

## Original Research Article

# Characterization of dentin morphology after application of ozone and sodium ascorbate by scanning electron microscopy and atomic force microscopy

João Batista de Souza<sup>1</sup>  
Daniel Rodrigues<sup>1</sup>  
Paula Cícilia Faquim Rodrigues<sup>1</sup>  
Lawrence Gonzaga Lopes<sup>1</sup>  
Tainah Costa Firmiano<sup>1</sup>  
Cynthia R. A. Estrela<sup>2,3</sup>  
Carlos Estrela<sup>1</sup>

### Corresponding author:

João Batista de Souza  
Department of Oral Rehabilitation, Dental School, Federal University of Goiás  
Faculdade de Odontologia  
Av. Universitária, esquina com 1.<sup>a</sup> Avenida, s/n – Setor Universitário  
CEP: 74605-220 – Goiânia – GO – Brasil  
E-mail: jbs.ufg@gmail.com

<sup>1</sup> Department of Oral Rehabilitation, Dental School, Federal University of Goiás – Goiânia – GO – Brazil.

<sup>2</sup> Dental School, Anápolis University Center – UniEvangélica – Anápolis – GO – Brazil.

<sup>3</sup> Dental School, University of Cuiabá – Cuiabá – MT – Brazil.

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### Abstract

**Objective:** The aim of this study was to evaluate dentin morphology after ozone gas and sodium ascorbate application by the Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) images. **Material and methods:** Seven freshly extracted human third molars were sectioned perpendicularly to the tooth long axis, 3 mm above the cemento-enamel junction and other section above the first. Dentin slices were obtained, 2 mm thickness, then the slices were sectioned vertically and horizontally into four equal parts, resulting in 28 samples. These were divided in 4 groups (n=7): (G1) phosphoric acid – control; (G2) ozone + phosphoric acid; (G3) phosphoric acid + ozone; and (G4) ozone + sodium ascorbate + phosphoric acid. Dentin morphology of six samples of each group was evaluated by SEM and one by AFM. **Results:** In Groups 2 and 4 there was a change in the effectiveness of acid etching in terms of removing the smear layer. In Group 3 there was a change in the

dentin microstructure, which can be a harmful factor regarding the bond strength of adhesive agents. **Conclusion:** After the qualitative analysis, the application sequences of ozone, phosphoric acid etching and sodium ascorbate application generated differences in the dentin morphology by SEM and AFM. Sodium ascorbate decreased the ozone effect before the acid etching but did not eliminate it.

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## Introduction

Ozone (O<sub>3</sub>) is a powerful oxidant used as an antimicrobial agent [1, 2]. It occurs naturally in the atmosphere or it can be artificially produced by electrical generators [1, 2]. The bactericidal efficacy of ozone is based on forming free radicals which damage the cell membranes by altering the osmotic stability and permeability [1, 2]. Due to these properties, it has been used in the stomatology, endodontics, periodontics, dental surgery and in the treatment of early and advanced carious lesions [4, 14, 19, 23, 24, 25].

In addition to the antimicrobial effect, its use in caries treatment is based on the ability to oxidize the proteins in carious lesions allowing the diffusion of calcium and phosphate ions through lesions and then resulting in remineralization of the affected dental tissues [3, 4]. Ozone is also capable of decarboxylating pyruvic acid, the strongest acid naturally produced through cariogenic bacteria, to acetic acid and it can also promote the remineralization of the early carious lesions [1, 3].

Because it is an allotropic variety of oxygen, O<sub>3</sub> is highly unstable and rapidly returns to oxygen [2, 18]. Presence of oxygen free radicals in the tooth can inhibit the polymerization of the resinous monomers, and therefore decrease the bond strength between the restorative material and the dentin substrates, as occurs with the bleaching agents [9, 21]. With the intention to neutralize the residual oxygen, the use of an antioxidant agent on the surface exposed to free radicals has been indicated [12]. Antioxidants, such as the ascorbic acid derivatives (sodium ascorbate 10%), act as free radical stabilizers neutralizing the effect of the oxygen in the bond materials polymerization [13, 15].

Oxygen released using bleaching agents alters the properties of both enamel and dentin [7, 8]. Because of this, it is necessary to verify if changes in the morphology of the dentin substrates occurs by the presence of free oxygen from the ozone gas application and if the sodium ascorbate combined to the gas interferes in the dentin morphology. Thus, the aim of this study was to evaluate the dentin morphology after ozone gas and sodium ascorbate

application by scanning electron microscopy and atomic force microscopy images.

