Comparative Evaluation of Disinfection on Elastomeric Impression Material using 2% Gluteraldehyde, Ultra Violet Radiation and Gaseous Ozone using Customised Disinfection Unit - An In-Vitro Study

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Abstract

Dental impressions is the preliminary step for any procedure and acts as a microbial source leading to contamination to the operator and lab technician. Hence, the disinfection of these impressions materials should be carried out unfailingly. The device was fabricated which helped in easy and effective disinfection.

Aim: The aim of the study is to Compare and Evaluate the Efficacy of Disinfection on Elastomeric Impression material using 2% Gluteraldehyde, UV Radiation Disinfection and Gaseous Ozone. Objective: The efficacy of the disinfectants was compared based on the ability of the disinfectants in reducing the microbial colonies on the impression material. Methodology: Microbiological analysis was done and the colony forming units were evaluated and compared. The disinfection was done with a customized disinfection chamber which can be readily installed in the clinic and has multitudes of use other than disinfection. Result: Dry Gaseous Ozone as a disinfectant proved to be efficacious as compared to the other disinfectants. Conclusion: Dry gaseous ozone can be used effectively for disinfection of impressions without altering its dimensional stability. The customized disinfection unit is portable and can be readily installed in the clinic.

Keywords: Disinfection, Ozone gas, UV Radiation, Colony forming Unit, Disinfection chamber

Introduction

Dental impression making is the Pre-requisite for majority of dental procedures. During this procedure, the impression materials contact with the saliva and blood, which are sources of contamination, and carries a high quantity of microorganisms of the oral flora upon the removal from the mouth. Dental impression can act as a means of transmission of infectious agents from patients to dental personnel who handle the impression or the casts.

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It has been a customary to rinse impression under running tap water to remove blood and saliva, but no routine method of sterilization or disinfection of dental impression has been accepted by dental profession. Difficulties in disinfecting impressions by traditional methods have led to chemical disinfection as an alternative, and some studies have shown that these disinfectants may adversely affect impressions.\textsuperscript{1}

The use of ultraviolet rays can be a good alternative choice for disinfection because Ultraviolet (UV) rays have long been recognized as an effective method for eliminating microorganisms without requiring chemicals or heat.\textsuperscript{2}

Gaseous ozone when used as disinfectant doesn’t affect the dimensional properties of the impressions after disinfection.

To simplify the procedures for disinfecting Elastomeric Impressions registered in the dental office, it may be advantageous to use the same disinfectant solutions that are used for dental Instruments.\textsuperscript{3} Therefore we fabricated a device with standard temperature pressure which can be used to provide ozone disinfection which is connected with ozone generator. The purpose of the study is to compare the efficacy of Gaseous ozone to other standard disinfectants

**Methodology**

An in-vitro study is to be conducted, to analyze bacterial colonization due to salivary contamination on elastomeric impression material (Photosil) and Effect of various means of disinfection on microbial growth. In order to evaluate & compare the efficacy of the disinfectants on elastomeric impression materials, the following materials and methodology was used. The study was carried out within the duration of 6months with the sample size of 45 (Figure 1A, 1B, 1C).

The materials used were divided into three groups (A, B, C) according to disinfection system(Figure 1 A,B,C). Disinfection systems are the commonly used gaseous ozone disinfection Using customized Ozone disinfection chamberwith attached ozone generator (K.H. Ozonators) (Group A), Ultraviolet chamber(Figure 5)(Group B) and immersion systems as: Korsolex [Glutaraldehyde 2%] 1:19 dilution (Group C) These were compared before and after disinfection. Impressions were made with Elastomeric Impression material and rinsed with distilled water for 15 s.

The Elastomeric Impression material is mixed according to the manufacturer’s instructions and the impression blocks were custom made. The Group A samples were disinfected by gaseous ozone (SH ozone -2ppm) in the ozone chamber for about 20 mins(Figure 6). The second group i.e, Group B sample was dried after rinsing and placed in ultra-violet light chamber at 254 nm wavelength for 3 min (PW 003, Himedia Laboratories Pvt. Ltd) (Figure 5). The third sample Group C is to be sprayed with 2% gluteraldehydeTotal there were 45 samples for one impression material. Disinfection was performed at room temperature. The control group samples(Figure 3) were tested for microbiological analysis using the Colony forming unit(CFU) The specimens were again rinsed with distilled water for 15 s to remove any traces of the disinfectant from the impression surface. These were then wiped with sterile cotton swabs. These swabs were then inoculated in petri dishes with agar media namely, blood agar media. These were incubated in an incubator for 24 hrs. at 37°C for aerobic organisms. The inoculation was done to test the viability of microorganisms that can persist after rinsing and disinfection for that long and can thus spread the infection. Microbial growth was identified from the colony characters seen in the culture media Colony forming units (CFU) were counted(Figure 2A,2B,2C) and the results documented(Figure 4 & Table 1,2).
Figure 1: samples categorized into 3 groups for varied modes of disinfection

