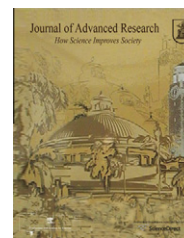




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ORIGINAL ARTICLE

Chemical analysis and surface morphology of enamel following ozone application with different concentrations and exposure times

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KEYWORDS

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Abstract This study aimed to determine the effect of different ozone concentrations applied with different exposure times on the chemical composition and the surface morphology of enamel. Twenty human mandibular molars were divided into four groups according to ozone concentration and exposure times. Group A received 90 μg of ozone/ml oxygen for 1 min, group B received 90 μg of ozone/ml oxygen for 2 min, group C received 120 μg of ozone/ml oxygen for 1 min and group D received 120 μg of ozone/ml oxygen for 2 min. The ozone source was from a medical ozone generator equipped with a device to adjust the concentration. Buccal surfaces of teeth were tested before and after ozone application so that each tooth served as a control for itself, using Environmental Scanning Electron Microscope (ESEM) connected to an Electron Dispersive Analytical X-ray (EDAX). Changes in calcium and phosphorus percentage levels were recorded and the Ca/P ratio was calculated. The values were statistically analyzed using the one-way ANOVA test with a level of significance set at $P \leq 0.05$. No statistical significant difference was found between the control and the tested groups in minerals content or ratio as $P > 0.05$. ESEM images showed enamel surface roughness with 2 min ozone exposure times. High ozone concentration with prolonged exposure time does not change the chemical composition of enamel. Applying ozone for 2 min alters the surface morphology of enamel causing variable degrees of roughness. Using high ozone concentrations with prolonged exposure times

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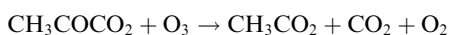
for caries reversal or prevention and for bleaching may be contraindicated if this changes the surface morphology of enamel.

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Introduction

A significant decline in dental caries has been observed over the last few decades in both industrialized [1] and developing countries [2]. However, caries occurrence is still high in some individuals. In high caries risk patients, developing new methods to prevent caries is extremely important to control the disease completely. In 1993, Anderson et al. [3] described the traditional treatment of caries as the surgical removal of the diseased parts of the tooth and the restoration of the cavity with an inert filling material. This concept has changed entirely to a more conservative approach, due to better understanding of caries as a disease and major developments in diagnostic and cutting tools [4].

Recently, the use of ozone in restorative dentistry has been employed for treating pit and fissure caries [5], non-cavitated occlusal caries [6], and primary root caries [5,7–9]. Ozone therapy has great potential as a non-traumatic approach. It has been reported that ozone, in either the gaseous or aqueous phases, has a strong oxidizing power with a reliable antimicrobial effect against bacteria, fungi, protozoa and viruses [10–12]. It is generally accepted that the oxidant potential of ozone destructs cell walls and cytoplasmic membranes of bacteria and fungi, attacks the amino acids and inhibits and blocks the enzymatic control system of these cells [13]. By oxidizing biomolecules in dental diseases, ozone has a severely disruptive effect on cariogenic bacteria [14]. Ozone can decarboxylate pyruvic acid, which is the strongest acid produced by acidogenic bacteria during cariogenesis, to acetic acid, as in the following equation [15]:



Aciduric (acid loving) microorganisms prefer an acidic ecological niche in order to prosper. Remineralization of incipient carious lesions can be encouraged by buffering plaque fluid by the production of acetate or other high pKa acids found in resting plaque [16]. Thus an effective alternative to conventional “drilling and filling” is to arrest primary caries non-operatively with ozone and remineralising products [14]. Using HealOzone, an ozone-generating device, for 10–20 s in vivo, killed 99% of microorganisms present in the lesions [8,9]. However, the total number of microorganisms was greatly reduced in small, non-cavitated lesions after ozone application than in larger lesions and lesions close to gingival margins [7], which suggests the need to increase time of application or ozone concentration.

