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# Effectiveness of Low-flow High-ozone Concentration Disinfection of Dental Impressions: A Comparative Study to Immersion Disinfection

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors NP and ND designed the study, author MK performed the statistical analysis, authors AK, ND wrote the protocol, and NP wrote the first draft of the manuscript and managed literature searches. The experiment part was performed by authors NP, AK, MK, EB. The ozone disinfection device was constructed by authors NP and AP. All authors read and approved the final manuscript.*

***Original Research Article***

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## ABSTRACT

**Aims:** To examine the effectiveness of low-flow high-ozone concentration disinfection of dental impressions, by means of an automated prototype device.
**Methodology:** Disc shaped dental addition-cured silicone was inoculated with *Klebsiella pneumoniae* and *Staphylococcus aureus*, 10 mm discs were removed and ozone disinfected for different time intervals, immersion disinfected or served as controls. Disinfection success was examined by using the viable plate count method, while the statistical analysis was conducted via one way-ANOVA ($p < 0.05$).
**Results:** Significant eradication was observed for selected Gram (+) and Gram (-)

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bacteria after 3 minutes of ozone exposure, leading to complete disinfection of the samples.
**Conclusion:** While immersion disinfection of dental impressions is currently the most widely accepted method of disinfecting dental impressions, low-flow high-ozone concentration disinfection provides a quick, efficient, fully automated alternative method, limiting liquid waste generation. Possible alterations of the materials' physical and chemical properties, like those of immersion disinfection, are not included in the present manuscript. A precise automated method for impression disinfection is established, relieving the dental team of possible cross-contamination.

*Keywords: Ozone; disinfection; impression; bacteria.*

## 1. INTRODUCTION

Dental impressions contaminated with microorganisms that are present in the patients' oral cavity can cross-infect the dental team and recommendations concerning disinfection have been published several decades ago [1-8]. Since the dental impression is removed from the patient's mouth, it is transformed into a carrier of potential pathogen bacteria, viruses and fungi [2,6,7,9-11]. These opportunistic pathogens may persist even on gypsum casts [7,10,12]. Disinfection of dental impressions is vital and should be performed prior to dental laboratory delivery [13]. However, impression disinfection has not been satisfactorily integrated in the impression taking procedure nor the dental laboratory procedures, while some colleagues do not even rinse dental impressions with water before sending them to the dental laboratory [7,14].

Liquid chemical immersion disinfection is currently the most widely accepted method of disinfecting dental impressions [1,15-19]. Although alternative disinfection methods such as microwave, ultraviolet light, etc. have been proposed, no notable outcomes were observed [20]. Disinfection by spray atomization is the only daily practice counter proposal, though not researched to the extent of immersion disinfection [21,22] and not preferred that much [23]. The need to overcome immersion disinfection drawbacks, as well as limiting the environmental hazardous liquid waste generation, led to investigation of new innovative disinfection methods.

Ozone, as a potent oxidizing agent [24], presents strong antimicrobial action, recognized since the 19th century [25], and is being used in a wide range of applications as a disinfecting agent [26]. Ozone is an unstable compound which decomposes relatively soon from the time it is produced (half-life 40 minutes at 20ºC) [27]. Ozone derives from ozone generators, which make use of either "corona discharge" technology, ultraviolet light or electrolysis [28,29].

The inactivation mechanism of microorganisms with ozone is based on its effect on their cell membrane, on vital proteins and unsaturated lipids, as well as on the intracellular enzymes. In addition, its action to the microbial DNA structure and viral capsid protein is of great importance [30-32]. The DNA degradation effect of ozone has been extensively studied in the past years showing great interest [31,33,34].

Despite the fact that ozone has many applications in modern Dentistry [26], it has never been used for dental impression disinfection. The aim of this study was to develop a novel, efficient, low-flow high-ozone concentration disinfection method of dental impression

materials, via a prototype automated ozone disinfection device, minimizing environmental hazard through liquid waste generation. The null hypothesis was that dental impression ozone disinfection would be as efficient as immersion disinfection. Possible alterations of the materials' physical and chemical properties, such as dimensional and surface stability under ozone exposure were also investigated but are not included in the present manuscript.