## Material and methods

Seven recently extracted caries-free human third molars stored in 0.2% thymol solution at 4°C for no longer than 3 months were used in this study. The research was approved in the Ethics Committee of Federal University of Goiás under the protocol number 85/2010. After disinfection and removal of soft tissues, flat middle/depth coronal dentin surfaces were exposed, and the roots were removed using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) under water irrigation to obtain dentin slices of 2 mm thickness.

Exposed dentin surfaces were manually polished using wet silicon carbide sandpapers with decreasing abrasiveness (600, 1000 and 1200). After that, each dentin slice was cut into four equal parts, thus producing a total of four samples per tooth [22]. These four samples of each tooth were divided into study groups (n=7): Group 1, acid etching (G1); Group 2, ozone application followed by acid etching (G2); Group 3, acid etching followed by ozone application (G3); Group 4, ozone and sodium ascorbate application followed by acid etching (G4).

Dentin was etched for 15 seconds with phosphoric acid, rinsed with deionized water for 15 seconds, and dried with absorbent paper. Ozone was applied to the flat dentin surface at a concentration of 5,0 g/L por 40 s [1, 2, 10, 16] in a flow rate at 5 g.h<sup>-1</sup>. of ozone [2, 21] produced by electric discharge through an oxygen current (Generator PXZ3507, Eaglesat Systems Technology Ltd., São José dos Campos, SP, Brazil) coupled to an autoclave. This part of the study was conducted in a dry environment (inside a modified autoclave to receive only the ozone gas). The specimens were then irrigated with 10% sodium ascorbate for 10 min, flow rate of 1 mL min<sup>-1</sup>, then washed with 10 ml of deionized water and right after the excess water was removed with absorbent paper [21, 26].

Samples were stored in deionized water at 37°C and morphological evaluation of dentin samples were performed as follows: six samples from each group

were evaluated under scanning electron microscopy (SEM) (JSM 6610 LV, JEOL, Tokyo, Japan) and one sample from each group were evaluated under atomic force microscopy (AFM) (Agilent Technologies 5500, Santa Clara, USA).

### Scanning Electron Microscopy (SEM)

For observation by SEM, samples were dehydrated through ascending grades of alcohol, (25%, 50% and 70% ethanol) for 15 minutes each step, 95% ethanol for 30 minutes and subsequently with 100% ethanol for 60 minutes [1]. Afterwards, the samples were mounted on aluminum stubs, gold-sputter coated and evaluated under SEM in secondary electron mode (SE). Serial SEM microphotographs of the surfaces of each specimen at 1,000, 5000 and 15000× original magnification were obtained [18].

### Atomic Force Microscopy (AFM)

One sample of each group were observed with an AFM equipped with a piezoelectric scanner, which can cover an area of  $100 \times 100 \mu\text{m}^2$  with a

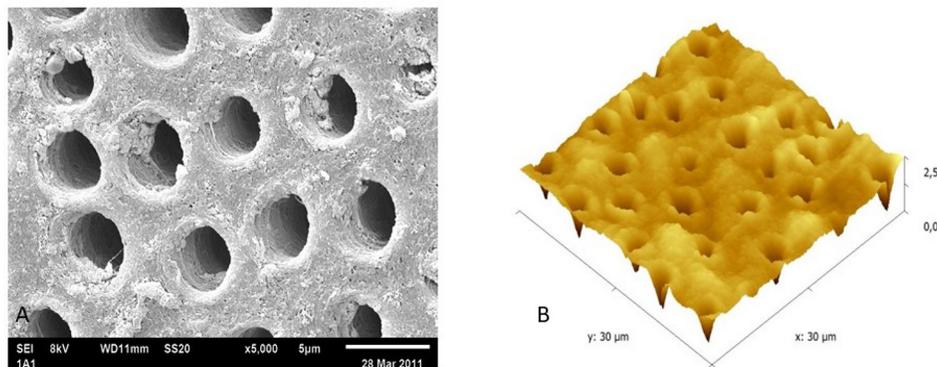
range of  $7 \mu\text{m}$  in the z-direction. For each sample, four images of three different sites of  $30 \times 30 \mu\text{m}^2$  were obtained with inclination of  $20^\circ$ . In the images, darker colors indicate a greater depth, while lighter colors indicate lower depth [16].

### Morphology analysis

Dentin morphology analyses were performed qualitatively. Samples obtained from the same slice were compared between the different treatments and samples from different slices were compared between the same treatment to verify a pattern.

## Results

In a qualitative analysis, the results showed different application sequences of the ozone and phosphoric acid, as the sodium ascorbate application after the use of the ozone generated differences in the dentin micromorphology in relation to the control group, with only the acid etching for 15 seconds (figures 1, 2, 3 and 4).



