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Ozone and microstructural morphological changes of tooth enamel

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Abstract

The paper aims to study the impact of ozone (O_3) treatment on the microstructural changes of the tooth enamel after the treatment at different time intervals. The ozonation was performed with gaseous O_3 produced by HealOzone X4, the demineralization level was measured with the DiagnoDent Pen 2190 device, and the microstructure changes of enamel surface were observed using scanning electron microscopy (SEM) analysis. The results showed the exposure to O_3 for 40–50 seconds enhanced enamel micro-hardness and ensures a rate of remineralization between 96.82–97.38%. In conclusion, in search of new minimally invasive solutions in the treatment of caries and to offer antimicrobial support of the oral cavity, the use of O_3 as an alternative therapy to classical solutions may be a viable solution in dentistry.

Keywords: enamel, ozone gas, scanning electron microscopy, remineralizing, caries.

Introduction

Ozone (O_3) is a highly reactive compound formed of three oxygen atoms that works as an oxidant and oxidizer [1]. As a non-invasive option for treating tooth decay, O_3 therapy has some distinctive features compared to other available modalities. The aim of treating carious lesions with O_3 is to decrease the causative microbiota and contributing risk factors, stop the caries process and stimulate remineralization. Enamel is the most complex and most mineralized hard tissue in the human body, with a content of 96% hydroxyapatite (HA) and 4% water and organic material [2]. Enamel can be damaged throughout life, as it is continuously exposed to the oral environment. The acidic environment created by acidic bacteria, acidic foods and drinks results in dental problems for four out of five temporary teeth in children and over half of permanent teeth in adults) [2, 3]. Because enamel is classified as a cellular tissue, it cannot regenerate or reshaped on its own; therefore, the lesions are irreversible and restored with artificial material.

The main issue of non-invasive approaches for preventing carious lesions and remineralization of enamel is the difficulty of controlling dental plaque. O_3 can remove proteins from carious lesions and allow calcium (Ca^{2+}) and phosphate (PO_4^{2-}) ions to diffuse through lesions, leading to remineralization [4]. In addition, O_3 has a significant environmental advantage, meaning a rapid degradation of O_3 clinically causes its low cytotoxicity after contact with organic compounds. All these features suggest that O_3 could be widely used soon in restorative and preventive dentistry [4].

Because of its high oxidizing power, O_3 can oxidize the bacterial cell wall, inducing its lysis and the pyruvic acid produced by the bacteria, turning it into acetic acid and carbon dioxide (CO_2), stopping or reversing the progression of caries. While the antimicrobial qualities of O_3 are well known [5–9], its remineralization potential, by increasing the permeability of the dentin tubules, is less studied. As a demineralizing agent, O_3 seems to improve the diffusion of salivary ions to the surface of degraded dentin, thereby promoting remineralization [10–13]. In this context, O_3 can neutralize acid proteins produced by cariogenic bacteria, representing the osmotic stimulus responsible for the movement of fluids in the dentin tubules leading to hypersensitivity [12–14].

Given all the therapeutic effects of O_3 mentioned above, the mechanisms of action are also diversified, such as: (*i*) as an antimicrobial agent, O_3 breaks down viral capsid and interrupts the reproductive cycle by peroxidation; (*ii*) as an immunomodulatory and antioxidant agent, it stimulates immunoglobulin synthesis, preserves cellular redox state and raises cellular levels of glutathione peroxidase (GPx); (*iii*) the anti-hypoxic effect occurs by increasing hemoglobin oxygen saturation; (*iv*) the anti-inflammatory action is due to the impact of O_3 on the synthesis of interleukins, prostaglandins and leukotrienes; (*v*) the bioenergetic function is exerted by activation of protein synthesis and an by enhancement of cellular metabolism; (*vi*) the detoxification activity occurs by activation of cellular aerobic processes (Krebs cycle, glycolysis and fatty acid oxidation); and (*vii*) the biosynthetic activity is achieved by enhancement of carbohydrate, protein and lipid metabolism [15].

O_3 can be applied to the tooth surface using either gas or water. It is a powerful bio-oxidation agent, is highly bactericidal and has been utilized in dentistry to treat dental caries (enamel and dentin), root caries and dental hypersensitivity [10].

Aim

The aim of this study was the analysis of the bio-oxidative effect and the microstructural changes of the tooth enamel following the use of O_3 at different time intervals.

