The Effect of Ozone Gas Using Different Remineralizing Materials on Non-Cavitated Caries-Like Lesions in Permanent Teeth

Inas Mahmoud Karawia[1], **Osama Safwat Mohamed**[2]

[1]Community Department, Faculty of dentistry, Pharos University, [2]Prothodontic Technology Department, Faculty of Medical Allied, Pharos University

Abstract

Objective: To evaluate *in vitro*, the effect of ozone gas followed by remineralizing materials on non-cavitated caries-like lesions in permanent teeth. Material and Methods: Sixty extracted sound premolars with standardized window on buccal and lingual surfaces were immersed in demineralizing solution. Each tooth was sectioned into two halves. The specimens were randomly divided into three equal groups (I, II, and III) (n=40), each group was subdivided into two subgroups (n=20), experimental [I(a), II(a), and III(a)] and control [I(b), II(b), and III(b)]. Subgroup I(a) was treated with ozone followed by fluoridated toothpaste, subgroup II(a) was treated with ozone followed by HealOzone remineralizing solution and oral hygiene patient kit, and subgroup III(a) was treated with ozone followed by fluoride varnish and fluoridated toothpaste. Their controls left untreated. After 4 weeks the specimens were evaluated using Scanning Electron Microscopy with Energy Dispersive X-ray Analysis (SEM-EDX) for identifying the elemental composition of the specimen. Results: There was significant increase in the mean Ca/P in the 3 test subgroups when comparing with their controls ($P<0.05$). The mean Zn values were significantly increased in subgroup II (a) ($P<0.05$) at confidence interval 95%. Conclusions: Ozone application followed by different remineralizing materials had the same effect on calcium uptake, although HealOzone remineralizing solution and patient kit had better effect on remineralization due to the presence of zinc.

Key Words: Ozone, Fluoride, Zinc, Remineralization

Introduction

Caries is a chronic, slowly progressive disease; its symptoms are not detected at the onset but generally much later. The initiation of dental caries is associated with demineralization, which is calcium and phosphate loss of subsurface tooth enamel, which is clinically manifested as a white spot lesion [1].

Demineralization and remineralization are dynamic processes, characterized by the flow of calcium and phosphate in and out of tooth enamel, which should be balanced in order to prevent the progression of dental caries [2].

The noninvasive intervention of non-cavitated carious lesions, by applying therapeutic agents for tissue healing is used, over the last 25 years; the decline in dental caries experienced can be attributed largely to the widespread use of fluoride. Fluoride affects the caries process by enabling the formation of high quality fluorapatite that aids remineralization and inhibits glycolysis of plaque microorganisms [3]. Fluoride varnish was originally developed to prolong the contact time between fluoride and the tooth surfaces, varnishes provide high concentration of fluoride, it is generally acceptable to patients, and does not require special preparation of the teeth or expensive equipment [4].

Ozone has been introduced to medicine in 1950 as a modern development application for treatment of wounds and other infections due to its anti-microbial efficiency. Ozone is a harmless, non-toxic, and environmentally safe material [5].

Regarding the antimicrobial effect, ozone blocks the enzymatic control system of the cell and increase membrane permeability, the key element of cell viability, leading to immediate functional cessation, then ozone molecules can readily enter the cell and cause the microorganism to die. This action is non-specific and selective to microbial cells [6].

In dental surgery, the ozone may be applied as a gas or dissolved in water to promote homeostasis, enhance local oxygen supply by improving the blood supply, and inhibit bacterial proliferation. By the same way, the ozone gas was used in treatment of infections such as herps, or aphthous ulcers. It shortened the clinical course of the illness and accelerated the healing process [7].

In addition to the antimicrobial effect of ozone, the oxidation of pyruvic acid in saliva by ozone to acetic acid, suppresses the development and progression of primary caries lesions [8], and enable calcium and phosphate ions to diffuse through the lesions, which result in remineralization of the lesion following ozone application [9]. Also ozone can oxidize fluoride into fluorine which is more active and more easily transported into enamel crystals raising its fluoride content [10].