## 2. MATERIALS AND METHODS

### 2.1 Ozone Disinfection Device

A prototype ozone disinfection device for dental impression materials was constructed (Greek patent office registration number: 20110100194/2013). Ozone is generated by a corona discharge ozone generator (OZV-4, Ozone solutions, Inc, Hull, Iowa) from ambient air which dries through an air dryer (MAG-600, Ozone solutions, Inc), it is directed through Teflon pipework into an 18x14x9 cm (LxWxH) sealed disinfection chamber and is removed from the chamber for destruction through a manganese dioxide-copper oxide catalyst (ODS-1P, Ozone solutions, Inc). Ozone flow is controlled by a high precision 0-20 L/min flow meter (EK-4BR, Kytola Instruments) via an adjustable flow valve (Fig. 1).

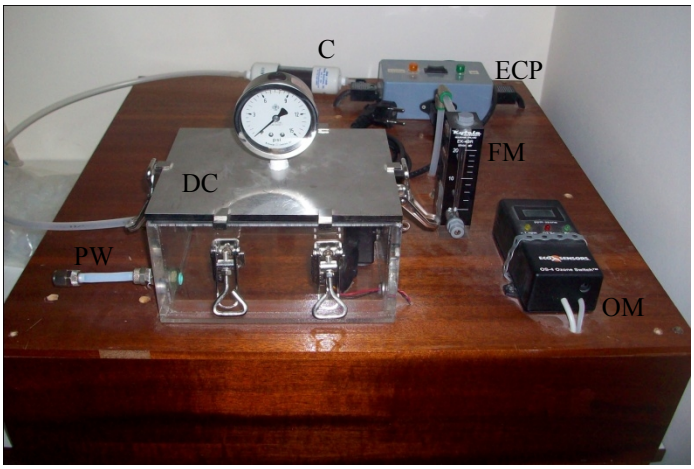


**Fig. 1. Prototype ozone disinfection device for dental impression materials.The ozone generator, the air dryer and other minor components, like pipework (PW), of the disinfection device lie beneath the wooden top. The disinfection chamber (DC), the manganese dioxide-copper oxide catalyst (C), the ozone meter (OM), the electronic control panel (ECP) and the flow meter (FM) are shown on top of the disinfection device**

The disinfection is conducted by constant flow of ozone with a flow rate of 4 L/min and ozone production of 2,61 g/h. During the experimental procedure, 2 min are added to the ozone exposure intervals aiming in reaching the ozone concentration upper threshold at the onset of the real exposure time. The completion of the exposure time follows the disruption of ozone supply and a subsequent feed of the chamber with ambient air for 10min, at a flow rate of 4 L/min, to completely wash it out of the ozone mixture. All manipulations of the ozone disinfection device are controlled by an electronic control panel.

## 2.2 Selected Bacteria

*Staphylococcus aureus* (ATCC 6538) and *Klebsiella pneumoniae* (Culture Collection of Microorganisms – Harokopio University) were used for this study. *Staphylococcus aureus* was cultured in Tryptic Soy Yeast Extract agar (Lab M Limited, Lancashire, UK) or broth at 37ºC for 24 h and *Klebsiella pneumoniae* in Nutrient agar or broth (Lab M Limited, Lancashire, UK) at the same conditions.

## 2.3 Inoculation and Disinfection Methods

Dental light-body addition-cured silicone (Aquasil Ultra LV-Regular Set, Dentsply, York, Pennsylvania) was loaded into the lid of a sterile 6 cm Petri dish and consequently the base of the Petri dish was pressed onto the silicone in such a manner that a uniform depth, flat surface of the impression material was formed. After material polymerization, the base of the dish was removed and 2 mL of the bacterial inoculum were delivered onto the impression mold. Following a 3 min submersion, the inoculum was carefully discarded from the impression material and the surface was dried for 5 min. Seven 10 mm diameter discs were removed from the inoculated impression material using a sterile dental copper ring (Fig.2).