Materials and Methods

Biological material

The study was performed on 15 permanent human teeth extracted for orthodontic reasons without harming them. Only fully erupted molars and whole molars without clinically detectable cavity lesions were included in the study, this being the main inclusion/exclusion criterion for molars studied. Teeth were collected and kept in saline until the start of the working method. All adherent soft tissues on the teeth were removed and cleaned by flushing. The samples were obtained using the same tooth on both sides to obtain the blank and working samples on the same enamel pattern by demineralization/ozonation (each tooth offered two samples). The experiment was done in duplicate. The 30 samples obtained were fixed in silicone material for better handling.

Demineralization and ozonation procedure

Teeth demineralization was performed with 35% phosphoric acid (H_3PO_4) (Gluma® Etch 35 Gel, Kulzer, Hanau, Germany), which was left on the enamel surface for 5 minutes for each half of the specimen separately for effective demineralization. The working specimens were successively subjected to ozonize with gaseous O_3 produced by HealOzone X4 (KaVo Dental & Co., Biberach, Germany), which releases O_3 at a fixed concentration of 2100 ppm, with a flow rate of 615 cm^2/min for 10, 20, 40 and 50 seconds, respectively. The ozonize device comprises an air filter, vacuum pump, an O_3 generator, a hand piece fitted with a sealing silicone cup, and a flexible hose. The procedure is usually between 20 and 120 seconds per tooth. According to the specifications, the device produces O_3 at a concentration of 32 g/m^3 at an exposure of 60 seconds. The initial mineralization degree of the enamel and the demineralization level was measured with the DiagnoDent Pen 2190 device (KaVo Dental & Co., Biberach, Germany). This equipment uses laser-induced fluorescence (LIF) technology to determine the quality of the structure of the tooth enamel, detecting the degree of its demineralization compared to healthy enamel. Fluorescent tooth enamel physiologically loses its properties with demineralization so that measurements with DiagnoDent Pen indicate on a scale of 0–12 healthy enamel, 13–24 enamel lesions and 25–99 dentin lesion (depending on the degree of demineralization).

The rate of mineralization was calculated with the formula:

$$\text{Rate of mineralization } [\%] = [(SD - SR)/SD] \times 100$$

where, SD – the DiagnoDent score after demineralization; SR – the DiagnoDent score after remineralization at different time.

The working (ozonized) and control (demineralized) samples were then analyzed with the scanning electron microscopy (SEM) using Quanta 250 FEG (FEI Company, Hillsboro, Oregon, USA) scanning electron microscope operating at 10 kV and 10 mm working distance to highlight the structural changes produced in the enamel. The applied procedure is presented in the Figure 1.

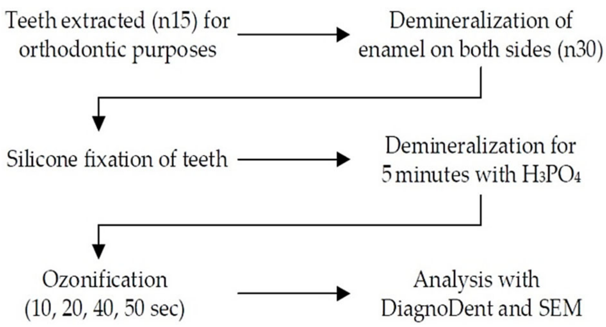

Figure 1 – ***The demineralization/ozonation procedure. H_3PO_4: Phosphoric acid; SEM: Scanning electron microscopy.***

Results

The remineralizing potential of ozone

In Figure 2 is presented the demineralized tooth (Figure 2A) compared with a tooth treated with O_3 after demineralization (Figure 2B). In Figure 3 is presented the score of DiagnoDent Pen 2190 measurement and in Figures 4 and 5, the microscopic aspect (SEM) of tooth enamel before and after exposure to O_3 for 10 seconds and 50 seconds, respectively.

