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Tubular occlusion of simulated hypersensitive dentin by the combined use of ozone and desensitizing agents

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Abstract

Objective. Ozone was suggested for treatment of hypersensitive dentin. The purpose of this study was to investigate the effect of ozone, with or without the use of desensitizing agents, on patency and occlusion of simulated hypersensitive dentin. ***Materials and methods.*** Sixty standardized dentin slabs were randomly divided into six groups: distilled water (Control), ozone treatment, fluoride desensitizer (ALLSolutions, Dentsply), oxalate desensitizer (D/Sense Crystal, Centrix), combined use of ozone/fluoride and combined use of ozone/oxalate. Ozone gas was delivered from OzonyTronX (Mymed). Specimens were evaluated using a scanning electron microscope and digital image analysis before and after treatment. ***Results.*** Statistical analysis using ANOVA and Mann-Whitney U-tests revealed significantly lower percentage of tubular occlusion with ozone treatment than distilled water at $p \leq 0.05$. Scanning electron microscope photomicrographs of oxalate desensitizer specimens revealed a thick homogenous precipitate with significantly higher percentage of tubular occlusion than fluoride desensitizer and distilled water. Combined use of ozone/fluoride resulted in a significantly higher percentage of tubular occlusion than fluoride desensitizer alone. However, no significant difference was found between oxalate desensitizer and combined use of ozone/oxalate. ***Conclusions.*** The use of ozone gas is a viable adjunct to fluoride-containing desensitizers in enhancing tubular occlusion, but is not effective with oxalate desensitizers.

Key Words: *desensitizing agents, fluoride desensitizer, hypersensitive dentin, oxalate desensitizer, ozone*

Introduction

Dentinal hypersensitivity is one of the most painful and least predictably treated chronic conditions in dentistry [1,2]. It originates from exposure of the dentinal tissue and is characterized by a painful sensation after thermal, chemical, mechanical or osmotic stimuli [3]. The sensitivity of the teeth is best explained on the basis of the hydrodynamic theory proposed by Brannstrom [4], who theorized that the movement of fluid within dentinal tubules stimulates pulpal nerve receptors and, thereby, causes pain. An understanding of this most widely accepted theory provides a basis for developing strategies to manage dentin hypersensitivity [5]. Thus, the therapeutic treatment for hypersensitive dentin aims at either blocking the sensitive mechanism through tubule occlusion, thus preventing stimuli from reaching nerve endings in the pulp, or interrupting the pulp neural response rendering it less responsive to stimulation [6].

Treatment of dentinal hypersensitivity can be categorized according to delivery and therapeutic aims into professional in-office treatments or at home using over-the-counter products [5]. In professional intervention, many dental products were suggested to occlude the dentinal tubules including ions/salts such as fluoride, oxalate, proteins/amino acid and resins [7]. Despite the great variety of available therapeutic methods, dentinal hypersensitivity remains an increasing and difficult-to-solve problem with uncertain prognosis [5,8]. Consequently, there is need for other clinical modalities for the treatment and management of dentin hypersensitivity. Ozone was suggested as a novel approach that could aid in treatment of dentin hypersensitivity [9].

Ozone is an elemental, active, trivalent form of oxygen that occurs naturally as a result of ultraviolet energy or lightening, causing a temporary recombination of oxygen atoms into groups of three. In a clinical setting, an oxygen/ozone generator simulates lightening via an electrical discharge field [9]. Ozone

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is a very powerful oxidizing agent. It also effectively kills bacteria, fungi, viruses and parasites at a low concentration without affecting human living cells [9]. These properties are the foundation of what makes ozone work so well in the medical field [10]. Ozone has many uses in operative dentistry; including sterilization, bleaching, treatment of dental caries and dentin hypersensitivity [9].

However, studies that examine the use of ozone as a desensitizing agent are rare in the literature [11,12]. In addition, the combined use of ozone and professional desensitizers has not, to our knowledge, yet been investigated. Thus, the aim of this study was to evaluate the effect of ozone alone or in combination with desensitizing agents on the patency and occlusion of dentinal tubules.