**Group A**- Using customized gaseous ozone disinfection unit

**Group B**- Using Ultraviolet Light Disinfection

**Group C**- Using 2% Gluteraldehyde

Figure 2 A: illustrates microbial colonies after disinfection of Group A using dry gaseous ozone by customized ozone disinfection unit

Figure 2 B: illustrates microbial colonies after disinfection of Group B using dry gaseous ozone by customized ozone disinfection unit

Figure 4: illustrates microbial colonies after disinfection of Group C using dry gaseous ozone by customized ozone disinfection unit

Figure 5: illustrates microbial colonies in Control Group before disinfection of samples.
Statistical Analysis

The data thus obtained was tabulated for statistical analysis. Data was analyzed using descriptive and analytical statistical methods. Statistical significance of the mean differences between the measurements tested using student paired t-test.

Observation & Result

Descriptive and analytical statistics were done.

The normality of data was analyzed by the Shapiro-Wilk test. As the data followed normal distribution the parametric tests were used to analyze the data. The one-way analysis of variance (ANOVA) test was used to check mean differences among the groups. Post hoc analysis was done using Tukey’s HSD test. Software: SPSS (Statistical Package for Social Sciences) Version 24.0 (IBM Corporation, Chicago, USA)

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>S.D.</th>
<th>S.E.</th>
<th>Min.</th>
<th>Max.</th>
<th>F-value</th>
<th>P-value#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Ozone Disinfection)</td>
<td>15</td>
<td>1.13</td>
<td>0.35</td>
<td>0.09</td>
<td>1.00</td>
<td>2.00</td>
<td>111.516</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Group B (UV light Disinfection)</td>
<td>15</td>
<td>3.26</td>
<td>0.88</td>
<td>0.22</td>
<td>1.00</td>
<td>5.00</td>
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<td></td>
</tr>
<tr>
<td>Group C (Glutaraldehyde spray)</td>
<td>15</td>
<td>1.40</td>
<td>0.63</td>
<td>0.16</td>
<td>1.00</td>
<td>3.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Group</td>
<td>3</td>
<td>24.33</td>
<td>9.29</td>
<td>5.36</td>
<td>18.00</td>
<td>35.00</td>
<td></td>
<td></td>
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</tbody>
</table>

#P-value derived from one-way ANOVA test; †significant at p < 0.05

The mean bacterial count (log10 CFU/ml) among the four groups was compared. The analysis done by one-way ANOVA showed statistically significant differences (p<0.001) in mean bacterial count (log10 CFU/ml). The ozone group had the least bacterial count (1.13 ± 0.35) followed by glutaraldehyde spray group (1.40 ± 0.63) and UV light Disinfection group (24.33 ± 9.29).

<table>
<thead>
<tr>
<th>Group</th>
<th>v/s Group</th>
<th>M.D.</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
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<td>Ozone Disinfection</td>
<td>UV light Disinfection</td>
<td>-2.13</td>
<td>0.036†</td>
</tr>
<tr>
<td></td>
<td>Glutaraldehyde spray</td>
<td>-0.26</td>
<td>0.985</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>-23.20</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>UV light Disinfection</td>
<td>Ozone Disinfection</td>
<td>2.13</td>
<td>0.036†</td>
</tr>
<tr>
<td></td>
<td>Glutaraldehyde spray</td>
<td>1.86</td>
<td>0.082</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>-21.06</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Glutaraldehyde spray</td>
<td>Ozone Disinfection</td>
<td>0.26</td>
<td>0.985</td>
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<tr>
<td></td>
<td>UV light Disinfection</td>
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<tr>
<td></td>
<td>Control</td>
<td>-22.93</td>
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<tr>
<td>Control</td>
<td>Ozone Disinfection</td>
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<td>&lt;0.001†</td>
</tr>
</tbody>
</table>

#P-value derived from Tukey’s HSD post hoc test; †significant at p < 0.05
The post hoc pair wise comparative analysis also showed significant differences in mean bacterial count (log10 CFU/ml) of the four groups. When ozone disinfection group was compared to other groups, significant difference was found with UV light disinfection (p=0.036) and control group (p<0.001). The ozone group performed better in reducing the bacterial count than UV light disinfection and control group. When ozone group and glutaraldehyde spray group was compared, NO significant difference was found (p=0.985). This implies that ozone group performed at par with glutaraldehyde spray group.