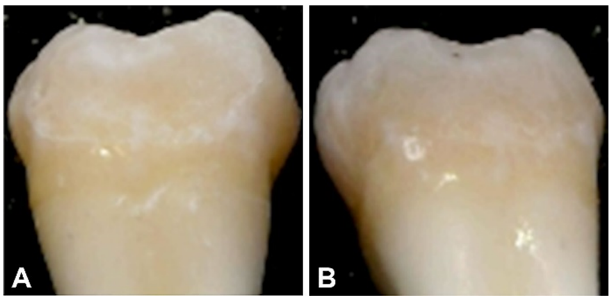

Figure 2 – ***The tooth aspect before and after exposure to the ozone treatment: (A) Demineralized tooth; (B) Tooth treated with ozone after demineralization.***

Values are reported as mean values ± standard deviations of total measurements. A *t*-test was applied to compare mean differences between samples; data within the same row with different superscripts are significantly different ($p<0.05$); data within the same row with the same superscripts are not significantly different ($p>0.05$).

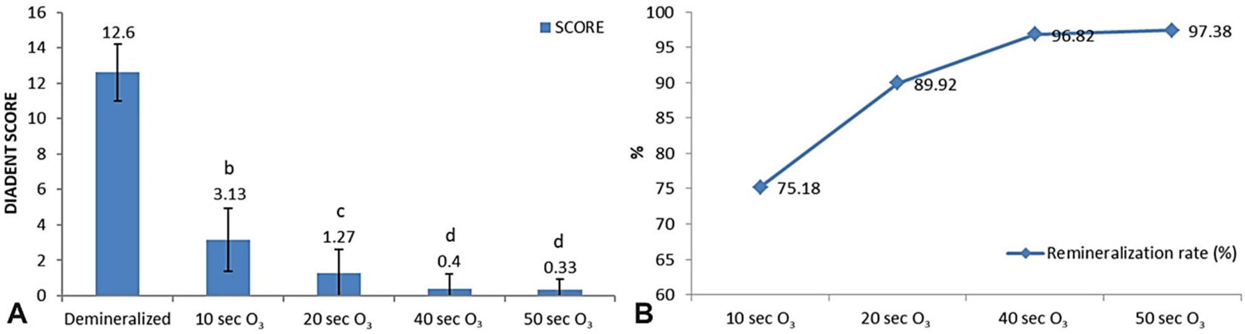

Figure 3 – ***DiagnoDent Pen 2190 measurement of the demineralized/ozonized enamel: (A) DiagnoDent score; (B) The remineralization rate. O_3: Ozone.***

