Ozone air levels adjacent to a dental ozone gas delivery system

ELISABETH JOHANSSON1, INGRID ANDERSSON-WENCKERT1,2, ANNIKA HAGENBJÖRK-GUSTAFSSON3 & JAN W. V. VAN DIJKEN1

1Dental Hygienist Education, Department of Odontology, Umeå University, Umeå, Sweden, 2Pediatric Dentistry, Department of Odontology, Umeå University, Umeå, Sweden, and 3The National Institute for Working Life, Umeå, Sweden

Abstract
Objective. Ozone (O₃) has been suggested as an anti-microbial treatment in dentistry, with an ozone gas delivery system introduced for the treatment of fissure and root caries. The aim of this study was to investigate the sealing capacity of the novel delivery system and its re-suction capacity during accidental displacement of the cup at different stages of ozone delivery. Material and methods. Ozone leakage was studied in vitro after application on a flat metal surface and on buccal and occlusal tooth surfaces. An ozone analyzer was used to measure ozone gas concentrations adjacent to the delivering cups when adapted to the target surfaces during and after 10–20 s application cycles. The measured levels were compared with the background concentrations in the room. Measurements were performed 1) after complete ozone application cycles, 2) within the cycle before the start of the suction period, and 3) after displacements of the cup during the cycles. Results. Ozone air values varied between 8 and 166 μg m⁻³ for the flat metal surface and between 0 and 108 μg m⁻³ for the tooth surfaces. Ozone leakage levels were 7.6 μg m⁻³ for the flat and 7.4 μg m⁻³ and 5.6 μg m⁻³ for the buccal and occlusal surfaces, respectively, and 5.2 μg m⁻³ and 9.8 μg m⁻³ for the premolar and molar surfaces, respectively. Cycles with displacement showed significantly higher leakage levels than continuous complete cycles (p = 0.03). Conclusions. Ozone application cycles with displacements showed significantly higher leakage levels than continuous complete cycles. The largest ozone delivery cups showed the highest leakage values. A change in background levels was seen with similar change in adjacent ozone levels. The overall measured ozone leakage values were low after normally functioning delivery cycles and after repeated displacements. The delivery system can be considered safe.

Key Words: Caries, micro-organisms, ozone, safety

Introduction
Ozone, a highly unstable form of oxygen, is known for its presence in the outer atmosphere. It absorbs dangerous ultraviolet radiation from the sun and is naturally present around us on Earth in small quantities. Long-term exposures to increased amounts of ozone have been shown to elicit negative effects on human health [1]. During inhaling, the gas readily penetrates the oral-nasal complex and the lungs, and repeated exposure to environmental and occupational gases increases the risk of airway irritation and asthma-like symptoms [2]. Still, ozone has also been recognized as a powerful sterilizing agent, and has been used in both medicine and dentistry with different indications. However, its use has been, and remains, controversial. Opponents are of the opinion that it is toxic and should not be used in medicine at all, while promoters claim that carefully selected dose levels of this agent are of potential therapeutic value and circumvent its toxicity [3]. Despite the decline of dental caries during recent decades, especially in industrialized countries, it is still a problem in pits and fissures of young people [4] and as root caries in the elderly [5]. Surfaces with higher microbial counts are more often associated with demineralization, and reversal of these lesions may in many cases be positively related with a reduction in cariogenic microorganisms [6–8].

Ozone has been advocated for use owing to its antimicrobial effects, and has been investigated as a therapy for treating fissure and root caries [9–12]. A novel ozone delivering apparatus has recently been introduced for use in dentistry with claims that it removes any remaining ozone during covering and sealing of the tooth [13], but there are areas where it
is difficult to apply, or to obtain a good seal, and this limits its clinical use. It is important that no repeat leakage of any remaining ozone occurs during the application in tooth treatment or during accidental displacement of the delivery system, thus exposing patient and operator to harm. On investigating the levels of ozone escaping during delivery, Millar & Hodson [14] found that the apparatus was safe to use during full application cycles, including final re-suction. However, they did not investigate situations where the delivery cycles were not completed.

The aim of this study was to investigate the sealing capacity of the novel delivery system and its re-suction capacity during accidental displacement of the cup at different stages of the ozone delivery. The hypothesis tested was that there were no differences in ozone air levels adjacent to the silicone cups compared with the background levels.

**Material and methods**

Ozone gas levels in air were measured continuously using a photometric ozone analyzer, which measures low ozone levels in air with a lowest detectable level of \( 2 \, \mu g \cdot m^{-3} \) \((=1\, ppb =0.001 \, ppm)\) (Dasibi model 1108; Dasibi Environmental Corp., Glendale, Calif., USA) [15]. The HealOzone (KaVo HealOzone, Biberach, Germany) delivers ozone at concentrations of \( 4,200 \, mg \cdot m^{-3} \pm 10\% \). Removable single-use silicone sealing cups, diameters ranging between 3 and 8 mm, are attached on a hand-piece to a console with a multi-lumen hose. The ozone gas can be delivered when a tight fit is achieved. The apparatus delivers ozone under vacuum only and has a re-suction system which the manufacturer claims removes any remaining ozone during the 10 s re-suction period that follows the ozone delivery period, while the cup still covers and seals the selected tooth.