Materials and methods

Specimen preparation

The specimens were prepared from 60 sound human premolars extracted for orthodontic reasons from patients of age group 18–25 years. The teeth were stored in phosphate buffer saline solution containing 0.2% sodium azide at 4°C for a maximum period of 1 month until use [13]. Each tooth was sectioned using a Bronwill cutting machine (E McGrath Inc., Denver, Colorado, USA) in mesiodistal direction to divide it into buccal and lingual halves. Standardized dentin slabs (4 × 3 mm) were obtained from the cervical third of the buccal halves of teeth. To expose the underlying superficial dentin at standardized depth, a central indentation was made using a round bur (ISO 005) (Komet 0197, GmbH, Besigheim, Germany) on the enamel side until dentin is reached. The enamel was then ground to the depth of the indentation on a water-cooled mechanical grinder (Red-wing, A.C. motor, Handler Co., Westfield, New Jersey, USA) using 180-grit abrasive paper. The thickness of the dentin slab was adjusted to 1 mm by grinding from the pulpal dentin side. Slabs were checked using a precise caliber (Mitutoya, Japan) to verify the width, height and thickness. The test surface was then identified by marking the opposite surface (pulpal side) with bur indentation. To remove the smear layer and prepare open dentinal tubules with simulated dentin hypersensitivity, the dentin slabs were immersed in 50% citric acid for 2 min and then rinsed with distilled water for 1 min [7].

Sample grouping and treatment modalities

The samples were randomly divided into six groups (n = 10). In Group 1 the samples were rinsed with distilled water for 1 min (control), in Group 2 the samples were treated with ozone gas which was delivered from OzonyTronX (Mymed GmbH, Rosenheim, Germany). This oxygen activation generator was adjusted to scale 5 to deliver high concentration ozone (100,000–300,000 ppm) to the dentin slabs for 40 s using a mushroom-tip probe (GI probe) supplied with the generator. When the tip of the probe gets in contact with the treated surface, it emits energy and splits environmental diatomic oxygen into singular atomic oxygen and ozone. In Group 3 fluoride-containing desensitizing agent (ALLSolutions, Dentsply, Milford, Delaware, USA) (1.23% acidulated phosphate fluoride) was immediately applied to specimens for 3 min according to the manufacturer's instructions. In Group 4 oxalate-containing desensitizing agent (D/Sense-Crystal, Centrix, Shelton, Connecticut, USA) (2.5% potassium oxalate and 2.5% nitric acid) was rubbed onto the surface for 20 s using a microsponge and left undisturbed for 2 min according to the manufacturer's instructions. In Group 5 samples were pre-treated with ozone prior to immediate application of fluoride desensitizer. In Group 6 ozone pre-treatment of samples was followed by immediate application of oxalate desensitizer. In treatment groups [3–6], specimens were washed with distilled water for 30 s after application of the desensitizer and blot dried to remove excess water.

Assessment of morphology and tubular occlusion

Environmental Scanning electron microscopy (SEM) (Quanta 200, FEI, Philips Electron Optics, Eindhoven, Netherlands) was used in combination with digital image analysis (XT document program, FEI) of the photomicrographs at 3000× magnification to provide both qualitative and quantitative assessments, respectively. The mean tubular diameter (μm) was calculated for each specimen before and after treatments. The results were expressed as a mean percentage of tubular occlusion per specimen and statistically analyzed using ANOVA and Mann-Whitney U-tests at a level of significance, $p \leq 0.05$.

To examine the extent of penetration inside the dentinal tubules, longitudinal fractured sections of specimens treated with fluoride desensitizer (n = 2) and specimens treated with oxalate desensitizer (n = 2) were also examined using SEM. Standardized dentin slabs were obtained from the cervical buccal third of premolars as previously described. After treatment, an indentation was made on the opposite (pulpal) side of specimens using a fine tapered diamond stone. The specimens were then fractured into two halves using pliers.

Results

The mean percentage of tubular occlusion for the six groups is summarized in Table I. ANOVA test revealed a significant difference between treatments ($p < 0.001$). The potassium oxalate, ozone followed by

Table I. Mean percentages of tubular occlusion and standard deviation values, in response to various treatment agents and combinations.