Ozone was also used in preventive dentistry: it was found that combined ozone-fluoride treatment significantly enhanced fluoride uptake within enamel surfaces and could offer a clinically promising and safe therapy with a higher efficacy for preventing initial and recurrent caries [17]. In cosmetic dentistry, ozone has successfully bleached stained teeth [18,19]. In addition, several research studies are currently addressing the use of ozone as an enamel and dentin conditioner prior to bonding procedures.

Despite these vast uses of ozone in restorative and preventive dentistry, little research effort has been applied to the effect of ozone on the chemical structure and surface morphology of hard tooth tissues. Therefore, the objective of this in vitro study was to determine whether different concentrations of ozone applied with different exposure times would alter the chemical composition and surface morphology of enamel. The null hypothesis tested was that ozone gas does not change chemical structure and surface morphology of enamel surface.

Material and methods

Twenty permanent, sound, human mandibular first molars, extracted for periodontal reasons, were selected for this study. All teeth were cleaned manually with a brush and water and then stored in saline at room temperature till use. The teeth were randomly divided into four groups each having five teeth. Teeth in group A received 90 µg of ozone/ml oxygen for 1 min, in group B teeth received 90 µg of ozone/ml oxygen for 2 min, in group C teeth received 120 µg of ozone/ml oxygen for 1 min and in group D teeth received 120 µg of ozone/ml oxygen for 2 min. Ozone was applied from a medical ozone generator (ozonelab OL80FS, ozone services company, Burton, British Columbia, Canada) adjusted on 7 V and 1/16 flow to receive 90 µg of ozone/ml oxygen for groups A and B; and it was adjusted on 10 V and 1/16 flow to receive 120 µg of ozone/ml oxygen for groups C and D. The source of ozone was pure medical oxygen and the generator was equipped with a device for exactly adjusting the concentration. This dose was selected to be compatible with the ozone application dose by HealOzone. Buccal surfaces of all teeth were selected and tested first, so that each tooth served as a control for itself, to show normal chemical composition and surface morphology. Teeth then received the ozone applications while they were immersed in water. After the completion of ozone application, the teeth were removed from water and washed with distilled water and kept for 30 min in a dry container before testing.

Teeth were examined for morphological and chemical characterization using an Environmental Scanning Electron Microscope (ESEM) (Quanta 200, FEI Company, Philips Electron Optics, Eindhoven, Netherlands) equipped with Electron Dispersive Analytical X-ray (EDAX). The surface levels of calcium (Ca) and phosphorus (P) were quantified as weight percent using EDAX. Each tooth was irradiated at the centre of the buccal surface and at two additional areas under the operating conditions of 20 kV, 1.00 Torr and at three magnifications; the mean was calculated for each tooth. Using the ESEM in this study allowed the examination of hydrated un-fixed teeth surfaces that reduces the occurrence of artifactual changes and gives clear images.

Changes in Ca and P percentage levels were recorded and the Ca/P ratio was calculated; the data were presented as mean and standard deviation values. The differences between groups were analyzed statistically using the one-way ANOVA test. The level of significance was set at $P \leq 0.05$. Statistical analysis

was performed with SPSS 14.0 (Statistical Package for Scientific Studies) for Windows.

Results

Chemical analysis

Changes in the mean concentrations of Ca, P and the Ca/P ratio in enamel are shown in Table 1 and Figs. 1–5. Mineral analysis revealed no statistically significant difference in content or ratio after ozone application with different concentrations and exposure times compared to untreated control enamel surfaces ($P > 0.05$, ANOVA). EDAX analysis revealed peaks of elemental constituents of the control and the tested groups. Elemental peak heights from the enamel surface were shown as a function of the X-ray energy.

