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Original Research Article

Effect of ozone and 10% sodium ascorbate on human dentin microhardness

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Abstract

Introduction: Several strategies have been tried out for the control and antimicrobial treatment of carious lesions such as the direct application of ozone. However, the the oxidation process after the use of ozone facilitates the permanence of residual oxygen, which can negatively influence the use of adhesive systems. The application of 10% sodium ascorbate on the dentin surface can neutralize the effect of oxygen. However, the influence of these substances on the coronary dentin microhardness must be studied. **Objective:** This study aimed to evaluate the microhardness of human dentin after the application of gaseous ozone and sodium ascorbate. **Material and methods:** Nineteen third molars were sectioned in order to separate the occlusal surface from the roots leaving a 4 mm thick specimen which was further divided into its mesial and distal parts. The mesial parts were allocated to Group A which was subdivided into two groups: control group, in which the initial microhardness was measured, and an ozone group, in which the application of gaseous ozone was performed for 40 seconds followed by the measurement of the final microhardness. The distal parts were allocated to Group

B, subdivided into two groups, a control group in which the initial microhardness was measured and an ozone + sodium ascorbate group in which the application of gaseous ozone was performed for 40 seconds and a solution of 10% sodium ascorbate for 10 minutes followed by measurement of the final microhardness. **Results:** The results showed that there was a statistically significant increase in dentin microhardness ($p>0.05$) in Group A (control and ozone) and in Group B (control and ozone + sodium ascorbate). However, there was no statistically significant increase in microhardness ($p>0.05$) between ozone in group A and ozone + sodium ascorbate in group B. Conclusion: The application of gaseous ozone increased the microhardness of human dentin and the application of sodium ascobate had no influence on the microhardness that has already been modified by gaseous ozone.

Introduction

Conventional treatment of dental caries consists of the mechanical removal of affected parts of the dental structure and restoration of the area with appropriate material. However, there is no definition of a criteria for judging whether caries has been completely removed from the cavity [19]. The remaining microorganisms in the tooth structure can become a key factor for the development of secondary caries and pulp problems after the therapy for the removal of caries [3].

Currently, with the strengthening of conservative dentistry, there is a search for less invasive and effective procedures to treat caries and a process of disinfecting the cavity during dental treatment would be important for the success of the restoration [19]. For this purpose, the use of sodium hypochlorite and chlorhexidine solutions have shown antimicrobial activity on oral microorganisms such as *Streptococus mutans* [15, 25]. Another alternative would be the treatment of caries lesions with the application of ozone, which eliminates microorganisms by inhibiting metabolic activity and rupture of the cell wall. Apparently, gaseous ozone has a more effective antimicrobial action than ozonized water [1, 20, 24].

After the mechanical cleaning of the cavity and the application of ozone gas is necessary to restore the tooth, but when the chosen restorative material is photopolymerizable resin, residual oxygen has a negative effect on the polymerization of adhesive systems [15, 18, 23]. However, this effect is transient and can be reversed if a period of two to four weeks is expected [4, 21], or with the use of an antioxidant substance. The antioxidant sodium ascorbate solution is used successfully when immediate neutralization of free oxygen is required [13, 22, 23].

The application of substances to dentin can alter some of its properties [5, 9]. Therefore, this study evaluated the effect of the application of ozone and sodium ascorbate 10% after the application of ozone on the microhardness of human dentin.

Material and methods

This in vitro study was approved by the Research Ethics Committee of Goias Federal University under protocol n. 083/2009. Nineteen recently extracted human permanent third molar teeth from patients ranging in age from 18 to 25 years were selected. These teeth had an indication of tooth extraction for orthodontic reasons and did not showed the presence of caries. After extraction the teeth were kept under refrigeration in a 0.2% thymol solution diluted in deionized water.

Soft tissue residues and periodontal ligament were removed by scraping with a periodontal curette (7/8) from all teeth, followed by cleaning with pumice paste and water, applied with the aid of a low-speed Robinson brush and finally, washing with deionized water. Sequentially, the teeth were examined in a stereoscopic magnifying glass, with a 10X magnification, to ensure the absence of defects or cracks and, again stored in deionized water.

The teeth were included, inside a silicone matrix, in orthophthalic resin. Dental surfaces were cut using double-sided diamond discs (KG Sorensen Ind. E Com, Ltda), under abundant cooling and low speed (420 revolutions per minute). To separate the occlusal surface, a cut was made 2 mm from the cemento-enamel junction. The second cut was made

4 mm from the first cut, obtaining a specimen 4 mm thick. This specimen was further divided into mesial and distal parts.

To differentiate the occlusal and cervical surfaces, the occlusal surfaces were marked with an overhead projector pen, making a point on the occlusal surface, close to the buccal surface. The mesial parts of each tooth were allocated to Group A and the distal parts to Group B. Therefore, each group was composed of 19 specimens.

Three control markings were made to assess the initial microhardness in the two Groups, A and B. The microhardness meter used was Shimadzu HMV II (Tokyo, Japan), with Knoop-type penetrator [26], and a static load of 25g applied for 30s. The markings were made 2 mm from the buccal surface, 2 mm from the lingual surface and a median point between these two. The teeth remained stored in deionized water until ozone gas was applied.

