EFFECTS OF THE TEETH DECONTAMINATION TREATMENT ON DENTIN COMPONENTS: A RAMAN SPECTROSCOPY ANALYSIS

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Abstract - Raman spectroscopy evaluated changes in the dentin components submitted to decontamination methods. Twelve human third molars were divided into two groups: six teeth were stored in thymol (group A) and six teeth were autoclaved (group B). The enamel was removed and the dentin components were evaluated before and after the 37% phosphoric acid etching. The evaluation of the dentin components was done by calculating the relative area of the 961, 1260 and 1670 cm^{-1} peaks. Statistical analyses were performed by ANOVA and Bonferroni test. Non-significant differences were found in the group A for the peak at 1670 cm^{-1}. However, a significantly reduction was observed in the group B. Non-significant differences were found in both groups for the peak at 1260 cm^{-1}. Statistical differences for the PO_4 component were found in both groups. Raman spectra showed that the components of the autoclaved teeth were more affected.

Key words: dentin, collagen, decontamination, Raman spectroscopy.
Knowledge area: III - Engenharias

Introduction

Currently, one of the main problems with dentin bonding is the presence of microleakeage at the restoration/tooth interface [1]. The formation of an adequate dentin-adhesive bond depends upon diffusion of the adhesive throughout the demineralized layer and into the unaffected dentin. If the adhesive does not penetrate into the demineralized dentin, then the naked protein within this layer will be exposed to oral fluids and vulnerable to degradation by bacterial enzymes. Such degradation can ultimately lead to premature failure of the composite restoration [2].

Another point to be mentioned is that little attention have been reported to the decontamination methods in the use of extracted human teeth for scientific research. Teeth are often grossly contaminated, difficult to sterilize because of their structure, and may be damaged or altered by the sterilization process [3].

Because of the very small thickness of the hybrid layer, methods to analyze this effect must have a very high resolution and sensitivity similar to that of electron microscopy. Raman micro-spectroscopy is a very useful analytical technique for studying the composition and structure of bonding of a sample [2, 4, 5]. The acquired spectra are attributed to molecules rather than to single elements. Dehydration of the sample is not required and the measurements can be done under room conditions [6].

The purpose of this in vitro study was to evaluate and compare by Raman spectroscopy changes in the mineral and organic dentin structures relative to the sterilization process and to the dentin etching with 37% phosphoric acid.

2. Materials and method

The specimens used in this study were obtained from twelve human non-carious third molars. All the teeth were recently extracted from patients needing extractions as a part of their dental treatment, as approved by the Ethic Committee from University of Paraíba Valley (Protocol n° L102/2003/CEP).

Teeth were cleaned and then divided into two groups: six teeth were stored in 9°C aqueous 0.1% thymol solution (group A) for one week and six teeth were autoclaved at 121°C for 15 min in a flask of sterile saline covered (group B) and stored in 0.9°C saline solution (Farmavale&Cia – LBS Laborasa Ind. Farm. Ltd., Brazil).

To prepare dentin slices, the teeth stored in aqueous thymol solution were washed for 24 hours with filtered water to eliminate thymol residues.

After these procedure, the occlusal one-third of the crown was sectioned perpendicular to the long axis of the tooth by means of a water-cooled low speed saw (Isomet 1000 - BUEHLER) with a diamond disc (11-1190) at 250 rpm and with 100 g load.

Deep dentin surface was grinded on wet 600-grit silicon carbide paper (3M) at 150 rpm (Knuth Rotor – Struers) under constant cooling with water for 1 minute each to produce an standard smear layer [6, 7]. Ultrasonic cleaning (Cole-Parmer 8891) with distillated water for 5 min was performed to remove the excess debris, and the specimens were washed and stored in saline solution at 0.9° C in a refrigerator. To prepare dentin disks and to include the specimens in the sampler holder, roots were removed with a water-cooled low-speed saw (Isomet 1000) producing one dentin disk with thickness of 4 mm for each tooth.

The chemical etching was performed using 37% phosphoric acid (FGM) for 15s. The etched surface was then rinsed with water with the syringe combination by 15s.

Dispersive Raman Spectroscopy analyzed the dentin surfaces before and after the treatment by the phosphoric acid. For the Raman spectra calibration, the spectrum of the Indine (C_9H_8,) substance was obtained [8]. Raman data of each dentin disk before the treatment were recorded as references. The samples were placed on a precision *X-Y-Z* stage to obtain four spectra per sample.