The sealing capability of the system was first studied on a surface with optimal possibilities of a tight seal to a flat metal surface, and then on more difficult to seal buccal and occlusal premolar/molar surfaces simulating the clinical situation. Molar and premolar teeth recently extracted for orthodontic or other reasons were used. Full ozone application cycles, including the re-suction period, and interrupted cycles with displacement of the cup during the delivery cycle were studied.

At the start of the study, background ozone levels were measured once an hour for 5 days when the ozone apparatus was not used. On the days of measurement, the background levels in the room were noted before the start of each ozone application situation and at regular intervals after a pause of 600 s. The pauses were made to minimize possible influence from the ozone applications performed.

The maximum ozone air levels were noted during the cycles adjacent to the delivery cups (within 2 cm of the cups). Ozone leakage was defined as the difference between the ozone levels in air adjacent to the cup minus the background levels in the room. The following ozone application situations were evaluated.

**Flat metal surface**

*Continuous complete 10 s ozone delivery cycles. No displacement of the cups between measurements*

Ozone was delivered by the HealOzone apparatus. Ozone air levels were measured during and after 10 s delivery cycles using five different cup sizes (3, 4, 5, 6 and 8 mm diameter) placed on a flat stainless steel surface. Each cup was used continuously over the course of 30 ozone delivery cycles each of 10 s. Each new cycle started 10 s after the end of the former re-suction period. The cups were not dislodged from the exposed surface during or after the measurements. Background ozone air levels were measured at the start and 600 s after the finish of each 10th application. Differences between the cup sizes were analyzed before the start of the following evaluations with the metal surface.

*Continuous 10 s ozone delivery cycles. Displacement of the cup directly after the ozone treatment and before the start of the re-suction period*

In the first part of the flat metal surface experiment, the 8 mm sized cups showed the highest leakage values and were therefore used in the following evaluations on the flat metal surface. After a 10 s ozone application, the cup was displaced before the re-suction period of the apparatus started. Ozone concentrations in air were measured during the following 15 s and the maximum value was noted. Two hundred measurements were taken. New cups were used after each 50th application measurement and ozone background levels were measured at the start and after a pause of 600 s after each 50th application.

*Displacement of the cup after 5 s during a 10 s delivery cycle*

To simulate non-intentional displacement of the cup during ongoing treatment, the cup was displaced after 5 s during the 10 s ozone delivery cycles. The ozone air measurements were taken in the 5 s after each displacement (i.e. during the re-suction period of the apparatus). Four hundred measurements were taken. Ozone background levels were measured as earlier described for displacement of the cup before re-suction.
Tooth surfaces

Premolars (4) and molars (4), newly extracted for orthodontic reasons, placed in plaster were thoroughly cleaned with a polishing paste before the experiments. Ozone gas was delivered with the HealOzone (model 2130C) using suitable cups for all occlusal and buccal surfaces. Ozone levels in air adjacent to the cups were measured afterwards.

Displacement of the cup after a full ozone delivery cycle of 20 s and the 10 s re-suction period

The measurements were repeated 100 times for each surface. Background ozone air levels were measured at the start and 600 s after the finish of each 10th application.

Displacements of the cup during 60 s ozone delivery cycles

The cup was displaced every 5 s within the cycle, and ozone concentrations were measured during the following 5 s. One hundred measurements were taken for each tooth and surface. Background ozone air levels were measured at the start and 600 s after the finish of each 10th application.

Statistical analysis

The data were processed using the Statistical Package for the Social Sciences (SPSS, version 12.1). Frequency distributions of ozone levels in air were described for each of the experimental groups. Ozone leakage levels were calculated, defined as differences between ozone air levels adjacent to the silicon cups and the background levels measured in the room. Differences in leakage with the cup sizes were tested using the Kruskal-Wallis test, and the Wilcoxon signed ranks test. The level of significance was set at \( p < 0.05 \).

Results

During the 5 days of measurements, when the ozone apparatus not was in use, the backgrounds values varied between 0 and 64 µg·m\(^{-3}\), i.e. day 1: 0 and 64 µg·m\(^{-3}\); day 2: 0 and 56 µg·m\(^{-3}\); day 3: 18 and 42 µg·m\(^{-3}\); day 4: 36 and 52 µg·m\(^{-3}\); and day 5: 6 and 52 µg·m\(^{-3}\). The variations were irregular throughout the day (Figure 1).