![Graphical Comparison of mean bacterial count (log10 CFU/ml) among the four groups post disinfection](image.png)

**Figure 4: Graphical Comparison of mean bacterial count (log10 CFU/ml) among the four groups post disinfection is depicted**

**Note:** The error bar represents standard deviation

**Discussion**

Impression materials, including synthetic elastomers, must be disinfected after removal from the oral cavity because of the risk of transmitting pathogenic. In the present study, the antibacterial efficacy of gaseous ozone on the light-body consistency PV impression specimens was compared with Ultra-Violet Radiation and 2% Gluteraldehyde, which are conventionally used for disinfection purposes.

WHO suggested Gluteraldehyde as the primary disinfecting agents for various infectious hepatitis world-wide. The Gluteraldehyde is used commonly in 2% concentration. It is used in the form of spray disinfection, immersion method. The major disadvantage of various other disinfectants is that; they produce irritating vapours. There are studies that compared the disinfectants.

In 2017 Zhang W et al conducted a study in which the objective was to evaluate the effect of ultraviolet ray...
combined with immersion method on the disinfection of silicone impression materials. 2% glutaraldehyde immersion or ultraviolet radiation disinfection alone failed to achieve high disinfection effect. Combined use of ultraviolet radiation and 2% glutaraldehyde immersion can eliminate both HBV and HIV.4

The use of ultraviolet rays can be a good alternative choice for disinfection because ultraviolet chambers are available in most of the dental clinics and are used to store sterilized dental instruments to avoid recontamination from dental operatory.5 Ultraviolet rays have long been recognized as an effective method for eliminating microorganisms without requiring chemicals or heat. When microorganisms are exposed to ultraviolet rays at a particular wavelength (200-280 nm), their reproduction capability is destroyed and inactivation occurs at a faster rate, so that they no longer pose threat to humans.

Ozone is an allotropic form of oxygen, which is effectively used in the treatment of different diseases for more than 100 years. In the present era of increasing antibiotic resistance, ozone therapy is an alternative medical treatment that rationales to increase the amount of oxygen to the body through institution of ozone into the body. Owing to its beneficial biological properties including antimicrobial and immune-stimulating effects, ozone therapy has opened new vistas in treatment modalities of dental pathologies for patients of all ages.

Ozone has anti-microbial action which leads to destruction of bacteria, fungi and viruses. Ozone also possesses immuno-stimulating effect as the immunocompetent cell proliferation and immunoglobulin synthesis is stimulated. Ozone shows anti-hypoxic effect, as it changes cellular metabolism by raising partial pressure of oxygen in tissues and improving the transportation of oxygen in blood. It also has biosynthetic effect as it causes activation of protein synthesis mechanism with increased amount of mitochondria and ribosomes in cells that leads to elevation of functional activity and regeneration potential of tissues and organs. 5,7

Celebi H. et al in an in-vitro study titled Disinfection of polyvinyl siloxane impression material by gaseous ozone compared the efficacy of gaseous ozone and sodium hypochlorite (NaOCl) in disinfecting light-body consistency hydrophilized polyvinyl siloxane (PVS). Gaseous ozone treatment was identified as a promising method of disinfecting polymerized PVS impression materials because of its positive effect on the wettability of the material. 8

In a review study by Issac AV et al, it was concluded that even though both ozonated water and ozonated gas are used for cleansing of dentures, gaseous ozone has been found to be more effective. In comparison with other medical modalities like antibiotics & disinfectants, it was found that the ozone gas was more conservative, predictable and inexpensive option. This option increases the patient compliance with minimal adverse effects.9