The samples were excited in the near infrared region by a Ti:Saphire laser (Model 3900S, Spectra-Physics, λ = 785nm, beam diameter = 1.5 mm) pumped by an Argon laser (Stabilite 2017/Spectra-Physics, λ = 514nm). The power of the Ti:Saphire laser was limited to 80mW in the sample holder.

The spectral slit was set to 200μm. A CCD detector cooled by liquid Nitrogen collected the Raman spectra of the dentin disks. For each measurement, 10 readings with an integration time of 1s were accumulated in a personal computer. After the dentin treatment, the same procedure was repeated. Averages of the Raman spectra were obtained from each sterilization treatment.

The spectra fluorescence was removed with a polynomial fitting from the average spectra, with varying degree in the Microcal Origin® software.

The calibration of wavelength was carried out using a calibration column generated by the pixels values of the Indine spectra in the Excel® software.

Relative areas of the peaks were calculated in the Microcal Origin® software. The semi-quantitative evaluation of the changes in mineral and organic structures were done by calculating the relative intensity ratio of the 961, 1260 and 1670 cm^{-1} regarding to the 1046cm^{-1} peak. This procedure is based on the assumption that the ratio of the integrated intensities of the antisymmetric and symmetric stretching mode of phosphate ion should not change [4].

The Raman results were subjected to the one-way ANOVA at the 95% level of confidence. The Bonferroni multi comparison post-hoc test compares all pairs of columns using the Instat® software.

Results

The typical Raman spectra of untreated human dentin are shown in Figures 1 and 2. The intense peak at 960 cm^{-1} is associated with the P-O stretching vibration in the mineral apatite component of dentin. Weak bands at 1260 and 1670 cm^{-1} are attributed to the organic components of dentin, such as type III and I collagen, respectively [2, 5].

Figure 1 and 2 shows the dentin Raman spectra of the specimens treated with Thymol (A) and autoclave (B) before and after the dentin acid etching. The mineral content showed by the intensity ratio of the peak at 960 cm^{-1} demonstrated more mineral reduction for the autoclaved specimens as indicated by the lower relative intensities (Fig. 1). The same aspect was observed for the organic components (Fig. 2).

The results of the peak area evaluation showed reduction in the relative area of the inorganic phase (960 cm^{-1}) and in the organic component (1260 and 1670 cm^{-1}) for all groups tested (Figures 3, 4 and 5). For the peak at 960 cm^{-1}, higher statistical significant differences (*** $p<0.001$) were found between the normal dentin (N) and treated dentin (T) in the group B specimens; however, in the group A this difference was less significant (* $p<0.05$) (Fig. 3).

Analysis of the area reduction for the peak at 1260 cm^{-1} (Figure 4) demonstrated non-significant statistical difference (ns $p>0.05$) for both groups. For the peak at 1670 cm^{-1}, non-significant statistical difference (ns $p>0.05$) was found after acid etching in the specimens treated by Thymol and a significant reduction (* $P<0.05$) in the area peak was evident for the autoclaved specimens (Figure 5).

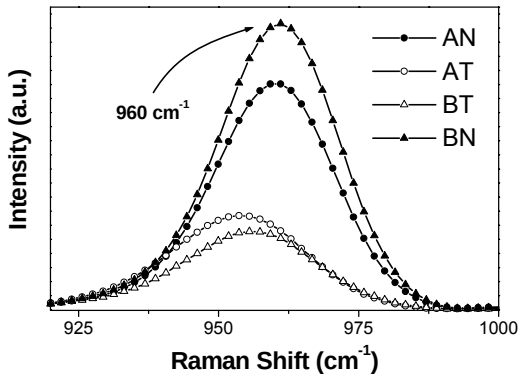

Figure 1. Raman spectra of normal (N) and treated (T) dentin for autoclaved (•) and Thymol (▲) specimens.

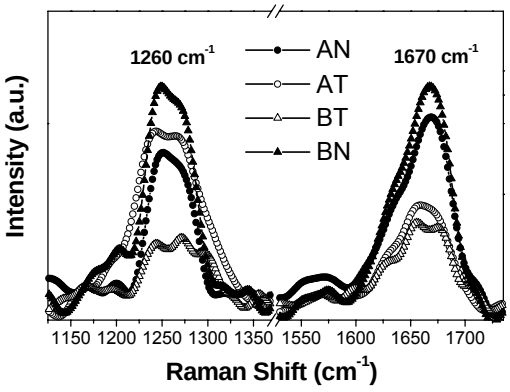

Figure 2. Raman spectra of normal (N) and treated (T) dentin for autoclaved (•) and Thymol (▲) specimens.