Treatment	*M* (SD)	*p*-value
Distilled water	9.1 (± 6)^{c}	<0.001*
Ozone	−10.1 (±28.1)^{d}	
Fluoride	20.2 (±11.2)^{b}	
Potassium Oxalate	67.8 (±7.3)^{a}	
Ozone + fluoride	57.7 (±11.9)^{a}	
Ozone + Potassium Oxalate	66 (±9.8)^{a}	

*Significant at $p \leq 0.05$.
^{a,b,c,d}Means with the same letter are not significantly different at $p > 0.05$.

potassium oxalate and ozone followed by fluoride groups showed the highest percentage of tubular occlusion of 67.8 ± 7.3, 66 ± 9.8 and 57.7 ± 11.9, respectively, with no significant difference between them. Fluoride-treated specimens resulted in significantly lower percentage tubular occlusion of 20.2 ± 11.2 than the other treatment groups, but were significantly higher than the control group treated with distilled water (9.1 ± 6). On the other hand, ozone-treated specimens resulted in a negative decrease in percentage of tubular occlusion (−10.1 ± 28.1), denoting widening of tubules than control group.

In the fluoride treated specimen, SEM examination of the surface topography (Figure 1) revealed a relatively smooth thin surface coating over some areas of the treated surface. Most of the dentinal tubules showed narrowing in the tubule aperture due to fine precipitates on the tubular walls (white arrows). However, full tubular occlusion was rare (black arrows). The fractured fluoride-treated specimen (Figure 2) revealed that this surface coating had fine deposits adhered to the surface (white arrow).

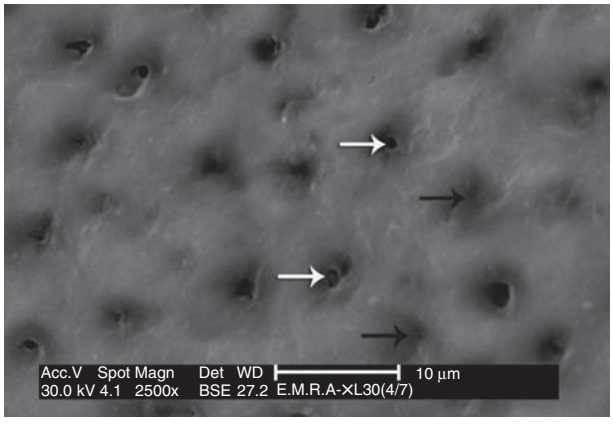

Figure 1. SEM photomicrograph representing surface topography of fluoride-treated dentin specimen (2500×). A continuous surface coating is revealed, with narrowing of some dentinal tubules apertures (white arrows) and obliteration of other dentinal tubules (black arrows).

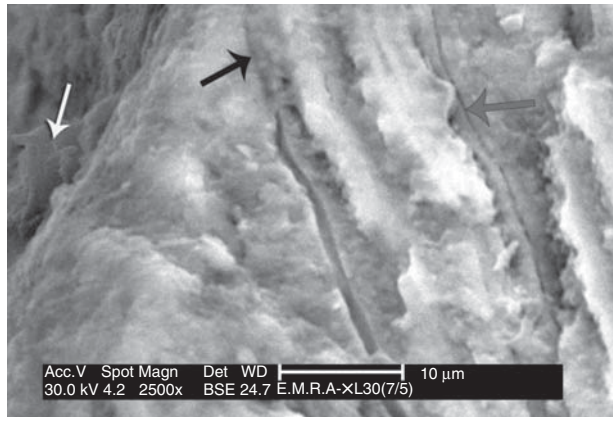

Figure 2. SEM photomicrograph representing a longitudinally fractured fluoride-treated dentin specimen (2500×). The fluoride coating is seen adhered to the specimen surface (white arrow). Some dentinal tubules were partially infiltrated by the precipitate (black arrow), while other dentinal tubules appear infiltrated to a deeper level leading to narrowing of the tubule (grey arrow).