Recent technical advances have made it possible for ozone to be applied to small areas of dental hard tissues using a device, known as the Healozone [11]. Healozone application on non cavitated carious lesion is recommended to be followed by Healozone remineralizing solution, and instruction given to the patient to use oral hygiene patient kit, which consists of toothpaste, oral rinse, and oral spray all containing ingredients required to allow effective remineralization, which are fluoride calcium, phosphorous, xylitol and zinc, to promote caries reversal and tooth remineralization [12].

Zn has the potential to enhance fluoride-induced remineralization in early caries lesion. Exposure to fluoride may arrest lesions but that the subsurface region will likely remain hypomineralized, as highly mineralized surface zone acts as a barrier to diffusion of ions into the subsurface lesion.

Corresponding author: Inas Mahmoud Karawia, Assistant Lecturer, Community Department, Faculty of Dentistry, Pharos University, Tel: 02-01151155229; E-mail: inas.karawia.ik@gmail.com

The possibility of substitutions among the elements Ca- Zn [13], delay the complete mineralization of the surface zone, allow more subsurface remineralization lesion [14].

Studies have been reported successful results on managing pits and fissure caries, as well as deep carious lesions with ozone gas [15,16]. Further studies are needed to examine the effectiveness of ozone on early smooth surface carious lesions.

Therefore, this study investigated the efficacy of ozone gas application followed by different remineralizing products on smooth enamel caries like lesions in permanent teeth.

Materials and Methods

This article was extracted from master thesis, the study was an experimental study, *in-vitro* design and it was approved from the Research Ethical Committee, Faculty of Dentistry, Alexandria University.

Sample selection

Sixty sound premolars extracted for orthodontic reasons were collected from the out-patient clinics of the Alexandria University hospitals as well as the Ministry of Health hospitals. The selected teeth had no or slight change in enamel translucency after prolonged air drying (5 seconds), that scores 0 according to ICDAS-II [17], and diagnosed with DIAGNOdent (DIAGNOdent KaVo CO, Biberach/Riss, GmbH, D-88400 Germany) showing a reading less than 10 indicating sound enamel [18].

Preparation of early artificial caries lesions

Teeth were thoroughly cleaned from periodontal tissues and extrinsic deposits with hand scalar and fluoride free pumice and kept in saline solution. The smooth surfaces of the 60 teeth were coated with a nail varnish leaving a 4 × 4 mm window on the buccal and lingual surface of each tooth [19]. The teeth were immersed in demineralizing solution with pH 4.4 (2.2 mM Potassium dihydrogen phosphate KH_2Po_4, 50 mM Acetic acid, 2.2 mM Calcium chloride ($CaCl_2$) [20] for 72 hours at room temperature, to create an early enamel lesion. After demineralization, the teeth were washed with deionized water and dried [19].

Enamel specimen preparation

The crowns were cut from the roots. The crown samples were further sectioned into two halves in mesio-distal direction along the central fossa; one half was treated and the other was left without treatment, each half was considered as a specimen.

Treatment and pH cycling

According to the treatment used, the specimens were randomly divided into three groups (I, II and III) (n=40), each group was subdivided into two subgroups (n=20): Experimental (Ia, IIa, and IIIa) and Control (Ib, IIb, and IIIb). Subgroup Ia; was treated with ozone gas application using The HealOzone device (HealOzone KaVo Co, GmbH, D-88400 biberach/Riss-Germany Germany) for 60 seconds, with appropriate size of sealed cup [21], followed by daily brushing with fluoridated tooth paste (Unilever Mashraq-Personal Care (S.A.E) 6TH October City, 4th Industrial Zone) for 4 weeks. Subgroup IIa; was treated with ozone gas application for 60 seconds, using appropriate size of sealed cup, followed by HealOzone remineralizing solution for 60 seconds and daily use of HealOzone patient kit: tooth paste and spray (CurazoneInc Usa, Imc) for 4 weeks [19]. Subgroup IIIa; was treated with ozone gas for 60 seconds, using appropriate size of sealed cup [19], followed by painting with a thin layer of 5% sodium fluoride varnish (Durashield, Sultan Healthcare, Hackensack, NJ 07601) [22], and daily brushing with fluoridated tooth paste (Unilever Mashraq-Personal Care (S.A.E) 6TH October City, 4th Industrial Zone). The specimens of all control subgroups were not treated. All procedures were done according to manufacturer instructions. During treatment all the specimens will be kept in artificial saliva with pH 7 (20 mM Sodium bicarbonate $NaHCO_3$, 20 mM Sodium bicarbonate $NaHCO_3$, 3 mM Sodium dihydrogen phosphate NaH_2Po_4, 1 mM Calcium chloride ($CaCl_2$) [20].