Ozone was applied using a PXZ3507 Generator (Eaglesat Tecnologia em Sistemas, São José dos Campos, SP, Brazil) coupled to an autoclave. The generator was kept on for an hour in a semi-closed environment at a temperature of 36 to 37ºC, so that the place was saturated with ozone gas at a concentration of 7.0 g / h. After this period, all specimens were introduced simultaneously into the autoclave and kept in contact with the gas for 40 seconds [5].

Soon after being removed from the autoclave, group B specimens were immersed and kept for ten minutes in a 10% solution of the antioxidant sodium ascorbate [8, 22]. The specimens were stored in an environment with 100% relative humidity.

The evaluation of microhardness was performed 12 hours after the application of ozone gas. The marking was made at 25 micrometers of the control markings in both groups A and B, using a Shimadzu HMV II microdurometer (Tokyo, Japan), with a Knoop penetrator, and a static load of 25g applied for 30s.

Results

The results were statistically evaluated by the t-Student test and demonstrated that there was a statistical difference between the initial microhardness of Group A and the final microhardness of Group A ($p = 0.0231$). The final microhardness of Group A (ozone) was higher than the values found for the initial microhardness of Group A (control). The same test defined that the means between the initial and final microhardnesses in Group B (ozone + sodium ascorbate) were statistically different ($p = 0.0015$). The final microhardness in Group B (ozone + ascorbate) sodium) had a higher mean (table I).

The t-Student test was applied to compare the final microhardness of Group A (ozone) and the final microhardness Group B (ozone + sodium ascorbate), however the result did not indicate a statistically significant difference between the two groups ($p = 0.3789$) (table I).

Table I – Mean and standard deviation between the groups Group A Ozone and Group B Ozone + sodium ascorbate ($p>0.05$)

Groups	Mean
Group A-Control	52.62 (5.23)[a]
Group A-Test	55.98 (4.24)[a]
Group B Control	48.42 (6.85)[b]
Group B Test	54.18 (7.65)[a]

Different letters show a statistically significant difference, $p<0,05$

Discussion

The dental enamel has a high hardness, ranging from 200 to 500 Knoop. It has a high modulus of elasticity, and a relatively low tensile strength, characteristic of a very fragile material, which only does not fracture easily due to the high compression strength of the underlying dentin. In turn, dentin is more rigid than bone, but less rigid than enamel. Unlike enamel, which is very hard and fragile, dentin is subject to slight deformation and is elastic [12]. The microhardness of enamel and dentin has been determined by several methods, with Vickers and Knoop impressions being the methods most used by many researchers [6, 16], with Knoop impression being used to determine the microhardness of thin regions due to the fact the penetration is narrower in relation to the Vickers penetration [7].

The application of ozone to the dentinal surface is due to the ability of this gas to eliminate bacteria present in human dentin after mechanical removal of the carious lesion [15]. However, there is no consensus on the time of application of the gas, varying between 40 and 120 seconds [5, 15, 19]. This study used 40 seconds, which is in agreement with Celiberti *et al.* [5]. After the application of ozone, residual oxygen may delay the preparation of the restoration that requires adhesive systems [15, 18, 23]. In order to eliminate this oxygen, it was used an antioxidant solution of 10% sodium ascorbate for a period of 10 minutes [10, 22].

Dentin is known to be the most abundant tissue in the dental structure. Consequently, knowledge of its physical properties is essential to predict changes that may occur in its structure through the application of some substance [12].

It was observed in the present study, that fter the use of ozone gas the final microhardness of the specimens was greater than the initial microhardness. However, when comparing the two test groups, ozone and ozone + 10% sodium ascorbate, there is no statistically significant difference in relation to microhardness. Studies indicate that the dentin microhardness is directly related to the mineral concentration and type I collagen present in it [11, 12].

Such observation is related to the fact that the mineral portion present in the dentin is located in two locations, intrafibrillar and extrafibrillar zone. The place where the mineral is inside or immediately adjacent to the open areas of collagen fibrils is called the intrafibrillary zone and the place where the mineral is located in the interstitial space, separated from the fibrils, is the extrafibrillar zone. It is estimated that between 70-75% of the mineral content of dentin is in the extrafibrillary space, although the intrafibrillary content is of fundamental importance for dentin microhardness [11].

Ozone in contact with organic fluids reacts immediately, forming signaling molecules for various cellular processes, for example, reactive oxygen species (ROS) capable of interacting with cellular components [2]. All living cells such as lymphocytes, neutrophils, monocytes, platelets, fibroblasts, osteoclasts and probably odontoblasts can generate these species during their normal metabolism [2].

The sodium ascorbate associated with ozone may also have some contribution to increase microhardness. Sodium ascorbate is a powerful antioxidant that donates its electrons to unstable molecules [22]. It is a non-toxic substance and, therefore, it is unlikely that its use will produce any adverse effect on periapical and / or dentin-pulp complexes, or even on the patient and / or professional during its handling [14].

Ascorbic acid is known as an innocuous remover of reactive oxygen species, but it oxidizes easily in the presence of oxygen and moisture. In order to make it more stable in oral biological tissues, a stabilized sodium ascorbate compound was formulated. It is known that ascorbic acid is a fundamental element in the synthesis of collagen, the most abundant component of the organic dentin matrix [17]. Therefore, it is suggested that there may be a greater stimulus for collagen synthesis and subsequent matrix mineralization.

Conclusion

Based on the methodology used, it was concluded that after ozone application on the dentin surface it had its microhardness increased and that the use of the antioxidant solution 10% sodium ascorbarte after ozone application did not produce changes in microhardness which had already been influenced by ozone.

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