It is also shown that this fine coating infiltrated dentinal tubules at various depths. Some tubules were infiltrated by the precipitate, but not to the full length of the dentinal tubule (black arrow). Other tubules appear to be narrowed to its full visible length by the infiltrate which appear to line the lumen of the tubule (grey arrow).

On the other hand, SEM examination of the surface topography of the oxalate-treated specimen (Figure 3) revealed crystalline rectangular, rod-shaped aggregates with different dimensions. Angular and cluster-like crystals are deposited on the dentin surface, hiding most orifices of the dentinal tubules. Some tubules are barely visible. A fractured longitudinal section (Figure 4) revealed that some oxalate crystals (black arrows) were found plugging the tubules but did not penetrate, however, deeply inside the tubules.

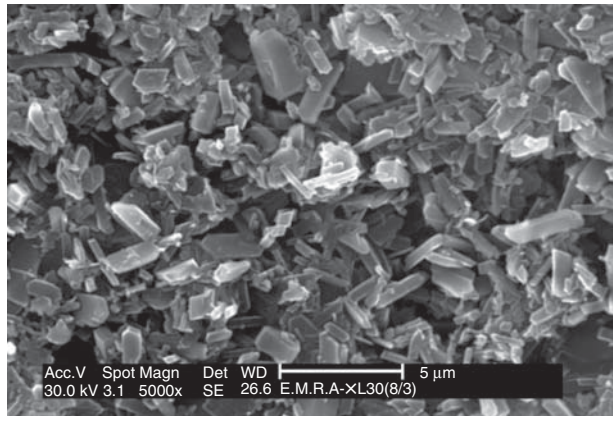

Figure 3. SEM photomicrograph representing surface assessment for oxalate-treated dentin specimen (5000×) revealing angular, crystalline oxalate crystals on the specimen's surface.

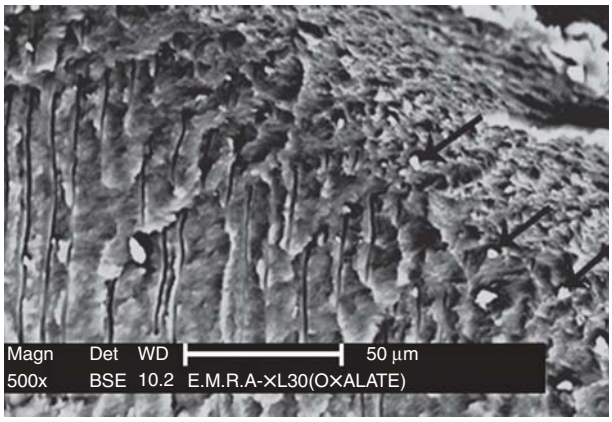

Figure 4. SEM photomicrograph representing side view assessment for oxalate-treated dentin specimen (500×). Some oxalate crystals are seen loosely attached to the dentinal tubules' apertures (black arrows).

Discussion

In vitro examination of desensitizing products using a reproducible model, such as dentin slabs, was proven useful in the initial screening and evaluation of available desensitizing agents [7,14]. In this study, dentin slabs were prepared from the buccal cervical third of premolar teeth, which is a common site for dentin hypersensitivity due to the presence of a thin layer of enamel in this area [3,15]. The dentin slabs were immersed in 50% citric acid for 2 min to ensure sufficient removal of dentinal plugs and smear layer to expose the dentinal tubules, thus clinically simulating dentin hypersensitivity. The smear layer is responsible for 86% of the total resistance to the movement of fluids [7,16].

Ozone gas used in this study was delivered from OzonyTronX. This is an oxygen activation generator which uses high frequency power (25 KHz) and high voltage (12,000 V) to produce an electromagnetic field that causes breakage of environmental molecular oxygen bonds, causing a temporary recombination of oxygen atoms into groups of three, leading to formation of highly reactive nascent ozone. Dentin slabs were treated by ozone at high concentration at scale 5 which is recommended by the manufacturer for treatment of dental lesions. A GI probe (mushroom-like tip) was used to cover the whole surface of the dentin slabs.