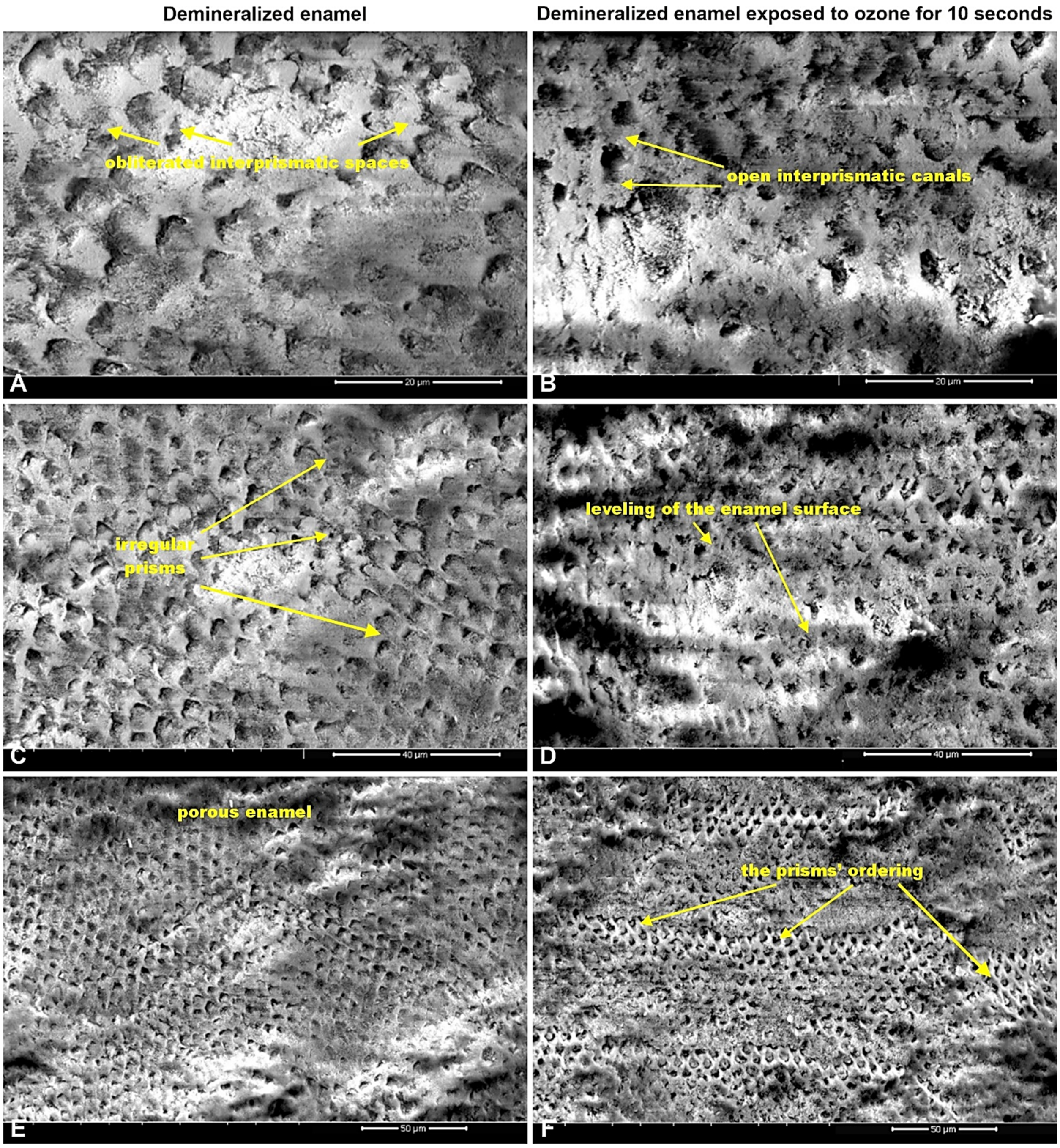

Figure 4 – ***Microscopic aspect of tooth enamel before (A, C and E) and after exposure to 10 seconds ozone (B, D, and F). Microscopic aspect of demineralized enamel: (A) ×2000; (C) ×1000; (E) ×500. Microscopic aspect of demineralized enamel exposed to ozone: (B) ×2000; (D) ×1000; (F) ×500. Scale bar: (A and B) 20 µm; (C and D) 40 µm; (E and F) 50 µm.***