The remineralizing and demineralizing solutions were freshly provided in biochemical laboratory, Faculty of Pharmacy, Alexandria University.

SEM examination (Appendix I)

After 4 weeks, the nail varnish was removed carefully from each specimen [23]. The specimens were evaluated by Scanning Electron Microscopy with Energy Dispersive X-ray Analysis (SEM-EDX) (FEL,Philips Elctron Optics and Micrion, USA.), a device used for identifying the elemental composition of the specimen by measuring re-emitted characteristics x-ray from elements [24]. The X-rays were analyzed to provide information on the elemental distribution (Ca, P and Zn) in the surface. The specimens were disposed into medical waste containers.

Statistical analysis

After data were collected, it ware revised, coded and fed to statistical software IBM SPSS version 20 (SPSS Inc. Chicago, IL, USA.) [25] Non parametric tests were applied, and descriptive statistics were displayed as means and standard deviations for quantitative variables (Ca/P, Zn in weight percentage). Comparisons between each test and control subgroup were done using Wilcoxon matched pairs signed ranks test. Comparison between the three groups was done using Kruskal-Wallis H test, followed by post Hoc test to compare between each 2 subgroups. The 5% was chosen as the cut off level of significance.

Results

Results show the change in enamel mineral content (calcium, phosphate and zinc) using SEM-EDX analysis.

In the comparison of mean Ca/P ratio between different test and control subgroups of the three groups there was significant difference (P=0.001) favor the three test subgroups (*Table 1*). There was no statistical significant difference between the three test subgroups regarding Ca/P ratio (*Table 1*).

Table 1. *Comparison of mean CA/P ratio in all groups between test and control subgroups.*

Ca/P ratio	**Test subgroups**	**Control subgroups**	**P value**
	Mean ± S.D	**Mean ± S.D**	
Group I	3.73 ± 0.37	1.32 ± 0.10	0.001*
Group II	3.61 ± 0.09	1.32 ± 0.10	0.001*
Group III	3.84 ± 0.27	1.33 ± 0.10	0.001*
*Statistically significant at P ≤ 0.05			

According to the comparison between different test subgroups [I(a), II(a) and III(a)] regarding Ca/P ratio. There was no statistical significant difference between different test subgroups for different groups regarding Ca/P ratio (P>0.05).

In the comparison of mean Zn weight % between different test and control subgroups of the 2 groups (I and III) there was significant difference (P=0.001) favor the control subgroups but for group II there was significant increase in test subgroup (P=0.0001) (*Table 2*). This is means that Zn increased only in the test subgroup II which used ozone gas followed by remineralizing solution and patient kit (*Table 2*).

Table 2. *Comparison of mean Zn weight % in all groups between test and control subgroups.*

Zn weight %	**Test subgroups**	**Control subgroups**	**P value**
	Mean ± S.D	**Mean ± S.D**	
Group I	0.31 ± 0.14	2.40 ± 0.17	0.0001*
Group II	9.70 ± 0.58	2.39 ± 0.15	0.0001*
Group III	0.25 ± 0.05	2.37 ± 0.17	0.001*
*: Statistically significant at P ≤ 0.05			

There was significant difference between different test subgroups [I(a), II(a) and III (a)] regarding mean Zn weight% (P=001). And also there was significant difference between subgroup II(a) and both test subgroups I(a) and III(a) (P=0.0001), there was no statistical significant difference between test subgroup I(a) and test subgroup III(a) (P<0.05).

Discussion

Prevention has become the cornerstone of dentistry. Since the caries process is an ever-present and natural phenomenon, but it can be controlled to the extent that a clinically visible caries lesion never develops. Ozone Therapy coupled with fluoride shows the potential to move toward this goal [10].

Quantitative data obtained from the SEM-EDX demonstrated that there was a significant increase in the mean Ca/P ratio from 1.32 in subgroup I (b) to 3.73 in subgroup I(a), indicating lesion remineralization in the specimens of subgroup I (a) which treated with ozone followed by fluoridated tooth paste. This present results are consistent with the suggested remineralizing mechanism of ozone which is based on the ability to decarboxylate acids leading to higher pH level and mineralization of carious lesions [9] and enhance the remineralizing potential of remineralizing material on artificially demineralized enamel which investigated by Snnivasan et al. [26].

