

# Influence of gasiform ozone on the micromechanical properties of dentin

Elisa Magni<sup>1,2</sup>, Reinhard Hickel<sup>2</sup>, Nicoleta Ilie<sup>2</sup>

## Abstract

**Objectives:** The aim of this in vitro study was to assess the effect of an ozone application on the micromechanical properties of dentin. **Methods and Materials:** Twelve sound human third molars were cut in order to obtain 1 mm-thick slabs, which included both coronal and root dentin. The buccal surfaces of the slabs were polished and each slab was further divided into two halves (mesial and distal). The mesial half of each specimen was subjected to a 60s application of ozone (HealOzone, KaVo), whereas the distal half remained untreated and served as control. The modulus of elasticity (E) and the Vicker's hardness (VH) of the dentin specimens were immediately measured with an automatic microhardness indenter (Fischerscope H100C, Fischer). In order to compare the E and VH means of the ozone-treated and control groups, the t-test for dependent samples was applied and the level of significance was set at  $p < 0.05$ . **Results:** No significant differences in E and VH of the dentin specimens were detected between the ozone-treated group and the control group ( $p > 0.05$ ). The means (SD) of E and VH were respectively 18.4(3.7) GPa and 71.7(19.8) N/mm<sup>2</sup> in the ozone-treated group and 18.1(2.5) GPa and 75.1(17.2) N/mm<sup>2</sup> in the control group. **Conclusions:** The application of gasiform ozone does not affect the modulus of elasticity and the Vicker's hardness of dentin. **Clinical significance:** When the disinfection of dentin is required, the dental clinician can apply ozone gas on the dental substrate without impairing its micromechanical properties.

**Short title:** Effect of ozone on dentin

**Key words:** ozone; dentin; micromechanical properties

## Introduction

Gasiform ozone was introduced in dental practice as an alternative and minimally invasive treatment for occlusal<sup>1,2</sup> and root caries.<sup>3,4</sup> More recently the use of ozone for root canal<sup>5,6</sup> and post-space disinfection<sup>7</sup> has been also investigated. Ozone has also been reported as not affecting the bond strength to dental hard tissues<sup>7,8</sup> nor impairing the micromechanical properties of dental

adhesives bonded to dentin.<sup>9</sup>

Several studies reported that the application of disinfectants<sup>10-16</sup> or oxidants, such as bleaching agents,<sup>17-22</sup> exerts mostly an alteration of the hardness and other mechanical properties of dental hard tissues. However, low concentrations of hydrogen peroxide have been reported as having no negative effect on dentin microhardness.<sup>23</sup>

Due to the high reactivity of the ozone molecules and to the substrate's dehydration possibly determined by the gas flow, an alteration of the properties of dental hard tissues might be induced by an ozone application. The impact of ozone treatment on some enamel's physical properties has been previously assessed and no impairment of the investigated properties has been found.<sup>24</sup>

No information is currently available on the interaction between ozone and dentin. Thus, the aim of this study was to investigate the effect of ozone gas on the modulus of elasticity and Vicker's hardness of dentin. The tested null hypothesis is that the application of gasiform ozone affects dentin's properties.

*1 Department of Fixed Prosthodontics and Dental Materials, University of Siena, Policlinico "Le Scotte", Viale Bracci, 53100 Siena, Italy.*

*2 Department of Restorative Dentistry and Periodontology, Dental School of the Ludwig-Maximilians-University, Goethestrasse 70, D-80336 Munich, Germany.*

### Corresponding author:

*Dr. Elisa Magni, DDS, MSc, PhD student, Department of Fixed Prosthodontics and Dental Materials, Policlinico "Le Scotte", University of Siena, Viale Bracci, 53100 Siena, Italy  
Telephone: +39-0577-233131  
Telefax: +39-0577-233117  
E-mail: elisamagni@hotmail.com*

**Table 1**

Means (SD) of the elastic modulus (E) and the Vicker's hardness (VH) of dentin in the experimental groups.

Group	E (GPa)	VH (N/mm <sup>2</sup> )
Ozone	18.4(3.7)	71.7(19.8)
Control	18.1(2.5)	75.1(17.2)

No significant differences of the micromechanical properties were detected between the ozone-treated and the control group (t-test for dependent samples,  $p > 0.05$ ).

## Materials and Methods

### *Specimens preparation*

Twelve sound human third molars extracted due to orthodontic reasons were collected. Any residual soft tissue was removed from the tooth surface with a hand scaler. The teeth were kept in 37°C saline solution (0.9% sodium chloride in water) for no longer than one month before being used in the experiment.

Each tooth was subjected to two cuts in its middle portion, parallel to its long axis, in a mesio-distal direction with a low-speed diamond saw under abundant water cooling (Isomet, Buehler, Lake Bluff, IL, USA), in order to obtain a 1 mm-thick slab, which included both coronal and root dentin. The buccal surfaces of the obtained slabs were polished with silicon carbide paper discs (1,000, 1,200 and 2,500 grit) and 1  $\mu\text{m}$ -polycrystalline diamonds particles (DP-Spray, P; Struers) under water rinsing. Each slab was further divided into two halves (mesial and distal) with a longitudinal cut through its centre (Isomet). The specimens were then kept at room temperature in deionised water.

The mesial half of each specimen was subjected to a 60s application of ozone (2,100 ppm equal to 4.2  $\text{g}/\text{m}^3$ ; HealOzone, KaVo, Biberach, Germany), whereas the distal half remained untreated and served as control. The specimens were immediately processed for the measurement of the micromechanical properties.