**Figure 1** - a) SEM image of the group 1 (control), exposition of the collagen fibrils; b) 3D model generated by AFM of the group 1 (control). Lower topographic standardization and smaller opening of the dentin tubules

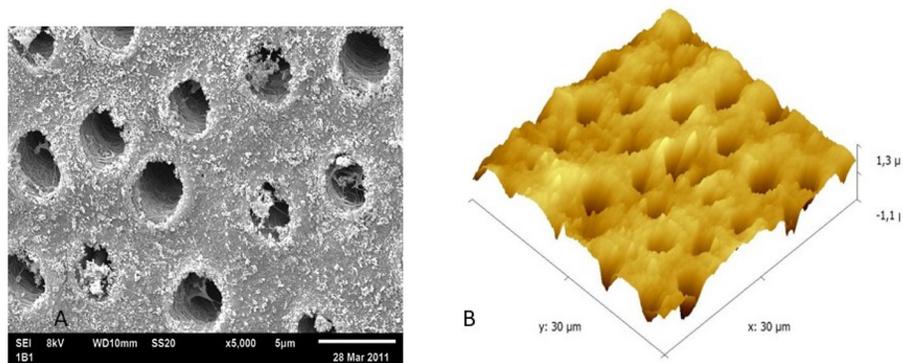


Figure 2 - a) SEM image of the group 2, exposition of the collagen fibrils, dentin tubules partially obliterated because of smear plug and smear layer; b) 3D model generated by AFM, pronounced irregularity in the opening of the dentin tubules and in the surface

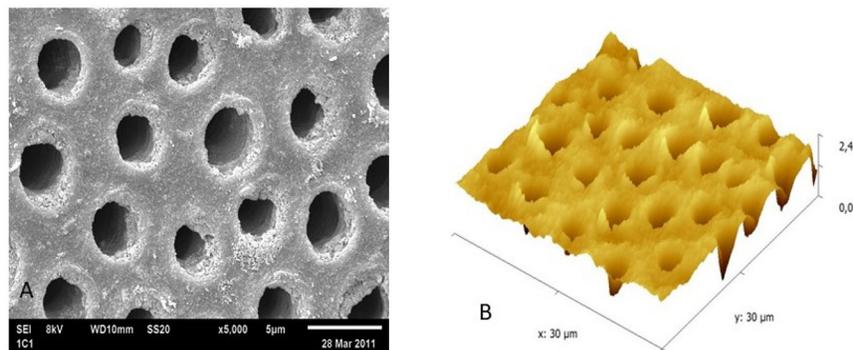


Figure 3 - a) SEM image of the group 3, "clean" surface aspect, it is not possible to observe the presence of collagen fibrils; b) 3D model generated by AFM. Darker peaks, uniform demineralization and increase in the diameter of the dentin tubules

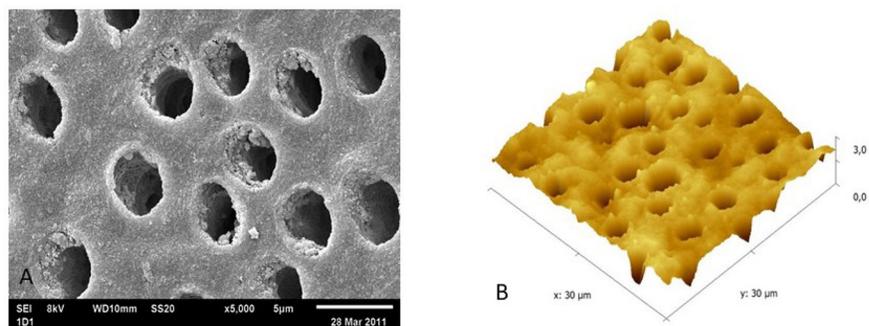


Figure 4 - a) SEM image of the group 4, exposed collagen fibrils, dentin tubules partially obliterated because of smear plug and smear layer; b) 3D model generated by AFM, increase in the diameter of dentin tubules, pronounced irregularity in the opening of the tubules and in the surface

In the SEM images of the groups 2 and 4 (figures 2a and 4a), in which the acid was used after the ozone, the dentin tubules remained partially obliterated because of smear plug and smear layer presence, with granules in the dentin surface of the group 2. Besides this, the intertubular dentin presented modified, with exposition of the collagen fibrils in these two groups cited, like in the control group (figure 1a). Therefore, the morphological pattern of the dentin remained the same, even with the acid etching after the ozone application and when the sodium ascorbate was used when compared to the control group. In the group 3 (figure 3a), when the acid was used before the ozone, demineralization of the dentin was observed, promoting one "clean" surface, however, there were changes in the intertubular dentin and it was not possible to observe in none of the images the presence of the collagen fibrils, which were observed in all the other groups (1, 2 and 4).