In subgroup II(a) the treated specimens with ozone followed by HealOzone remineralizing solution and patient kit showed significant increase in the mean Ca/P ratio in comparison with untreated specimens of subgroup II(b). These findings can be attributed to the capability of ozone in protecting the enamel against demineralization or promoting remineralization when combined with the HealOzone remineralizing solution and Patient Kit, as demonstrated by Tahmassebi et al. [27].

When the specimens treated with ozone followed by fluoride varnish and fluoridated toothpaste, in subgroup III(a), there was a significant increase in the mean Ca/P ratio from 1.33 in subgroup III(b) to 3.84 in subgroup III(a).These current results are in agreement with those of El-Sayed et al. [10], who used the ozone followed by topical fluoride application. They found that ozone induced significant increase in fluoride uptake. In addition, the scanning electron microscope revealed calcium fluoride deposits on enamel surface.

When comparing the three subgroups I(a), II(a) and III(a), there were no significant differences regarding Ca/P ratio. However, the lowest mean value (3.61) was related to group II which was treated by ozone followed HealOzone remineralizing solution and patient kit. This result may be due to the presence of Zn in the content of HealOzone remineralizing solution and patient kit, and with the possibility of substitution among the Ca-Zn elements in the dental enamel [28] lead to decrease in Ca content on the enamel surface and subsequently decrease in Ca/P ratio. Zn maintains little surface-zone porosity for longer time, facilitating diffusion of ions into the lesion, enhance remineralization in subsurface lesion as concluded by Lynch et al. [14].

For the specimens of subgroups I(a) which treated with ozone followed by fluoridated toothpaste, and subgroup III(a) which treated with ozone followed by fluoride varnish and fluoridated toothpaste, the mean Zn value significantly decreased to be less than their controls. This may be due to hyper-mineralization of the surface of the lesions following the use of fluoridated materials as explained by Matsuyama et al. [13]. With complete remineralization of enamel surface, Zn replaced by Ca, and this would prevent further remineralization occurring through the lesion depth.

However, specimens treaded with ozone followed by HealOzone remineralizing solution and patient kit, in subgroup II(a), there was a significant increase in the mean Zn value from 2.39 in subgroup II (b) to 9.70 in subgroup II(a). Also, when comparing the 3 subgroups I(a), II(a) and III(a), there was statistical significant difference in the mean Zn content with a trend favoring subgroup II(a) Lynch et al. [14] discovered that Zn maintains greater surface-zone porosity, facilitating diffusion of Ca, P and F into the lesion; allow more subsurface remineralization to take place. And according to the manufacture, Zn is one of the component of HealOzone remineralizing solution and patient kit [12], consequently in the present study enamel surface showed greater mean Zn value in subgroup II(a), in comparison to subgroup II(b) and the other two test subgroups I(a) and III(a).

Although, this study methodology tried to simulate the oral condition as closely as possible, saliva, plaque and many other confounding factors may affect the action of remineralizing agents used. Thus further studies employing clinical trials are important.

Within the limitation of the present study, it can be recognized that, the results of the current investigation together with the aforementioned literature showed that the use of ozone followed by remineralizing material can actually remineralize subsurface enamel lesions in permanent teeth, and it is believed that this would be effective in preventive dentistry.

Conclusion

From the results of the present study, it was concluded that:

- Ozone application with fluoridated toothpaste, fluoride varnish, and HealOzone remineralizing solution and patient kit have the same effect on calcium uptake in enamel caries-like lesion of permanent teeth.
- Ozone gas followed by HealOzone remineralizing solution and patient kit has better effect on remineralization of subsurface lesion due to the presence of zinc.

References

1. Lata S, Varghese NO, Varughese JM. Remineralization potential of fluoride and amorphous calcium phosphate-casein phospho peptide on enamel lesions: An *in vitro* comparative evaluation. *Journal of Conservative Dentistry.* 2010; **1**: 42-46.

2. Abbas HM, Hamza HM, Ahmed HM. Minimal intervention approaches in remineralizing early carious lesions. *Journal of American Science.* 2012; **8**: 709-717.

