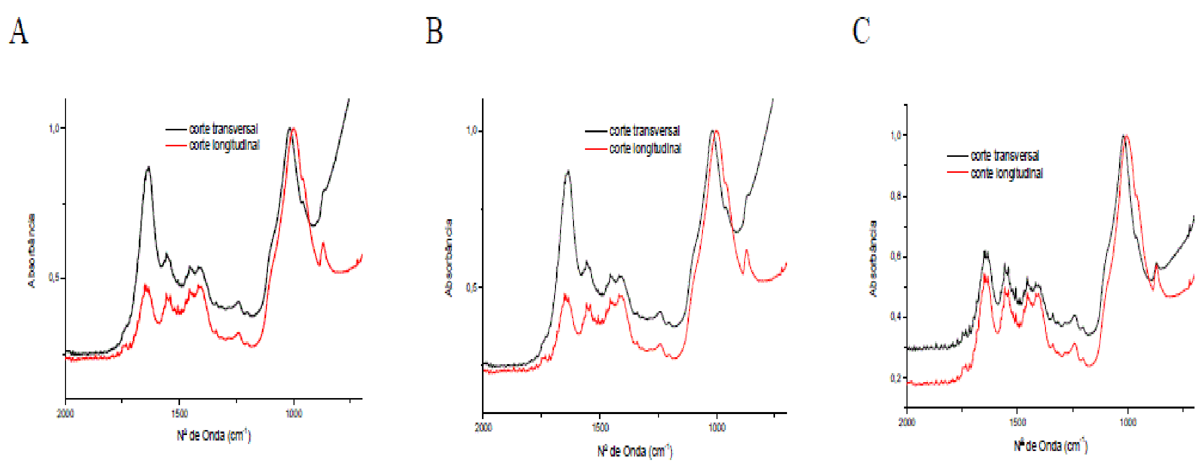

Figure 3: Comparison of the spectra obtained from samples cut in the transverse and longitudinal direction of the regions (A) Cervical, (B) Average and (C) Apical.

Push-out results

The laser irradiation with Er,Cr:YSGG inside root canal before post cementation with dual curing luting adhesive composites, significantly affect the adhesion ($p < 0.05$) in the laser conditions of this study.

The use of RelyX Unicem Aplicap in irradiated samples statistically improves the push-out bond strength results in all thirds among all groups ($p < 0.02$). (Tabela 4.5 e fig 4, grupo VI)

The use of laser irradiation associated with Panavia F has improved the push-out bond strength in the medial third in comparison with non-irradiated samples ($p = 0.034$) (tabela 3, fig 4 grupo II)

The use of Variolink II statistically improves the push-out bond strength in apical third in irradiated samples in comparison to the Panavia F irradiated apical third results ($P<0,05$). (tabela 5e figura 4)

For groups luted with Variolink II there is no statistically significant difference among control group and irradiated group, in any third.

For control groups, Panavia F in comparison to Variolink II, there is no statistical difference in any post third. The Unicem control group the bond strength at middle post third was statistically higher then Panavia F ($P<0,01$).

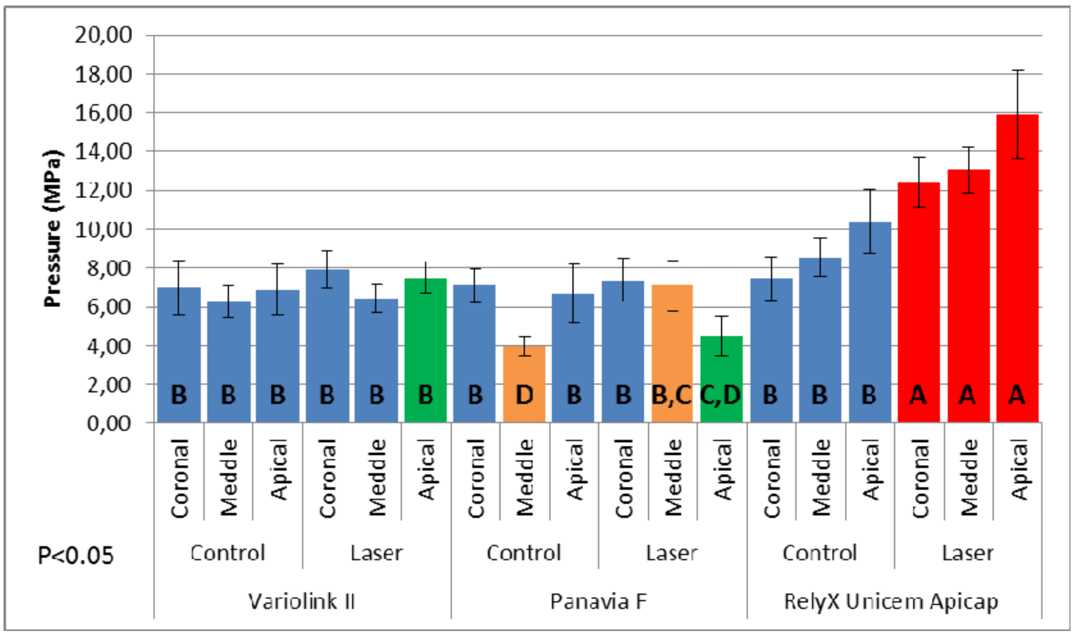

Figure 4. Push-out bond strength for coronal, middle and apical thirds per cement irradiated or control (no irradiation). Mean values and standard deviations. Different letters in columns indicate significant differences ($p<0.05$)