The results of the present investigation revealed that ozone showed a significant increase in the diameter of dentinal tubules. This could be attributed to the powerful oxidizing property of ozone. This oxidation potential is greater than that of hypochlorite acid or chlorine and ~5-times more oxidizing than oxygen [17]. Ozone can readily react with a substrate on its own, whereas oxygen usually requires a catalyst to initiate a reaction [18]. This oxidizing property is likely to be responsible, to varying extents, for the changes observed in dentin after ozone treatment. Mature dentin is formed of ~76% minerals, 20% organic matrix and 10% water as a weight basis. The main component of the organic matrix is collagen [19]. Since ozone is known to readily react with most species containing multiple bonds (such as C = C, C = N, N = N) [20], it is expected to react with the double bonds present in the organic part of dentin.

The oxidation mechanism of ozone is based on the effect of direct and indirect reaction mechanisms. One kind of reaction would dominate, depending on various factors such as temperature, pH and chemical composition [21]. The direct oxidation of organic matter by ozone is quite a selective reaction mechanism, during which ozone reacts quickly with organic matter that contains double bonds, activated organic groups or amines. However, the indirect reaction of resulting oxidators, such as free hydroxyl radicals, is sequential to the reaction of ozone. These radicals are very short living compounds that have an even stronger oxidation mechanism than that of ozone. Hydroxyl radicals do not influence the inorganic tissue but attack the organic components of dentin [22]. These direct and indirect reactions are likely to be responsible for the preferential attack on demineralized peritubular dentin leading to an increase in diameter of dentinal tubules.

Accordingly, the usage of ozone only is not expected to reduce the pain sensation in hypersensitive dentin, at least not by occlusion of dentinal tubules. This result was in agreement with Dähnhardt et al. [12], who stated that ozone therapy did not reduce dentin hypersensitivity. However, other studies suggested that this increased patency of the dentinal tubules could facilitate the entrance of minerals, either from saliva or from other desensitizing agents, into the dentinal tubules [9,18]. On the contrary, Azarpazhooh et al. [11] found that clinical application of ozone had an immediate relief of pain after treatment. However, this relief was insignificant compared to a placebo agent.

Dentin specimens treated with the oxalate desensitizer without ozone pre-treatment resulted in a significant increase in the percentage of tubular occlusion compared to distilled water (Control group). SEM examination of these specimens revealed the presence of precipitates of oxalate crystals covering the treated dentin surface and occluding the orifices of the dentinal tubules. This could be attributed to the chemical reaction between the potassium oxalate and the calcium ions (Ca++) that resulted in the production of calcium oxalate crystals covering the surface and occluding the orifices of dentinal tubules. In addition, the low pH of <1 of this desensitizer was probably able to liberate more calcium from the dentin, leading to increased formation of insoluble crystals of calcium oxalate. This finding is in agreement with many previous studies [7,23–27].

The amount of pressure as much as time plays an important role in the process of tubular obstruction [26]. Calcium oxalate loosely arranged crystals might be further displaced toward the tubule opening by hydraulic pressure during the filtration procedure. The fluid pressure tends to force these crystals against each other to create a plug that actually reduces fluid filtration as time elapses [26]. In addition, the insoluble calcium oxalate crystals occluding the dentinal tubules might bind to dentinal proteins, increasing the adhesion to the dentin surface [13].

Despite the low percentage of tubular occlusion in specimens treated with fluoride desensitizer without ozone pre-treatment, the results of this group were significantly high compared to distilled water. SEM examination of these specimens revealed some areas of tubular infiltration and narrowing of dentinal tubules. This was supported by the results of previous studies [5,7,28]. The exact mechanism of fluoride compounds is not very clear despite the great number of clinical *in vivo* and *in vitro* investigations. Its mechanism is probably referred to the action of sodium fluoride preparations. The acidic property of the fluoride gel is able to etch peritubular dentin and form a funneled portion of the tubule at the dentin surface. Thus, the ionized calcium in the tubular fluid, liberated during acid attack, reacts with the active ingredient of the sodium fluoride gel leading to formation of calcium fluoride crystals, which are deposited into the dentinal tubules [29,30].