Morphological changes

Teeth were examined at magnifications of ($\times 600$, $\times 800$ and $\times 1200$) with the ESEM. Figs. 1–5 show representative ESEM images and the EDAX mineral surface levels of the different groups. In Fig. 1 the ESEM image shows the normal enamel prisms' ends with some surface deposits. In Fig. 2 the ESEM image from group (A) shows normal prisms' ends with few surface deposits and no surface enamel roughness. In Fig. 3 the ESEM image from group (B) shows surface deposits with mild roughness of the enamel surface. In Fig. 4 the ESEM image from group (C) shows scarce surface deposits and no surface roughness. In Fig. 5 the ESEM image from group (D) shows severe roughness of enamel surface with no surface deposits.

Discussion

Ozone is a chemical compound consisting of three oxygen atoms (O_3 – triatomic oxygen), which are in a higher energetic form than normal atmospheric oxygen (O_2). It is one of the most potent oxidants and has a great capability for oxidation [20]. Ozone therapy, as a non-invasive alternative for treating dental caries, has several distinguishing characteristics compared with other available modalities. The goal of treating carious lesions with ozone is to reduce the causative microbiota and contributing risk factors, to halt the caries process and to stimulate remineralization, and this goal is achieved markedly. The main problem of non-invasive pharmaceutical approaches for caries reversal and remineralization of lesions is the difficulty in suppressing or eliminating micro-organisms for the time that is required for remineralization; this is omit-

ted with ozone use. Ozone enables the shifting of microbial flora from acidogenic and aciduric microorganisms to normal oral commensals, which will allow remineralization to occur [7]. Ozone has the ability to remove proteins in carious lesions, and to enable Ca and P ions to diffuse through the lesions, leading to remineralization [9]. In addition, ozone has a major environmental advantage, its low cytotoxicity, which is clinically caused by a rapid degradation of ozone after contact with organic compounds [21]. All of these characteristics suggested that ozone might be widely used in the near future in restorative and preventive dentistry.

In this *in vitro* study, the EDAX analysis was carried out to identify changes in minerals percent or ratio due to the oxidation effect after ozone application. The results of this study showed that there was no evident change in the peak profiles of Ca and P between the control and the different tested groups. This suggested that the application of ozone on human enamel did not evidently alter the mineral profile of enamel. This may be attributable to the major content of inorganic elements in enamel, while ozone attacks amino acids and disrupts proteins. This is in agreement with Celiberti et al. [13] who found that ozone application from HealOzone for 40 s, did not affect the physical properties of enamel. In contradiction, Abu-Naba'a et al. [22] found that pit and fissure carious lesions showed significant reduction in hardness. This might suggest that several ozone applications within a period of six months adversely affects enamel physical properties and is contraindicated.

From the SEM images of different groups, it can be observed that changes in surface morphology, in terms of roughness, were evident with prolonged ozone exposure time for 2 min in groups B and D, while no surface morphology changes occurred in groups A and C with ozone exposure time for 1 min. This could be because the longer ozone exposure times enabled more opportunity to disrupt enamel matrix proteins with the subsequent loss of embedded materials by oxidation. Nascent oxygen is thought to penetrate enamel structure much easier across the organic phase [23]. Thus, the changes on enamel surfaces probably occur disproportionately in the enamel containing proteins or other organic materials; this could be expected for ozone too. The null hypothesis for this study should be partially rejected, as the ozone gas did not change the enamel's chemistry; but long ozone exposure time for 2 min did alter the enamel's surface morphology. Although the enamel surface was markedly altered in group D, it was difficult to determine if these changes were reversible or not, as no further treatment and testing was done. Further investigations should address the action of dental plaque on ozonated enamel surfaces.

The EDAX analysis is based on bombarding specimens with a beam of high voltage electrons that are refracted at different energy levels from the individual minerals. The

Table 1 Mean concentration (percent), standard deviation values of Ca, P and Ca/P ratio in enamel of different groups.