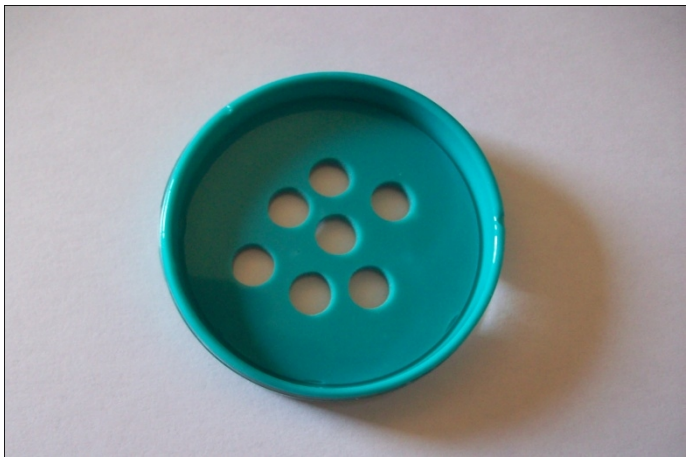

**Fig. 2. Polymerized, uniform depth, flat impression surface where the removed inoculated discs can be distinguished**

Four discs were exposed to the ozone chamber for time intervals of 3 min ($O_3$-3), 5 min ($O_3$-5), 10 min ($O_3$-10) and 15 min ($O_3$-15), respectively. Two discs served as control samples, one at the start of the ozone disinfection procedure (C) and the other one at the end of the experimental procedure (C+). An additional disc (A) was disinfected with immersion in a disinfectant solution containing 0.3% benzalkonium chloride as the active ingredient for 2 min (Prosept® Impression, OCC, Fehraltorf, Switzerland) and was depleted in isotonic solution PBS (10 mL).

## 2.4 Viable Plate Count Method

Each disc was placed into a 1.5 mL safe lock tube (Eppendorf, Germany) containing 1 mL PBS and was vortexed for 1 min (Autovortex SA6, Stuart Scientific, Surrey, UK). To determine the viable bacterial cells after different treatments, the viable plate count method

was used (in triplicate), by plating 0.1 mL of serially diluted inoculum. Petri dishes were incubated at 37ºC for 24h aerobically and colonies were enumerated. The experiments were repeated 5 times for *Klebsiella pneumoniae* and *Staphylococcus aureus* species.

## 2.5 Statistical Analysis

The statistical analysis of the disinfection effectiveness of the various treatments was conducted via one way-ANOVA, with significance threshold at 5% ($p$<0.05), based on the $\log_{10}$ transformations of the colony-forming units (CFU). Pairwise comparisons were conducted by the Tukey's test. All statistical analyses were calculated by the Sigma Stat v3.5 statistical software package (Systat software, Inc, Chicago, Illinois). A sample was considered as disinfected if the reduction of the bacterial population was higher than 3 logarithmic units [35-37].

## 3. RESULTS

After treatment of sample A with liquid disinfectant solution, no bacterial cells were found for both species. In the case of the samples inoculated with *Staphylococcus aureus,* the bacterial densities of the controls (C, C+) differ significantly compared to the various ozone exposure interval samples ($O_3$-3, $O_3$-5, $O_3$-10, $O_3$-15) and the immersion disinfected sample (A) ($p$<0.05), while there is no significant difference between the bacterial density of the control (C) and the bacterial density of the control (C+). A higher than 3 log reduction of the bacterial population was achieved after the first 3 min of exposure to ozone ($O_3$-3). The four different ozone exposure time samples do not differ significantly between them and the population levels remain rather stable, even though the time of ozone exposure was gradually increased from 3 to 15 min (Fig. 3).