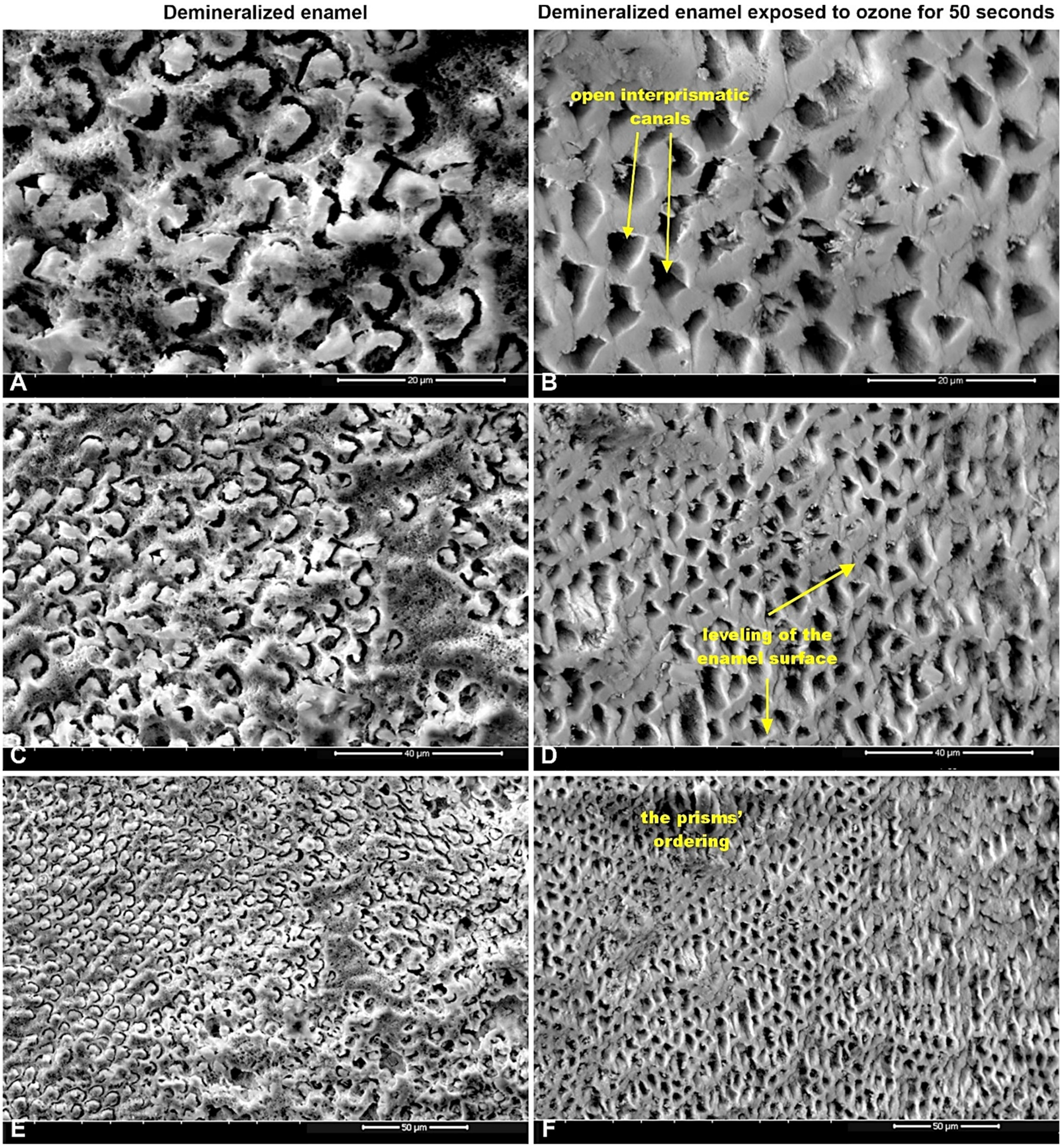

Figure 5 – ***Microscopic aspect of tooth enamel before (A, C and E) and after exposure to 50 seconds ozone (B, D, and F). Microscopic aspect of demineralized enamel: (A) ×2000; (C) ×1000; (E) ×500. Microscopic aspect of demineralized enamel exposed to ozone: (B) ×2000; (D) ×1000; (F) ×500. Scale bar: (A and B) 20 µm; (C and D) 40 µm; (E and F) 50 µm.***

The results of the test performed using DiagnoDent Pen 2190 equipment before and after O_3 exposure (Figure 2, A and B) shown that initially, after demineralization, the scores obtained are between 11–14, recommended intensive prophylaxis measures, including ozonized. After a reduced exposure of only 10 seconds to the O_3 treatment, a significant decrease of DiagnoDent Pen 2190 scores is observed, with values between 1–5, respectively after 20 seconds the values obtained decrease between 0–3, and after 40–50 seconds the scores obtained be less than 1. The exposure to O_3 gas for 40 seconds dehydrated the enamel and increased its micro-hardness.

The statistical analysis using *t*-test shows statistical relevance ($p<0.05$) between demineralization sample (after acidic treatment and before O_3 exposure) and experimental trials (with O_3 treatment at different exposure time). Thus, it can be said that the dental structures were remineralized in proportions compared to the control samples depending on the O_3 exposure. After 10 seconds of exposure, the remineralization rate was 75.18% and increased at 89.92% after 20 seconds. Higher rates (96.82% and 97.38%) were recorded after 40 seconds, respectively 50 seconds of O_3 exposure. According to *t*-test, there is a dominant difference between the values recorded at the exposed sample at 10 seconds from the initial sample. No significant differences are recorded between the score obtained after 40 and 50 seconds to O_3 exposure.

Tooth enamel, like bones, is the hardest tissue in the body, being even more mineralized than the skeleton and having a complex three-dimensional structure due to the presence of HA crystals. Under normal conditions, mammals have a uniform and orderly spatial arrangement of these tightly packed crystals. Thus, the HA crystals of the enamel are organized in the form of prisms (aligned crystals) and in the form of an interprismatic substance [16, 17].