Group	Element %					P-value
	Control Mean (SD)	Group A Mean (SD)	Group B Mean (SD)	Group C Mean (SD)	Group D Mean (SD)	
Ca	43.85 (5.85)	45.84 (6.18)	44.73 (2.20)	41.65 (7.97)	44.73 (7.12)	0.898
P	22.95 (5.58)	23.99 (2.76)	22.84 (1.38)	22.41 (5.61)	21.00 (3.10)	0.992
Ca/P	1.984 (0.42)	1.912 (0.15)	1.958 (0.02)	1.922 (0.45)	1.986 (0.13)	0.983

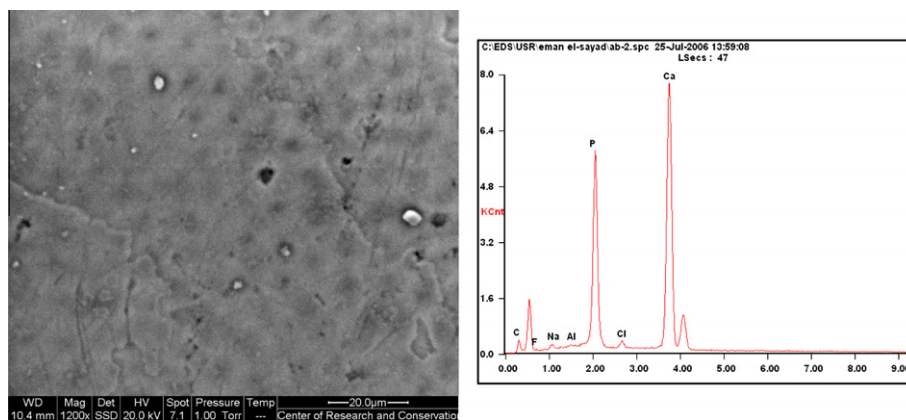


Fig. 1 SEM image (1200×) and EDAX surface levels of Ca and P in the control group.

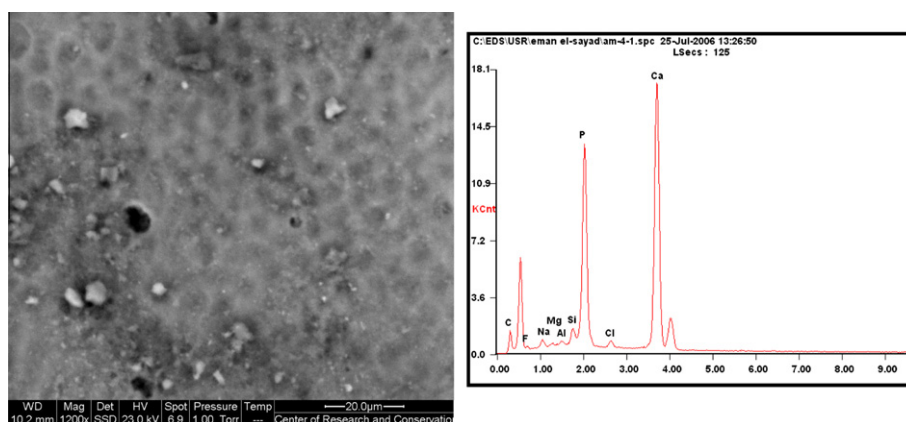


Fig. 2 SEM image (1200×) and EDAX surface levels of Ca and P in group A.