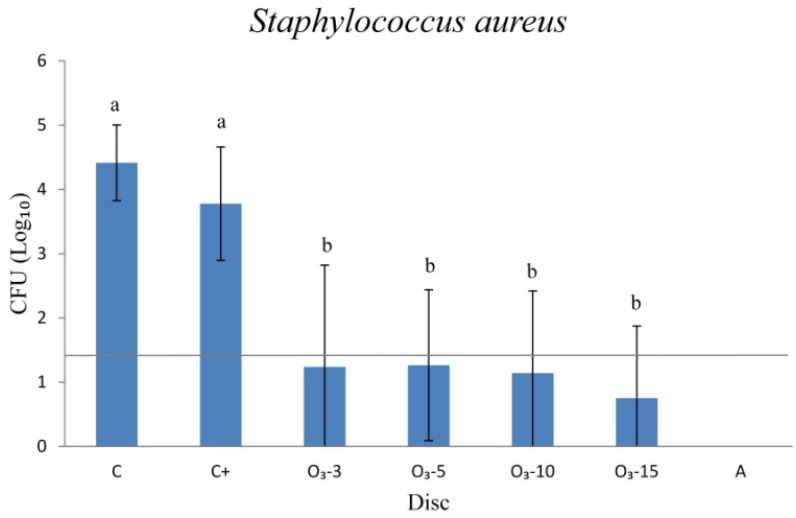


**Fig. 3. Ozone effect on *Staphylococcus aureus* species. Vertical bars correspond to standard deviation. Different letters represent significant differences between the means (Tukey's test, p<0,05, n=5). Horizontal line represents the disinfection threshold [35-37]**

Similar results are repeated with *Klebsiella pneumoniae,* where after treatment with ozone for 5, 10 or 15 minutes ($O_3$-5, $O_3$-10, $O_3$-15), no bacterial cells survived in petri dishes (Fig. 4).

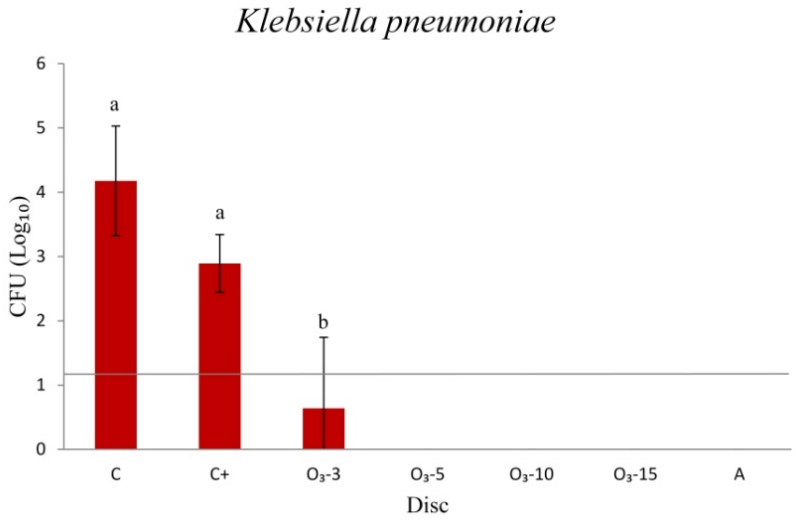


**Fig. 4. Ozone effect on *Klebsiella pneumoniae* species. Vertical bars correspond to standard deviation. Different letters represent significant differences between the means (Tukey's test, p<0,05, n=5). Horizontal line represents the disinfection threshold [35-37]**

*Klebsiella pneumoniae* was found to be more sensitive to ozone than *Staphylococcus aureus.*

Pairwise comparisons' P values for both species obtained via one way-ANOVA (Tukey's test) are presented in Table 1 and 2.