Flat metal surface

Ozone air concentrations during and after the 10 s ozone delivery cycles, as well as the background levels in the room, are given in Table I. The concentrations for all tested situations are given as mean (min-max) values in µg·m\(^{-3}\) ozone gas. Low

Leakage (measured ozone air levels minus background levels)

The maximum ozone leakage value measured on flat metal was 120 µg·m\(^{-3}\). On premolars and molars combined, the maximum leakage values after full
ozone cycles and after accidental displacements were 50 µg m⁻³ and 88 µg m⁻³, respectively. The mean ozone air values differed in general very little from the background air ozone values observed in the room. In 26 of the 33 test groups, the ozone air concentrations adjacent to the cups were lower than the background concentrations. For the metal surface groups, a mean difference value of 7.6 µg m⁻³ (4.4–10.2) was observed. In the tooth surface groups, the observed differences for the buccal and occlusal surfaces were 7.4 and 5.6 µg m⁻³, respectively. Differences in ozone leakage values between full delivery cycles and disrupted cycles were significantly higher for disrupted cycles (p = 0.03).

**Table I. Ozone applications on flat metal surfaces. Ozone air levels (µg m⁻³) adjacent to the different cups measured after ozone delivery cycles without and with displacement of the cup**

<table>
<thead>
<tr>
<th>Cup diameter (mm)</th>
<th>n</th>
<th>Displaced before start of re-suction period</th>
<th>Displaced during delivery cycle</th>
<th>Mean (SD)</th>
<th>Min-max</th>
<th>Background values</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>30</td>
<td>13.8* (4.2)</td>
<td>8.0–24.0</td>
<td>22.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>19* (4.8)</td>
<td>16.0–30.0</td>
<td>30.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>16.0* (3.6)</td>
<td>12.0–26.0</td>
<td>26.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>16.2* (3.8)</td>
<td>12.0–26.0</td>
<td>26.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>22.0* (1.4)</td>
<td>18.0–26.0</td>
<td>24.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>100</td>
<td>Yes</td>
<td>54.6 (11.4)</td>
<td>38.0–84.0</td>
<td>40.0</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>100</td>
<td>Yes</td>
<td>87.0 (25.2)</td>
<td>26.0–166.0</td>
<td>26.0</td>
<td></td>
</tr>
</tbody>
</table>

b = p = 0.001 Kruskal Wallis test.

**Table II. Ozone air concentrations (µg m⁻³) measured (n = 100) after 20 s ozone delivery cycles including re-suction period adjacent to occlusal and buccal surfaces of 4 molar (M1–M4) and 4 premolar (P1–P4) teeth**

<table>
<thead>
<tr>
<th>Tooth</th>
<th>Mean (SD)</th>
<th>Min-max</th>
<th>Background</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>18.0 (7.4)</td>
<td>0.0–42.0</td>
<td>20.0</td>
</tr>
<tr>
<td>M2</td>
<td>28.2 (4.4)</td>
<td>20.0–42.0</td>
<td>38.0</td>
</tr>
<tr>
<td>M3</td>
<td>19.8 (7.2)</td>
<td>4.0–38.0</td>
<td>26.0</td>
</tr>
<tr>
<td>M4</td>
<td>21.4 (6.0)</td>
<td>10.0–40.0</td>
<td>38.0</td>
</tr>
<tr>
<td>P1</td>
<td>13.6 (9.8)</td>
<td>0.0–42.0</td>
<td>8.0</td>
</tr>
<tr>
<td>P2</td>
<td>14.2 (9.8)</td>
<td>0.0–42.0</td>
<td>8.0</td>
</tr>
<tr>
<td>P3</td>
<td>16.4 (10.2)</td>
<td>0.0–44.0</td>
<td>16.0</td>
</tr>
<tr>
<td>P4</td>
<td>23.2 (13)</td>
<td>0.0–54.0</td>
<td>24.0</td>
</tr>
<tr>
<td>Total</td>
<td>19.4</td>
<td>0.0–54.0</td>
<td>22.0</td>
</tr>
</tbody>
</table>

**Table III. Ozone air concentrations (µg m⁻³) measured (n = 100) adjacent to occlusal and buccal surfaces during and after accidental displacements of the cup every 5 s, during 60 s ozone delivery cycles. P1–P4 = premolar teeth and M1–M4 = molar teeth**