The SEM analysis of the O_3-exposed enamel reveals morphological changes in its roughness depending on the exposure times. Microscopically aspect of demineralized enamel recorded at ×500 magnification shown porous aspect of enamel (Figure 4A), respectively irregular prisms when the magnification was increased at ×1000 (Figure 4C) and obliterated interprismatic spaces at ×2000 (Figure 4E). The exposure at O_3 treatment for 10 seconds leads prisms ordering, leveling of the enamel surface and to the opening of interprismatic canals (Figure 4, B, D and F). The phenomena of enamel quality improvement are even more pronounced after exposure of 50 seconds to O_3 treatment (Figure 5, A–F).

These changes depend on the exposure time and the opportunity to disrupt the proteins embedded in the enamel matrix by bio-oxidation. Oxygen penetrates more easily into the structure of the enamel in the organic phase. On the surface of the demineralized enamel, the appearance of porous enamel was noticed because of the dissolution of the HA crystals around the enamel prisms. Also, irregular prisms and obliterated interprismatic spaces with the appearance of rough enamel was highlighted (Figure 4, A–C). After ozonized, the SEM images show a leveling of the enamel surface, open interprismatic canals, and the prisms' ordering.

At the higher the exposure, the better the appearance of the honeycomb with an orderly structure of the HA prisms and the cleaning of the interprismatic spaces was noticed (Figures 4 and 5).

Discussions

In this study, the remineralizing potential of O_3 was evaluated using two methods: (*i*) the measurement of fissure carries using DiagnoDent Pen 2190; and (*ii*) analysis of the microscopic aspect (SEM) of tooth enamel before and after exposure to O_3 treatment. The use of DiagnoDent Pen 2190 offers advantages in minimally invasive therapy and completes the clinical diagnosis and dental radiography. The smallest changes can be established and treated accordingly, which cannot be seen with the naked eye up to a depth of up to 2 mm from the dentin. The scores obtained in case of demineralization diagnosis using DiagnoDent Pen 2190 recommend the prophylactic treatment, as follows: (*i*) for values between 0–12, normal prophylaxis measures are recommended (*e.g.*, fluoride toothpaste); (*ii*) for values between 13 and 24, intensive prophylaxis measures are recommended (egg fluoridation or ozonizing); (*iii*) for values above 25, minimally invasive restorative procedures are recommended using filling materials and intensive prophylaxis, respectively for large lesions, classical restoration, depending on the risk assessment and on the diagnosis. The results analysis using DiagnoDent Pen 2190 measurement of the demineralized/ozonizing enamel shown that the scores decreased after the application of O_3 (Figure 2, A and B). The level of reduction, compared to the score obtained after demineralization was 75.18% after 10 seconds, 89.92% after 20 seconds, respectively 96.82% after 40 seconds and 97.38% after 50 seconds of remineralization. The results confirm the research obtained by other authors who showed that O_3 was effective on remineralization [15, 18].

SEM was used by several studies to assess the demineralization/remineralization effect, SEM analysis is often used to evaluate enamel processes, both in cariology, the adhesion of enamel materials, orthodontics [19, 20].

Baysan *et al.* (2007), evaluated the clinical inversion of root caries by exposure for 10 seconds to gaseous O_3 at 2100 ppm, succeeded by application for 5 seconds of xylitol and fluoride. The study found that after six months, O_3-treated lesions were significantly reduced compared to controls. Similar results were reported after 18 months, when primary root caries lesions were treated with gaseous O_3 (2100 ppm for 10 seconds), followed by daily use of the remineralization kit with patent [21]. The experimental studies recommend the exposure to O_3 gas for 40 seconds to enhance the enamel micro-hardness [15, 22]. Our study confirms the maximum efficiency of exposure for 40 seconds to O_3 on the remineralization of the enamel, but significant effects can be registered at an exposure of only 10 seconds.

The role played by O_3 in this process as a powerful oxidant and proteolytic agent that can disrupt the organic content of demineralized enamel and enhance the diffusion of remineralizing agents. O_3 oxidizes pyruvic acid (responsible for lowering pH and the carry process) into acetate and CO_2 and promote remineralization because acetate has a greater pKa and promotes pH buffering in the resting plate [23].

Conclusions

In search of new minimally invasive solutions in the treatment of caries and to offer antimicrobial support of the oral cavity, the use of O_3 as an alternative therapy to classical solutions may be a viable solution in dentistry. Even though the use of O_3 in routine dental medicine is not yet universally accepted due to lack knowledge, limited training and evidence in the literature, the results obtained in this study are encouraging and offer new approaches in dental technology.

Conflict of interests

The authors declare no conflict of interests.

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