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Research Paper

Effect of laser activated bleaching on the chemical stability and morphology of intracoronal dentin

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ABSTRACT

Objectives: To evaluate the effect of the bleaching with 35% hydrogen peroxide either activated or not by a 970 nm diode laser on the chemical stability and dentin surface morphology of intracoronary dentin.
Methods: Twenty-seven slabs of intracoronary dentin specimens (3 × 3 mm) were distributed into three groups (n = 9), according to surface treatment: HP – 35% hydrogen peroxide (1 × 4'), DL – 970 nm diode laser (1 × 30"/0,8W/10 Hz), HP + DL – 35% HP activated with 970 nm diode laser (1 × 30"/0,8W/10 Hz leaving the gel in contact to the surface for 4′ after activation). Three Raman spectra from each fragment were obtained to calculate the mean intensity of peaks of inorganic component (a.u.), organic collagen content (a.u.), and the ratio of inorganic/organic content, before and after treatment. Analyses of the samples by confocal laser microscopy were performed to evaluate the surface roughness, percentage of tubules, perimeter and area percentage of tubules, before and after treatment. Data were analyzed by Kruskal-Wallis, Dunn's, and Wilcoxon test ($P < 0.05$).
Results: Data analysis showed that HP + DL did not change the inorganic content peaks 8.31 [29.78] or the inorganic/organic ratio 3.37 [14.67] ($P > 0.05$). Similarly, DL did not affect the chemical stability of the dentin surface ($P > 0.05$). However, HP significantly increased inorganic content peaks 10.87 [22.62], as well as the inorganic/organic ratio 6.25 [27.78] ($P < 0.05$). Regarding the morphological alterations, all surface treatments increase tubules exposure; HP treatment significantly increases perimeter and area percentage; and HP + DL increases surface roughness.
Conclusions: Bleaching HP combined with DL offers an improvement in terms of intracoronal dentin surface protection, yielding better maintenance of dentin chemical stability and morphology.

1. Introduction

Aesthetic dental problems often result in stained teeth as result of pigment incorporation into tooth structure (Plotino, Buono, Grande, Pameijer, & Somma, 2008). The discoloration process occurs by formation of chemically stable structures on dentin (Tredwin, Naik, Lewis, & Scully, 2006). In a non-vital tooth, this process may be associated with pulp tissue decomposition, blood pigments from an intrapulpal hemorrhage, and iatrogenic endodontic treatment, such as roof remains or pulp tissue leftovers in the pulp chamber, insufficient debridement and irrigation, or the presence of restorative and filling materials left in contact with the pulp chamber for long periods of time (Plotino et al., 2008; Baratieri, Ritter, Monteiro, Caldeira de Andrada, & Cardoso Vieira, 1995).

Among the cosmetic restorative treatment used to remove these pigments and recover the compromised esthetics, the intracoronal bleaching procedure is the least invasive alternative. This method is widely used due to its relative simplicity and effectiveness, in addition to its low cost and preservation of the dental hard tissue, as compared to prosthetic treatment (Amaral et al., 2008; Joiner, 2006). Despite the advantages offered by this procedure, the underlying effects of bleaching agents on dental hard tissues remain controversial.

Bleaching is based on substances that have a high potential for oxygen release. Hydrogen peroxide is the most commonly used bleaching agent in dental practice (Joiner, 2006; Joiner, 2007). Used in high concentrations, hydrogen peroxide whitens teeth simply by oxidation of their transparent organic matrices (Eimar et al., 2012).

Contemporary approaches have focused on accelerating peroxide

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bleaching treatment by shortening the chemical redox reactions with different sources of light such as, lasers, light-emitting diodes (LED), plasma arc lamps, and halogen curing lights, in a variety of wavelengths and spectral power (De Moor et al., 2015; Toledano, Yamauti, Osorio, & Osorio, 2011; Berger et al., 2010; Buchalla & Attin, 2007; Carrasco-Guerisoli et al., 2009).

However, the possibility of adverse effects on tooth structure from the use of bleaching agents cannot be dismissed. Bleaching agents can cause changes in micro-hardness (De Oliveira, Teixeira, Ferraz, & Teixeira, 2007), elasticity modulus (Chng, Ramli, Yap, & Lim, 2005), and morphology of the dental substrate (Ferreira, Souza-Gabriel, Silva-Sousa, Sousa-Neto, & Silva, 2011), all caused by modifications in chemical and morphology of dental hard tissues (De Moor et al., 2015; Eimar et al., 2012; Plotino et al., 2008; Toledano et al., 2011).

Effectiveness and safety are two critical aspects of tooth surface treatment. In order to better understand the effect of bleaching and laser activation, further studies are necessary to investigate the mechanism of dentin whitening with hydrogen peroxide (Eimar et al., 2012). The aim of the present in vitro study was to assess the influence of a bleaching agent containing 35% hydrogen peroxide, or this solution followed by 970 nm diode laser activation, on morphology and chemical stability of the intracoronary dentin. The following null hypotheses were tested: 1st) the activation of the bleaching gel with 970 nm diode laser will not affect the morphology of intracoronary dentin, and 2nd) the activation of the bleaching gel with 970 nm diode laser will not affect the chemical stability of intracoronary dentin.

2. Materials and methods

The experimental protocol was approved by the ethical committee on human research.