<table>
<thead>
<tr>
<th>Tooth</th>
<th>Mean (SD)</th>
<th>Min-max</th>
<th>Background</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>29.6 (23.4)</td>
<td>0.0–108.0</td>
<td>20.0</td>
</tr>
<tr>
<td>M2</td>
<td>28.0 (6.0)</td>
<td>18.0–36.0</td>
<td>38.0</td>
</tr>
<tr>
<td>M3</td>
<td>29.6 (3.6)</td>
<td>24.0–40.0</td>
<td>38.0</td>
</tr>
<tr>
<td>M4</td>
<td>29.8 (8.6)</td>
<td>12.0–54.0</td>
<td>46.0</td>
</tr>
<tr>
<td>P1</td>
<td>31.2 (18.8)</td>
<td>0.0–84.0</td>
<td>16.0</td>
</tr>
<tr>
<td>P2</td>
<td>21.8 (13)</td>
<td>0.0–52.0</td>
<td>16.0</td>
</tr>
<tr>
<td>P3</td>
<td>16.2 (8.8)</td>
<td>0.0–32.0</td>
<td>18.0</td>
</tr>
<tr>
<td>P4</td>
<td>26.6 (13.6)</td>
<td>0.0–52.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Total</td>
<td>26.6</td>
<td>0.0–54.0</td>
<td>26.0</td>
</tr>
</tbody>
</table>
Discussion

During daily life, humans are continually exposed to low concentrations of ozone in the air. At high concentrations, such as during summertime photochemical smog, ozone is known to elicit a spectrum of symptoms in human subjects characterized by impairment of lung function [15–17]. On investigating the effect of 6 h of exposure to 0–1000 μg·m⁻³ ozone on bronchial epithelial cells *in vitro*, Ruznak et al. [16] indicated that ambient concentrations of ozone could induce airway inflammation through release of proinflammatory mediators from airway epithelial cells. The World Health Organization air quality updated guideline gives values to 100 μg·m⁻³, 8 h mean. The guideline is meant to limit the risks of repeated daily exposure rather than occasional peak levels [18].

Ozone has been used in a few dental application issues in dentistry. Ozonated water has earlier been used to clean dental units and as a denture cleaner [19–21]. Recently, it has been suggested as an alternative antimicrobial therapy for treating fissure and root caries [9–12]. Still, there is limited evidence of the benefits of ozone in its application in dentistry, and therapeutic use of ozone must be coupled with awareness of the risks.

The apparatus tested is an electrical medical appliance. According to the manufacturer, the pathway for the ozone is under negative pressure, which means that the ozone generator is shut down if an incomplete seal occurs during treatment. When the ozone treatment has been completed, only air is supposed to be supplied through the system. Owing to limited accessibility of the target tooth or to non-cooperating patients, slippage of the resin cup from the teeth can easily occur during different clinical situations. As stated, directly after any accidental loosening of the cup seal, the production of ozone will stop as a result of the negative pressure that occurs. Owing to the inherent dangers of ozone gas, equipment for use in the oral cavity must be completely safe for both the patient and the operator.

In 2006, Millar & Hodson [14] compared the safety of two ozone delivery systems, the Ozi-cure and the HealOzone, in regard to leakage. The Ozi-cure apparatus allowed high levels of ozone to build...
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2.44 mg m⁻³. No high ozone levels were detected when using the HealOzone, even without additional suction. The ozone sensor used in that study was accurate in detecting ozone between 0 and 20 mg m⁻³ compared to accuracy in the present study at the μg m⁻³ level. However, Miller & Hodson did not investigate situations where the delivery cycle was not completed and did not report background levels.

In the present study, possible leakage of ozone was observed during application periods with a tightly fitted cup and after displacement of the cups during the delivery cycles. We investigated both longer application times than indicated and situations that can readily occur in the clinic, e.g. the cups being lifted accidentally. Comparing data from days when the apparatus was not used with data from ozone application days showed the same kind of irregular variation in ozone background levels. An important question is whether background levels could have been influenced by the ozone applications. Background levels were used as base for our leakage values. A change in background levels was seen with a similar change in ozone levels adjacent to the cups. Despite maximum use of the ozone apparatus with repeated disrupted cycles, no gradual increase in background levels during the days was observed (Figure 3). This may have been expected if ozone applications had influenced background levels, but neither the detectable ozone levels adjacent to the cups nor the ozone leakage levels exceeded the guideline value in any situation [18], despite displacements of the cups during ongoing ozone delivery. Some moderate peak values were registered, but these were randomly dispersed among the readings. Comparisons between separate series did not show decisive significant values. The measured ozone air values were close to those detectable by the photometric O₃ analyzer, which may explain these variations in the readings, often below the registered background values. No significant differences were found between the air ozone levels adjacent to the cups and the background levels. The hypothesis was therefore accepted.

In conclusion, ozone application cycles with displacements showed significantly higher leakage levels than continuous complete cycles. The largest ozone delivery cups showed the highest leakage values. A change in background levels was seen with similar change in adjacent ozone levels. The overall measured ozone leakage values were low after normally functioning delivery cycles and after repeated displacements and the delivery system can be considered a safe system.

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References