In the AFM images, darker peaks and with greater depth were observed in the group 3 (figure 3b), this group presented a more uniform demineralization when compared to the other groups. While in the groups 1, 2 and 4 (figures 1a, 1b and 4b respectively) there is a more pronounced irregularity, both the opening of the tubules and in the topography of the surface presenting lower topographic standardization. Besides this, smaller openings of the dentin tubules were observed in the groups 1 and 2. Whereas, when one acid was used before the ozone, like in the group 3, an increase in the diameter of the dentin tubules was observed, suggesting greater removal of the smear layer, because of the use of phosphoric acid. Same can be observed in the group 4, when two-acid application at the dentin were made, although the ozone gas was applied prior to the acid.

## Discussion

Although it is known the effectiveness of the ozone in carious lesion treatments [1-4, 26] the high oxidation activity of this molecule can modify some physical and mechanical properties of the enamel and dentin [6]. It was observed in this study that the ozone gas was able to modify the dentin morphology, that is, the application changed physical properties of the dentin.

Studies of Rodrigues *et al.* [21] and Dalkilic *et al.* [10] showed the microtensile bond strength decreased when the ozone gas was applied to the dentin when compared to the application only of the phosphoric acid or the self-etch adhesive system. In

the experimental group of this study in which the acid etching was performed followed by the ozone application there was a lack of exposed collagen fibrils, in other words, the low bond strength value can be result of the collagen fibrils collapse because of the ozone gas application, impairing the dentin hybridization to an adequate adhesion.

Cadenaro *et al.* [6] implied some effects of the oxygen free radicals produced by bleaching agents based on hydrogen peroxide during the use of ozone. Additionally, Cortez *et al.* [8] and De Carvalho [11] verified after the dental bleaching, the sodium ascorbate 10% application was effective in reversing the bond strength, whose values had decreased comparing to when the tooth was only bleached. Briso *et al.* [5] observed that 10% sodium ascorbate after the dental bleaching, both with carbamide peroxide and hydrogen peroxide increased the hybrid layer thickness and the length of the resin tags, improving the bond strength values. Whereas in the groups where the sodium ascorbate was used after the ozone application, both in the present research and in the study carried out by Rodrigues *et al.* [21], the results were like those found on the control group, consequently, the antioxidant was able to stabilize the oxygen free radicals resulting of the ozone oxidation

Besides this alteration in the dentin morphology because of the free radicals, the ozone in the gaseous form can change the architecture of the collagen fibrils exposed due to the acid etching [5]. In an adhesive procedure, for efficient hybridization of the dentin tissue, the acid etching promotes dentin demineralization and expose the collagen fibrils, which remain expanded by the water presence [5] preserving the interfibrillar spaces for the subsequent infiltration of the adhesive agent [5, 6]. Thus, to prevent the over dryness of the dentin, the removal of water excess should not be performed with air jets directly at the etched dentin, because it may cause a collapse of the collagen fibrils network and thus impair the adhesion [5, 20]. This fact can explain the non-visualization of the collagen fibrils exposed in the group in which the ozone gas was applied with the air of the triple syringe right after the acid etching, leading to the probable collapse of these collagen fibrils.

Smear layer debris and the lack of standardization in the diameter of the dentin tubules observed in the use of the ozone before the acid etching, even when the antioxidant was present, suggest a lower effectiveness of surface etching, which may have occurred through the interaction of ozone with phosphoric acid. The literature is scarce about the oxidation reaction of

the phosphoric acid. Mill in 1979 [17] proposed the use of hydrogen peroxide like source of oxygen free radicals to the oxidation of some acids and other substances, between them the phosphoric acid, the research revealed the hydrogen peroxide, in the concentration used, is efficient in the oxidation of many compounds, such as phosphorus acid salts, therefore is possible that in this research the ozone interacted with phosphoric acid and reduced the effectiveness of the acid etching.

Finally, due to the limitations of this research, more studies are necessary to evaluate the ozone effect on the dentin surface, the interaction with the phosphoric acid, self-etching and universal adhesive systems.

## Conclusion

Results of this study support that the use of ozone before acid etching changes its efficacy in removal of the smear layer. The same effect can be observed when sodium ascorbate is used, but with less intensity and when ozone is used after the phosphoric acid, the dentin microstructure has changed, probably due to collapsed collagen fibrils and this can be a harmful factor to the bond strength of adhesive agents.

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