3. Elsayad I, Sakr A, Badr Y. Combibibg casein phosphopeptide-amorphous calciumphosphate with fluoride: synergistic remineralization potential of artificially demineralized enamel or not. *Journal of Biomedical Optics.* 2009; **14**: 044039.

4. Nalbantgil D, Oztoprak MO, Cakan DG, Bozkurt K, Arun T. Prevention of demineralization around orthodontic brachets using two different fluoride varnishes. *European Journal of Dentistry.* 2013; **7**: 41-47.

5. Seidler V, Linetskily I, Hubàlkovà H, Staňkovà H, Šmucler R, et al. Ozone and its usage in general medicine and dentistry: A Review Article. *Prague Medical Report.* 2008; **1**: 5-13.

6. Bocci V. Ozone. A new Medical Drug. The Netherlands: Springer Publishing Co; 2005: 1-3.

7. Macedo SB, Cardoso CC. The use of ozone in Dentistry. 16o Campinas International Conclave. 2005; 115: Quoted from Nogales CG, Ferrari PH, Kantorovich EO, Lage-Marques JL. Ozone therapy in medicine and dentistry. *The Journal of Contemporary Dental Practice.* 2008; **9**: 75-84.

8. Grootveld M, Silwood C, Sim J, Siddiqui N, Glaxon A, et al. High resolution NMR investigation of the mechanisms of action of ozone in the oral environment: oxidative consumption of salivary, plaque and carious dentine biomolecules. In: Lynch E (ed.). Ozone: The revolution in dentistry. Quintessence publishing Co; London. 2004: 39-48.

9. Knezevic A, Tarle Z, Mandie VN, Preskalo K, Panduric V, et al. Primary fissure carious lesion reversal using ozone. *Acta stomatologica Croatica.* 2007; **41**: 31-38.

10. El-Sayed I, Abo El-Magd D, El-Baz G. Effect of ozone on fluoride uptake in enamel. *Egyptian Dental Journal.* 2007; **53**: 1423-1430.

11. Kumar A, Bhagawati Sh, Tyagi P, Kumar P. Current interpretations and scientific rationale of the ozone usage in dentistry: A systematic review of literature. *European Journal of General Dentistry.* 2014; **3**: 175-80.

12. Atabeka D, Oztasa N. Effectiveness of ozone with or without the additional use of remineralizing solution on non-cavitated fissure carious lesions in permanent molars. *European Journal of Dentistry.* 2011; **5**: 393-399.

13. Matsuyama T, Ishizaki H, Tanabe S, Hayashi Y. Synchrotron radiation microbeam x-ray fluorescence analysis of zinc concentration in remineralized enamel in situ. *Archives of oral Biology.* 2009; **5**: 420-423.

14. Lynch RJM, Churchley D, Butler A, Kearns S, Thomas GV, et al. Effect of zinc and fluoride on the remineralization of artificial carious lesions under stimulated plaque-fluid conditions. *Caries Research.* 2011; **3**: 313-322.

15. Galal AM. Comparative study of clinical outcomes and cost-effectivness analysis off ozone gas and different approaches in managing active non-cavitated carious lesions in first permanent molars. PhD thesis in dental public health and preventive dentistry, Faculty of Dentistry, Alexandria University, 2009.

16. Safwat O. Clinical and microbiological evaluation of the effct of ozone gas on deep carious lesions in young permanent molars using the stepwise excavation. PhD thesis in pediatric dentistry, Faculty of Dentistry, Alexandria University, 2011.

17. Ismail AI, Sohn W, Tellez M, Amaya A, Sen A, et al. The International Caries Detection and Assessment System (ICDAS): an integrated system for measuring dental caries. *Community Dentistry and Oral Epidemiology.* 2007; **35**: 170-178.

18. Lussi A, Imwinkelrieed S, Pitts NB, Longbottom C, Reich E. Performance and re producibility of a laser fluorescence system for detection of occlusal caries in vitro. *Caries Research.* 1999; **33**: 261-266.

19. Huang SB, Cao SS, Yu HY. Effect of nano-hydroxyapatite concentration on remineralization of initial enamel lesion in vitro. *Journal of Biomedical Materials Research.* 2009; **4**: 1-6.