**Table 1. Pairwise comparisons' P values for *Staphylococcus aureus* experiment obtained via one way-ANOVA (Tukey's test)**

| | ***Staphylococcus aureus*** | | | | |
|---|---|---|---|---|---|
| **Sample** | **$C^+$** | **$O_3$-3** | **$O_3$-5** | **$O_3$-10** | **$O_3$-15** |
| C | 0,962 | 0,001 | 0,001 | 0,001 | 0,000 |
| $C^+$ | | 0,012 | 0,013 | 0,008 | 0,002 |
| $O_3$-3 | | | 1,000 | 1,000 | 0,990 |
| $O_3$-5 | | | | 1,000 | 0,987 |
| $O_3$-10 | | | | | 0,997 |

**Table 2. Pairwise comparisons' P values for *Klebsiella pneumoniae* experiment obtained via one way-ANOVA (Tukey's test)**

| | *Klebsiella pneumoniae* | |
|---|---|---|
| **Sample** | **$C^+$** | **$O_3$-3** |
| C | 0,136 | 0,000 |
| $C^+$ | | 0,003 |

## 4. DISCUSSION

For the ozone disinfection device efficiency consideration, a Gram (+) *(Staphylococcus aureus)* species and a Gram (-) *(Klebsiella pneumoniae)* species were selected. Bacterial load reduction for *Klebsiella pneumoniae* and *Staphylococcus aureus* is observed even during the time needed for the completion of all samples exposure to ozone ($C^+$ sample compared to C), but this reduction is not significant. In addition, greater resistance of *Staphylococcus aureus* to ozone can be seen in relation to *Klebsiella pneumoniae* for the various time intervals, due probably to the different structure of Gram (+) cell wall [38].

The population of the Gram (-) species (*Klebsiella pneumoniae*) was significantly decreased, almost eradicated after 3 min treatment with ozone. After 5 min exposure and as for the longer time intervals (10, 15 min), there were no viable counts. In contrast to Kowalski et al. (2003), who studied the exposure of *E. coli* to ozone and proposed that the decline of the bacterial population fits to a two-stage curve [39], a significant decrease of more than 3 log of *Staphylococcus aureus* colonies was observed, already, after only 3 min exposure to ozone, while the *Staphylococcus aureus* population remained constant throughout the 5, 10 and 15 min ozone exposure. Such differences may be due to the different experimental design e.g. the bacterial cells were plated onto petri-dishes, not permitting to form clumps.

The high standard deviation observed leads to the conclusion that initial bacterial loads are not equally distributed throughout the Petri dish. This resulted in a variability of bacterial load for all discs that were cut off the dish impression material. This is confirmed by the fact that during the removal of the inoculum from the impression material, the surface was drying asymmetrically. Some surfaces were drying faster compared to others. Possibly, areas that delay to dry enclose higher bacterial load and probably clumped bacterial cells. This concept is reinforced by the fact that clumped bacteria may self-protect against the corrosive action of ozone much better and tend to survive covered in between ozone eliminated and solitary bacteria [40], as seen in the case of *Staphylococcus aureus.*

Finally, a pilot experiment that was conducted on the bacterial strain *Staphylococcus aureus* showed that if the 3 min ozone disinfected sample is re-disinfected immediately after the completion of the initial disinfection, no viable cells are observed. The fact that bacterial ozone elimination increases proportional to ozone concentration up to a plateau, above which there is no increase in killing action [40] urged us to use high ozone concentration, which combined with a 2 step 3+3 min disinfection would possibly lead to complete sterilization of the inoculated samples (not shown in the current study).

## 5. CONCLUSIONS

Within the limitations of this study, the following conclusions were drawn:

1. The study revealed that the possibility of cross-contamination from potential pathogenic bacteria of oral flora, via dental impression materials, can be efficiently eliminated by using low-flow high-ozone concentration disinfection.
2. The use of low-flow high-ozone concentration disinfection of dental impressions promotes waste management through reduction of liquid waste production to achieve maximum environmental and human protection.
3. A quick, efficient and fully automated dental impression disinfection method can be established.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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