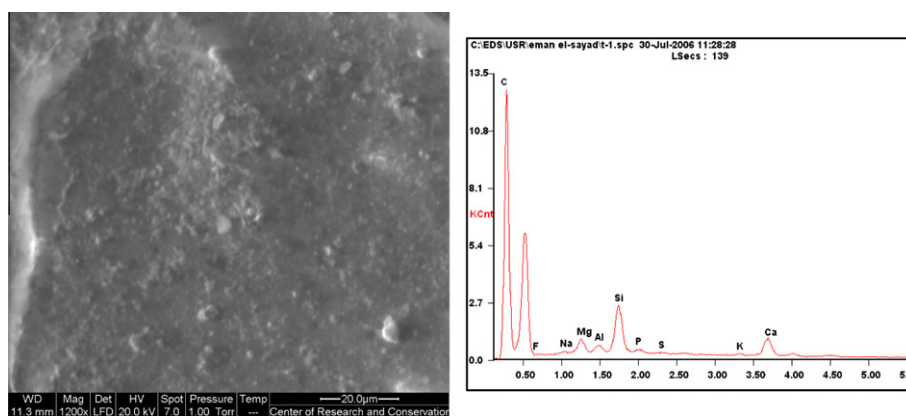


Fig. 3 SEM image (1200×) and EDAX surface levels of Ca and P in group B.

change in the energy returned from the specimen reflects the change in its mineral content. This technique allows the analysis of specimens accurately and non-invasively. However, this study faced some limitations, as the EDAX may be a method that is much less informative than XPS or Raman spectroscopy.

Conclusions

It is concluded that high ozone concentration with prolonged exposure time does not change the general chemical composition of enamel. In addition, applying ozone for 2 min alters the

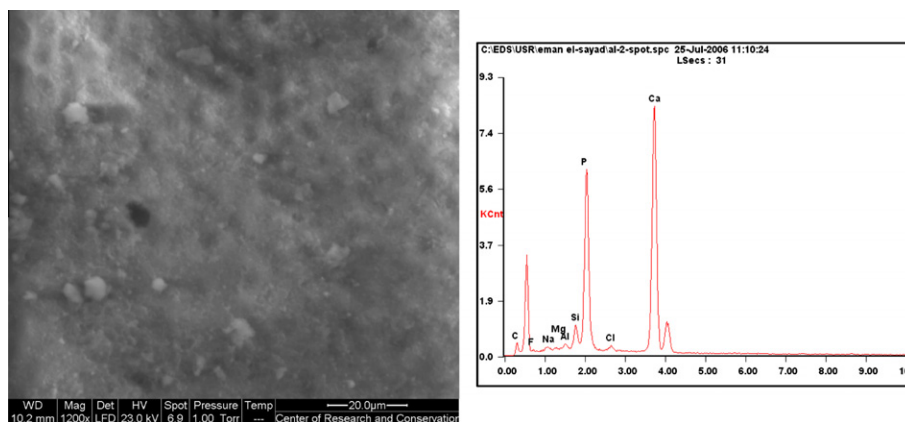


Fig. 4 SEM image (1200 \times) and EDAX surface levels of Ca and P in group C.

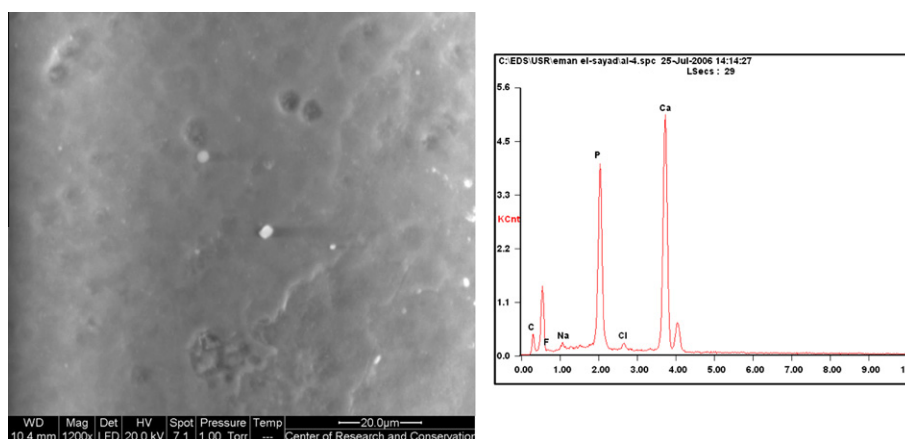


Fig. 5 SEM image (1200 \times) and EDAX surface levels of Ca and P in group D.

surface morphology of enamel, causing variable degrees of roughness.

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