20. Pullido MT, Wefel JS, Hernanez MM, Denehy GE, Guzman-Armstrong S, et al. The inhibitory effect of MI Paste, Fluoride and a combination of both on the progression of artificial caries-like lesions in enamel. *Operative Dentistry.* 2008; **33**: 550-555.

21. http://www.kavo.com/sg

22. http://www.sultanhealthcare.com/sw/swchannel/productcatalogcf_v2/internet/productcatalog.asp

23. Nasab NK, Davalloo R, Avali FN. The effect of NaF mouthrinse, GC tooth mousse and GC MI paste plus on white spot inhibition: An invitro study. *Dentomaxillofacial Radiology.* 2012; **1**; 19-25.

24. Barbour Me, Rees JS. The laboratory assessment of enamel erosion: a review. *Journal of Dentistry.* 2004; **32**: 591-602.

25. Moor DS, MeCabe GP. Introduction to the practice of Statistics (3rd edn.). WH Freeman Company, New York. 1998.

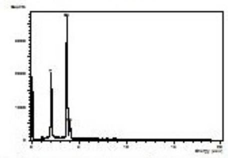
26. Srinivasan S, Prabhu V, Chandra S, Koshy S, Acharya S, et al. Does ozone enhance the remineralizing potential of nanohydroxyapatite on artificially demineralized enamel? A laser induced fluorescence study. 2014.

27. Tahmassebi JF, Chrysafi N, Duggal MS. The effect of ozone on progression or regression of artificial caries-like enamel lesions in vitro. *Journal of Dentistry.* 2014; **2**: 167-174.

28. Duggal MS, Nikolpoulou A, Tahmassebi JF. The additional effect of ozone in combination with adjunct remineralization products on inhibition of demineralization of the dental hard tissue in situ. *Journal of Dentistry.* 2012; **40**: 934-940.

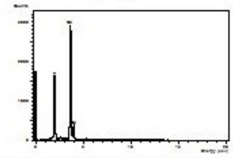
Appendix

Subgroup Ia



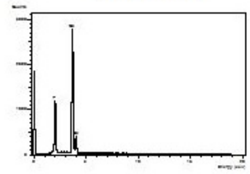
Label	Range (keV)	Gross	Net	% total
PKa	1.908 to 2.128	13308	9090	28.4
CaKa	3.568 to 3.807	27406	20126	63.0
CaKb	3.888 to 4.148	4352	2749	8.6

Subgroup Ib



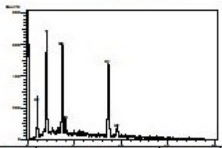
Label	Range (keV)	Gross	Net	% total
PKa	1.908 to 2.128	23308	14090	38.4
CaKa	3.568 to 3.807	17406	15126	53.3
CaKb	3.888 to 4.148	4349	2745	8.3

Subgroup IIIa



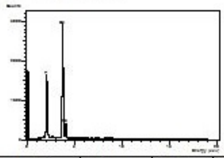
Label	Range (keV)	Gross	Net	% total
PKa	1.908 to 2.128	8207	5531	24.3
CaKa	3.568 to 3.807	20489	15172	66.7
CaKb	3.888 to 4.148	3157	2030	8.9

Subgroup IIa



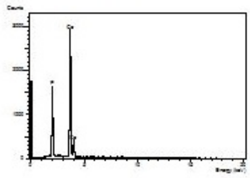
Label	Range (keV)	Gross	Net	% total
ZnLa1	0.928 to 1.107	1619	969	8.2
PKa	1.908 to 2.128	5042	3380	28.6
CaKa	3.568 to 3.807	19560	18987	60.3
CaKb	3.888 to 4.148	1122	387	3.3
ZnKa	8.467 to 8.807	4644	3528	25.9

subgroup IIb



Label	Range (keV)	Gross	Net	% total
PKa	1.908 to 2.128	25708	15990	39.5
CaKa	3.568 to 3.807	17359	15114	52.7
CaKb	3.888 to 4.148	4570	2643	7.8

Subgroup IIIb



Label	Range (keV)	Gross	Net	% total
PKa	1.908 to 2.128	256788	15986	37.9
CaKa	3.568 to 3.807	17389	15154	54.7
CaKb	3.888 to 4.148	4352	2749	8.2

Appendix 1