However, other studies [31–33] stated that fluoride acts more superficially to seal the dentinal tubules, making it less effective in occluding dentin tubules. As the crystal size of calcium fluoride (~0.05 μm) is small, a single application of sodium fluoride might not be effective in narrowing the diameter of dentinal tubules and multiple applications would thus be required [29].

Without ozone pre-treatment, the results showed that the potassium oxalate had a more significant effect on occluding the dentinal tubules of hypersensitive dentin as compared to the fluoride desensitizer. The potassium oxalate formulation used in this study has a more acidic pH (less than 1) than the fluoride desensitizer (~3.6). Thus, the oxalate desensitizer probably caused more release of calcium ions available for the reaction. This finding is in agreement with Pereira et al. [34], who confirmed the superior effect of oxalate over fluoride in occluding the tubules. The authors indicated that fluoride does not demonstrate the same obstruction ability as do the potassium oxalate-based materials. In our study, SEM examination of surface topography and longitudinal sections confirmed the partial blocking of dentinal tubules by aggregated oxalate crystals versus the different mechanism of fluoride infiltration into tubular walls, narrowing the lumen of dentinal tubules rather than occluding them. Arrais et al. [30] reported that potassium oxalate and acidulated phosphate fluoride were able to occlude dentinal tubules by different modes, but the effectiveness of reduction of dentin hypersensitivity depends on the longevity of the precipitates in dentinal tubules and their ability to resist acid challenge. Paes Leme et al. [28] agreed that potassium oxalate produces immediate reduction in the diameter of the dentinal tubules, but this effect was short-lived, due to the high solubility of calcium oxalate after immersion in artificial saliva. However, the tubules may have remained occluded with intratubular calcium oxalate crystals below the surfaces. In the current study, neither aging of specimens or the acidic challenge were performed. However, it could be revealed from SEM examination of fractured oxalate-treated specimen that some oxalate crystals were found loosely plugging the dentinal tubules. It is worth noting that, after fracturing of oxalate-treated dentin specimens, it was not always possible to find evidence of sub-surface oxalate inclusions, since their large size may have resulted in their loss during specimen preparation.

In this study, the combined use of ozone and fluoride desensitizer enhanced the treatment effectiveness when compared to either treatment alone. On the other hand, ozone pre-treatment with oxalate desensitizer showed insignificant tubular occlusion compared to oxalate alone. Unfortunately, no papers were found in the literature investigating or discussing the interaction of ozone with desensitizers for treatment of hypersensitivity. It can be hypothesized, however, that ozone pre-treatment 'paved the way' for fluoride precipitation inside the dentinal tubules. As discussed before, the powerful oxidative effect of ozone leads to an increase in the inorganic content as well as an increase in the patency and diameter of dentinal tubules. When applying fluoride desensitizer, calcium fluoride crystals are thus formed whose fine particle size can benefit from the increased patency and precipitate into the lumen of dentinal tubules. On the other hand, the large-sized calcium oxalate crystals that blocked the apertures of dentinal tubules were probably still larger than the patency of ozone-treated dentinal tubules. In addition, oxidation of the organic part of dentin by ozone leads to subsequent exposure of hydroxyapatite. Thus, when fluoride desensitizer was applied after ozone, an increased precipitation of fluoroapatite crystals occurred in addition to calcium fluoride crystals, thereby doubling the precipitation effect with fluoride desensitizer.

The findings of the current research also suggest that the use of ozone could enhance the effectiveness of topical fluoride in reducing root caries. Further research work is required, however, to investigate the possible interaction of nascent oxygen atoms from ozone treatment with the chemical components of desensitizing agents. Whether the formed chemical

compounds would antagonize or synergize the action of desensitizers is an interesting point to be investigated.

Conclusions

The use of Ozone gas enhances the tubular patency of dentin and is thereby not indicated as a sole treatment in dentinal hypersensitivity. However, Ozone could be considered a viable adjunct to fluoride-containing desensitizers in enhancing tubular occlusion, but not with oxalate desensitizers.

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