### *Measurement of the micromechanical properties*

The modulus of elasticity (E) and the Vicker's hardness (VH) of the dentin specimens were measured with an automatic microhardness indenter (Fischerscope H100C, Fischer, Sindelfingen, Germany). Fifteen indentations were performed in the dentin of each specimen, resulting in

180 indentations for each experimental group. The indentation procedure was carried out force controlled. A load application time of 50s was set and subdivided as follows: the force increased at a constant speed from 0.4 mN to 30 mN in 20s, the maximal force of 30 mN was kept constant for 5s, then the force decreased at a constant speed from 30 mN to 0.4 mN in 20s and the minimal force of 0.4 mN was kept constant for 5s. The load and the penetration depth of the indenter (Vicker's pyramid: diamond right pyramid with an angle  $\alpha = 136^\circ$  between the opposite faces at the vertex) were continuously measured during the load-unload cycle.

The Universal Hardness is defined as the test force divided by the apparent area of the indentation at maximal force. From a multiplicity of measurements stored in a database supplied by the manufacturer, a conversion factor between Universal Hardness and Vicker's hardness (VH) was calculated and implemented into the software, so that the measurements were expressed in Vicker's hardness units.

The indentation modulus was calculated from the slope of the tangent of the indentation curve at maximal force and is comparable with the modulus of elasticity of the substrate (E).

### *Statistical analysis*

The normal distribution of the E and VH data was verified with the Kolmogorov-Smirnov test ( $p > 0.05$ ) and a preliminary regression analysis was performed in order to verify that the measured properties were not affected by the tooth within each experimental group ( $p > 0.05$ ). In order to compare the E and VH means of the ozone-treated and control groups, the t-test for dependent samples was applied and the level of significance was set at  $p < 0.05$ .

## Results

According to outcome of the statistical analysis no significant differences in E and VH of the dentin specimens were detected between the ozone-treated and the control group ( $p > 0.05$ ). Table 1 reports means and SD of the measured properties in the experimental groups.

## Discussion

The application of gasiform ozone does not affect the modulus of elasticity and the Vicker's hardness of dentin. Thus, the tested null hypothesis was rejected.

A detrimental effect on the mechanical properties and on the microhardness of dentin has been reported to occur after the application of some common disinfectants<sup>10-16</sup> or bleaching agents.<sup>17-22</sup> Ozone gas was introduced in dental practice due to its antimicrobial potential,<sup>25-28</sup> which is enabled by the high oxidative effect of this gas. The effect of ozone on the physical properties of some enamel has been previously investigated.<sup>24</sup> The study by Celiberti et al.<sup>24</sup> reported that ozone did not affect the hardness of enamel compared to the application of dry air for the same duration of the ozone treatment. On the contrary, a reduction of the enamel microhardness was detected when both ozone-treated and air-treated specimens were compared with an untreated baseline group. The authors speculated that this difference was probably due to the dehydration of the substrate which occurred during the exposure to ozone or dry air.<sup>24</sup>

The application of ozone on both coronal<sup>8,9</sup> and root<sup>7</sup> dentin prior to adhesive procedures has been also proposed. Due to its higher moisture and organic content compared to that of enamel, dentin could be more sensitive to the highly reactive ozone molecule and to dehydration. If an excessive substrate's dehydration occurs, the exposure of dentin to gasiform ozone might cause a reduction of dentin wettability<sup>29</sup> and could also interfere with bonding procedures.<sup>30</sup>

In order to obtain a simulation of clinical conditions, in the present study the specimens of the control group were not subjected to a dry air flow. In fact, an unmotivated sustained drying of dentin is not indicated clinically. This way a direct comparison of ozone-treated dentin with an unaltered dentin substrate was allowed. As previously observed for enamel's properties,<sup>24</sup> the results of the present investigation showed that the modulus of elasticity and the Vicker's hardness of human dentin were also not impaired by a 60s ozone gas application.

Previous studies reported that ozone does not impair bonding procedures to coronal<sup>8,9</sup> and root<sup>7</sup> dentin, but no

clear information was provided on the interaction between ozone and dentin. The results of this study suggested that ozone does not modify the dentinal substrate. It might be speculated that the absence of a substantial alteration of dentin due to ozone treatment could account for the observed lack of any negative effect of ozone on bonding to dentin. These observations differentiate ozone from those disinfectants<sup>10-16</sup> and oxidants,<sup>17-22</sup> which have been reported to alter dentin and also impair the bonding to dentin.<sup>31-39</sup> Since common disinfectants or oxidants, such as bleaching agents, used in the dental practice are available mostly as solutions or gels, it could be hypothesized that also the type of formulation could be a critical factor affecting the interaction of these substances with the substrate. A liquid or gel formulation might be responsible for a sustained persistence of the substance on dental hard tissues, which could act as reservoirs, whereas a gaseous formulation, such that of ozone, might have a milder interaction with the substrate. Nevertheless, more studies should be necessary to better clarify the mechanism of interaction between gasiform ozone and dentin.

The same indentation procedure for the measurement of elastic modulus and Vicker's hardness used in this study has been previously applied to assess the micromechanical properties of enamel and coronal dentin.<sup>40</sup> The E and VH values reported for dentin in the present investigation are slightly higher than those previously reported,<sup>40</sup> probably because the measurement of these properties were also performed in deep coronal and root dentin.

## Conclusions

Within the limits of this *in vitro* study, it might be concluded that the application of gasiform ozone does not affect the modulus of elasticity and the Vicker's hardness of dentin. Thus, the application of ozone on dentin could be performed by the dental clinician without impairing the micromechanical properties of the substrate.

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