4. DISCUSSION

Notwithstanding biomechanical considerations, once using a post inside root canal with adhesive cement, the interface to radicular dentin is always the weak point [1,7,11,12]. This vulnerability may cause a collapse and jeopardize all prosthetic procedure. In this sense, making this interface stronger is an important achievement to pursue. Adhesion on dentin is a complex matter and the reduction or removal of the smear layer (depending on which technique is used) [2-11] is an important concern. Paradoxically there is always production of smear during space post preparation or endodontic treatment.

The results of this study showed that after irradiating the root canal dentin it significantly affected the FRC post adhesion (when dual curing luting adhesive is used) by enhancing the bond strength values ($p<0.05$). The findings in control groups of this experiment are in accordance with specialized literature [i] with is no statistical difference on bond strength values, among the adhesive luting cement used, unless the comparison is made onto two-population intra group on the root third.

Many procedures have being suggested in order to diminish or even eliminate the presence of smear layer inside root canal. Among many methods in current use, the chemical irrigants may interfere with post adhesion to dentin [7 ,8,10,11]. The use of Er,Cr:YSGG laser inside the root canal is a substantiate procedure were different laser parameters causes distinct results on dentin morphology [13,15,16-19,28,30] and may interfere on adhesion [21,28,29]. It was also reported that the laser irradiation inside root canal with water spray cooling, prevents temperature rise [13,14,15] and increases ablation depths in dentin [13,15,16,19]. Relate to laser energy parameter used it may ablate the smear layer accumulated over the dentin [13,14,15,20,28][ii] inside root canal.

One goal of this study was to reduce debris produced inside root canal due to prosthetic procedures. A previous pilot experiment was performed to seek the irradiation parameter that better removes the smear layer, letting the dentinal tubules unobliterated. Several laser irradiation parameters were tested inside the root canal. Scanning electron microscopic (SEM) images from three standardized sites of each portion of the irradiated root (2mm, 4mm and 6mm from apex) were analyzed relating each laser parameter to the number of opened tubules.

The energy of 45 (± 4) mJ per pulse, at 20 Hz, was very adequate to remove debris, letting tubules widely opening and yet the temperature rise was lower than 1˚C. These settings were checked with an energy meter. The Er,Cr:YSGG laser irradiation parameters used on this experiment removed smear layer and others debris from the root canal dentin leading to an etched surface by the laser with most of tubules opened.

The highest bond strength on the irradiated group cemented with RelyX Unicem Aplicap had among all others groups ($p< 0.02$) may be explained mainly because of better interaction of self-adhesive system with the dentin surface free from extraneous matter, as long as there was no other procedure before the post placement, just laser.

The phosphoric acid at 37% used in total etching technique (etch & rinse) removes the debris and peritubular dentin, opens the dentin tubules [iii] aiming to strength the interaction with adhesive coating. The Er,Cr:YSGG laser in this experiment has etched by ablation process the dentin surface inside root canal, opening the tubules as well. In spite of both removed smear layer, the self-adhesive system (Unicem Aplicap) had far superior bond strength values then total etching (Variolink II). However, there is no statistically significant using Variolink II in any third, neither in control group nor in irradiated group. The adhesive coating (Excite DSC) feasibility has a negative impact over a lased surface or etching the lased surface with phosphoric acid is effectiveness. Further studies are necessary.

When self-etching adhesive is applied, the reaction occurs with smear layer and not removes it. This reaction produces dentin sealed with the smear layer. The weak acidity of the primer let a shallow etching depth and few dentinal tubules open [31]. As the irradiated groups had few amount of smear layer so the primer of the self-adhesive (Panavia F) may had not enough substrate leading to weaker bond strength into irradiated groups then total etching (Variolink II) irradiated groups.

The use of Variolink II statistically improves the push-out bond strength in apical third in irradiated samples in comparison to the Panavia F results ($P<0,05$).

The clinical prosthetics procedures involves endodontics, post fixation, temporary prosthesis and even more issues taking a long period of time to be finished. Delayed fiber post cementation is not only a reality in clinical situation but also because it shows higher interfacial strengths then immediate placement[iv]. This delay in time may lead to contamination. The bacterial reduction effect with the Er,Cr:YSGG laser [13,22-27] in root canal is established and is used in clinical procedures is in accordance with the parameters used in this study, and also is satisfied using Er,Cr:YSGG laser before prosthetic post placement.

For that reason this procedure could be regarded as a clinical routine once there is an enhance on mechanical behavior of laser groups.

6- ACKNOWLEDGMENTS

Brazilian Government financial support for this study (CEPID/FAPESP 05/51689-2, PROCAD/CAPES Edital 2013, and CNPq/INFO 573.916/2008-0).

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