2.1. Experimental design

This study was factorial 3 × 1. Three different types of intracoronal surface treatment were utilized in this study: 1 – 35% hydrogen peroxide, 2 – 970 nm diode laser and 3 – 35% hydrogen peroxide activated by 970 nm diode laser. Twenty-seven intracoronary specimens were randomly assigned into these three experimental groups (n = 9). The quantitative response variables were the analysis of inorganic (hydroxyapatite) and organic (collagen) content by Raman spectroscopy and analyses of surface roughness, percentage of tubules, perimeter percentage, and area percentage of the tubules as measured by confocal laser microscopy.

2.2. Dentin sample preparation

Many human maxillary canines stored in 0.1% thymol solution at 4 °C were washed in running water for 24 h to eliminate thymol residues. Subsequently, teeth were radiographically examined to verify absence of calcification or resorption in the pulp chamber and examined with a 20× stereomicroscope to eliminate structurally damaged teeth (Leica, Model M165C Microsystem, Wrtzlar, Germany). Twenty-seven teeth were selected for further study.

Roots were sectioned 1 mm below the cementoenamel junction with a saw machine (Isomet 1000, Buehler, Lake Forest, USA). Crowns were embedded in wax and bisected longitudinally. The buccal face of each crown was further sectioned in incisal, mesial, distal, and cervical orientation to obtain square samples 3-mm wide and 3-mm high (9 mm^2), ultimately yielding 27 samples.

These were embedded in self-cure acrylic resin surrounded by a polyvinyl chloride (PVC) cylinder (1.5-cm diameter and 1.5-cm high) with the intracoronary dentin facing up. After resin polymerization, the PVC cylinder was removed and the dentin surfaces were polished with #280 and 400 grit silicon carbide (SiC) paper. Additional grinding was accomplished with #1200 grit SiC paper for 1 min to produce a standardized smear layer. Then specimens were stored at 37 °C in 95% relative humidity.

The samples were randomly assigned to three groups (n = 9), according to the surface treatment: HP: bleaching with 35% hydrogen peroxide; DL: 970 nm diode laser; and HP + DL: bleaching with 35% hydrogen peroxide activated by the 970 nm diode laser.

For the HP group, the red bleaching gel (JW Power Bleaching Gel, HeyDent GmbH, Kaufering, Germany − composition: hydrogen peroxide 35%, glycerol and potassium nitrate) was mixed at the moment of use with the colorless gel, by means of the manufactureŕs premix syringes. The 1.5 mm thick bleaching gel was evenly spread over teeth and left on the specimen for 4 min. The gel was removed using high-speed suction, then flushed with an air and water spray to remove any residual gel.

According to the JW Power Bleaching Gel manufacture, the combination with diode laser systems providing a wavelength between 810 nm and 980 nm or an Nd:YAG laser with a 1064 nm wavelength could be employed to activate it. Thus, in the DL group, the specimens were positioned so that the distance between the laser tip and the irradiated surface was a standardized 2 cm. The 970 nm diode laser device (SIROLaser Advance, Sirona Dental Systems, Bensheim, Germany) was used with a 200 μm diameter tip with a fixed frequency of 10 Hz, and 0.8 W. The specimens were irradiated for 30 s. The laser tip was analyzed between applications, removed when necessary, and the new tip moved to the standard position.

The HP + DL group followed manufacturer's recommendation protocol. The whitening procedure conducted for the HP group was followed by laser activation protocol performed in DL group. The laser activation was performed immediately after the whitening gel application, and removed from the surface after 4 min.

2.3. Analysis of the organic and inorganic composition by Raman micro spectroscopy

According to the method used by Lopes et al. (2016), the following protocol was performed. The contents of organic and inorganic phases of the intracoronal dentin surface were characterized by a Raman microscope (Horiba Jobin Yvon, Edison, USA). The intensity of PO_4^{-3} (960 cm^{-1}) and Amide III (1248–1273 cm^{-1}) peaks in the Raman spectrum are proportional to the amount of inorganic (hydroxyapatite) and organic (collagen) content, respectively, scattering Raman signals very strongly. The organic phase of dentin is predominantly composed of collagen. Further analyses of the ratio between inorganic and organic compounds were also performed.

The light generated by the laser source had a wavelength of 785 nm. The resulting excitation point was about 10 μm in diameter, and the laser penetration was about 100 μm. Initially, the system was calibrated using the known peak of a Si wafer at 520.7 cm^{-1}. In order to obtain the average mineral and collagen content within each region, three measurements were performed on each specimen.

Measurements were obtained using 1200 lines/mm grating, which provided a wavenumber resolution of 1.25 pixels/cm^{-1}. Each spectrum was the average of 20 consecutive spectra, each collected for 4 s. After implementing the surface treatment, the same analysis was performed in order to compare the data obtained before and after treatment. The percentage of the difference between before and after treatment were calculated.

2.4. Analysis of surface morphology by confocal laser microscopy

A three dimensional (3D) laser confocal microscope (LEXT, Olympus Corporation, Tokyo, Japan) was used to obtain images of the intracoronal dentin surface. First, the samples were marked to ensure the positioning and standard measurements. 3D images were obtained in two different amplifications (1074× for roughness and 2131× morphology analysis), at each moment (before and after surface