In a review article published by Garg R et al, it was mentioned that 99 percent of all the bacteria causing tooth decay have been eliminated after 10 seconds of ozone exposure and even 99.9 percent bacteria after 20 seconds exposure. Thus, treating patients with ozone cuts off the treatment time with a great deal of difference, it eliminates the bacterial count more precisely and moreover, it is completely painless, so increasing the patients’ acceptability and compliance.10

Zhao H et al in 2000 in their study, the disinfecting efficiency of five disinfecting methods to three bacterial: Staphylococcus epidermidis, Streptococcus sanguis and Bacillus subtilis were evaluated. 2% glutaraldehyde immersion, spray, 5% immersion, spray are all effective disinfecting methods for impressions and ozone treatment is an effective method in disinfecting the gypsum casts. However certain amount of dimensional changes were observed due to disinfecting techniques like immersion and spraying when compared with ozone therapy in the gaseous form. 9

Gaseous ozone could be administered topically either by an open system or by a sealing suction system to avoid inhalation and adverse effects. It can be installed in the dental clinics for its multitude application apart from disinfection.

In an invitro study conducted by Manju Sharma et al in the year 2007, efficacy of a portable ozone-generating machine, equipped with a catalytic converter and an accessory humidifier, to inactivate 15 different
species of medically important bacteria was evaluated. An ozone dosage of 25 ppm for 20 minutes, with a short burst of humidity in excess of 90% relative humidity, was able to inactivate more than 3 log10 colony-forming units of most of the bacteria, including Acinetobacter baumannii, Clostridium difficile, and methicillin-resistant Staphylococcus aureus, in both in a laboratory test system and simulated field conditions. In many cases, complete eradication was achieved. Dried and wet samples were equally vulnerable to the ozone. The ozone generator can provide a valuable decontamination tool for the removal of bacteria in many institutional and communal settings, including hospitals and other health care institutions. 12

Impression materials especially, hydrocolloids should be disinfected for a limited time period. Immersion is more secure than spraying and self-disinfecting materials are efficacious, but better accompanied by immersion. Polyethers, on the other hand, can be effectively disinfected by spraying. Although this seems to be the preferred method for disinfection of these water friendly materials, modern polyethers seem to withstand immersion, even long-term. Little information could be traced considering the stability of hydrophilic silicones upon prolonged immersion disinfection. In such situations, ozone therapy can be utilised in the form of gas for dimensional stability of impression materials. Hydrophobic elastomeric materials cannot be safely immersed in disinfectants and left for a long period.13

In this study the Efficacy of Disinfection on Elastomeric Impression material using 2% Glutaraldehyde, UV Radiation Disinfection(Figure 5) and Gaseous Ozone was evaluated and compared. The process of production of ozone gas and disinfection was carried out using a customized disinfection unit. The superiority of ozone gas over other conventional disinfecting materials and the efficacy of customized disinfection unit was carried out with this study(Figure 6). It was done on the basis of microbe evaluation Colony forming units (CFU). Significant reduction in CFU Count was seen after Gaseous Ozone disinfection(Figure 2A & 4) when compared with any other disinfecting materials. The Specifications of the device are that it can provide chairside disinfection
to the the dental instruments which are regularly used as well to the impressions and casts obtained with dry gaseous ozone at 2 ppm concentration for a time period of 10 minutes. No change in the surface characteristics of the disinfected impressions or instruments is seen. The customized device and the technique for disinfection of Impression material is copyrighted.(Table 1,2& Figure 2A,2B,2C, 3,4)

**Figure 5- Disinfectant UV Lamp**

**Figure 6- Customised Dry gaseous Ozone Disinfection Unit.**

**Conclusion**

There are in-numerous options in the medical field in context of disinfection techniques and related equipment. The only part that lies questionable is the efficacy of the disinfectant material or technique used and its deleterious effects on the material disinfected. Many disinfectants used alters the physical properties and surface characterization on the instruments or materials. The exposition of molecular mechanisms of ozone further benefits practical function in dentistry.
Treating patients with ozone therapy lessens the treatment time with an immense deal of variation and it eradicates the bacterial count more specifically. Dry gaseous ozone can be used effectively for disinfection of impressions without altering its dimensional stability. The customized disinfection unit is portable and can be readily installed in the clinic. The only shortcoming of the ozone being its short half-life. Therefore, needs to be freshly used in case of ozonated water used as any treatment adjunct. Further research is needed to avail more benefits from the unexplored applications of ozone.

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