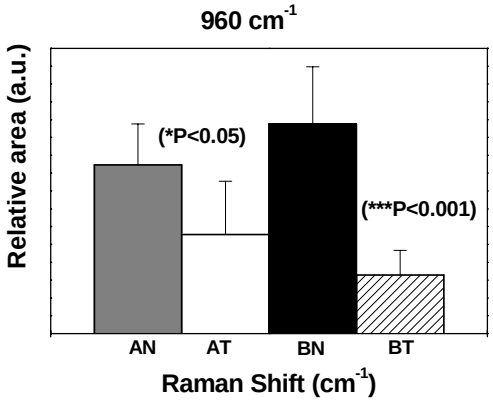

Figure 3. Relative area of the peak at 960 cm^{-1} according to the teeth decontamination process (A – Thymol; B – autoclaved) for normal (N) and treated (T) dentin specimens.

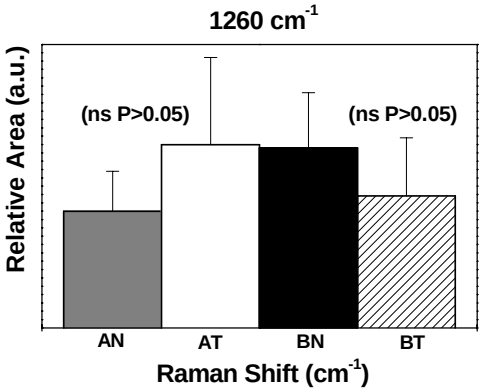

Figure 4. Relative area of the peak at 1260 cm^{-1} according to the teeth decontamination process (A – Thymol; B – autoclaved) for normal (N) and treated (T) dentin specimens.

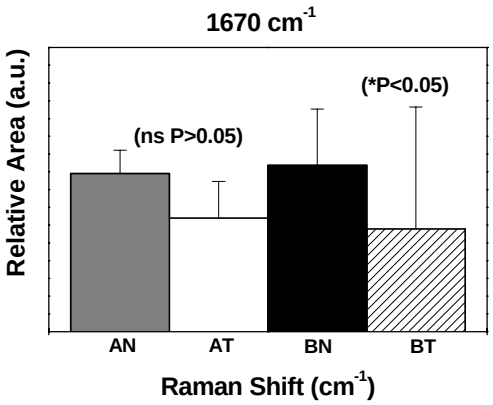

Figure 5. Relative area of the peak at 1670 cm^{-1} according to the teeth decontamination process (A – Thymol; B – autoclaved) for normal (N) and treated (T) dentin specimens.

Discussion

The requirements of an effective dentin adhesive system include the ability to thoroughly infiltrate the collagen network and in the partially demineralized zone, as well as to commingle and encapsule the collagen and hydroxyapatite crystallites at the front of the demineralized dentin, and to produce a well-polymerized durable layer [5].

Several concerns about the methods of teeth decontamination have also been reported in order to avoid contamination [3], but the damages or alterations on teeth structures by the sterilization procedures have not been well studied yet. On the present study, it was observed by Raman spectroscopy how the sterilization process affects the teeth components that are involved in the

adhesion process. Reductions in the inorganic and organic components were observed by the changes in the relative area of the peaks at 960, 1260 and 1670 cm^{-1}. It was observed that autoclaved specimens showed more reduction in the area of the peaks at 960 and 1670 cm^{-1}. However, non-significant statistical differences were found for organic component between the final peak area of the specimens treated by Thymol and autoclave after the dentin treatment.

Mineral content changes associated to the hydroxyapatite showed that the specimens autoclaved were more affected by the dentin treatment than the specimens treated by Thymol. The specimens of the Thymol group treated with phosphoric acid by 15s etched dentin in a more conservative way, removing less mineral from the dentin.

The organic component evaluated by the peak area at 1260 cm^{-1} presented area reduction without statistical significance for both decontamination treatments. However, a significant reduction was found for the autoclaved specimen in type I collagen area peak, showing thus, a substrate more favorable to the adhesion process in the group decontaminated with Thymol where the collagen fibrils were less affected.

Raman spectroscopy showed the changes that occurred in dentin produced by the decontamination process and by the chemical etching. Sample preparation is fairly simple, since no specific sample dimensions or sample translucency is required. Raman measurements can be carried out in normal atmospheric conditions without the need of a high vacuum. Finally, since this technique is non-destructive, samples can be used for multiple analyses.

Conclusion

Raman spectroscopy measurements showed that teeth autoclaving process affected more the mineral and organic dentin content than the specimens treated by Thymol. The adhesion could be harmed in autoclaved specimens.

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