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A COMPARATIVE EVALUATION OF THE EFFECT OF OZONATED WATER AND 2% GLUTARALDEHYDE AS DISINFECTANTS ON THE PHYSICAL PROPERTIES OF POLYVINYL SILOXANE IMPRESSION MATERIAL- AN INVITRO STUDY"

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https://doi.org/10.55231/jpid.2022.v06.i01.02

Abstract:

Aims: *To compare the efficacy of ozone water and 2% glutaraldehyde in disinfecting polyvinyl siloxane impression and the effect of both disinfectants on the wettability, dimensional accuracy and surface detail reproduction of the material.*

Settings and Design: *In vitro – comparative study*

Material and Methods: *Samples (N =108) were subjected to disinfection either with 2% glutaraldehyde (group 1) or ozone water (group 2) for 15 minutes. To evaluate the efficacy, the samples were inoculated with Streptococcus mutans [ATCC 25175], Staphylococcus aureus [ATCC 25923] and Candida albicans [ATCC 10231]. Reductions in the number of microbial populationafter disinfection were determined by counting the colony forming units (CFU). The contact angle measurements on the impression surface were used to determine the wettability. Dimensional accuracy and surface detail reproductions of the samples were assessedwith a stereomicroscope of 4X magnification.*

Statistical analysis used: *paired t- test and chi-square test were used.*

Results: *The efficacy of both disinfectants in reducing the microbial count found to be comparable. Both the disinfectants reduced the contact angle which improved the wettability of the material. Disinfection with 2% glutaraldehyde showed significant alteration in dimensional accuracy and surface detail reproduction ($p<0.05$) when compared to samples disinfected with ozone water, which preserves the dimensional accuracy and surface details.*

Conclusions: *Ozone water treatment was identified as a potential method for the disinfection of polyvinyl siloxane impression as it increased the wettability of the material, showed least dimensional changes and preserved surface details more accurately.*

Key words: Disinfection, ozone, infection control, polyvinyl siloxane impression.

Introduction

Prosthodontics is one field of dentistry where prevention of cross contamination seems to be a serious dilemma. The increased awareness of the dangers of cross contamination with infectious disease such as Hepatitis B, Hepatitis C, Herpes, Human immunodeficiency virus disease (HIV), Tuberculosis and recently Corona virus disease (COVID- 19) during dental procedures is creating a growing impact on attitude towards infection control in dental clinics and dental laboratories. So infection control is one of the primary facets of dental care. The increased incidence of infectious and/or contagious diseases of various etiologies has forced dental professionals to adopt precautions for the control of the spread of microorganisms for the protection of both the dentists and the patients. Every patient who seeks treatment should be considered a potential carrier of an infectious and/or contagious disease1. Therefore, daily routine measures for the control of microorganisms should be followed rigorously in dental practice.

Dental impressions are considered potentially infectious medium as they are contaminated with patient's saliva and blood. Pathogens, if present in high enough number, can survive several days on impressions and then can be transferred onto set gypsum material. Impression disinfection is now considered a routine procedure in dental offices and laboratories[1, 2]. Disinfection of the impression immediately after removal from the oral cavity is recommended by American Dental Association and Centers for disease control and prevention to avoid possible transmission of infectious diseases[2].

Prosthodontics treatment procedures begin with the making of dental impression, which forms the first link in the microbial contamination during dental care. Poly vinyl siloxane impression material is one of the most favoured impression materials in dentistry because of their excellent physical properties, favourable handling characteristics and good patient acceptance[3]. Many disinfectant agents are used to disinfect dental impression which may alter the physical properties of the impression material.

The action of environment friendly ozone as an oxidizing agent is well known and is being used in a wide range of applications as a disinfecting agent[4], killing both gram positive and gram negative organisms. The disinfection activity of ozone is due to its ability to attack the cell membrane and intracellular enzymes of microorganisms, as well as the viral capsids and DNA.

The purpose of this in vitro study is to compare the efficacy of ozone water and 2% glutaraldehyde in disinfecting polyvinyl siloxane impression and the effect of both disinfectants on the physical properties of the material such as wettability, dimensional accuracy and surface detail reproduction.

Materials and Methods:

The study was approved by institutional ethical committee, ref no. AEC/REV/2018/26.

The poly vinyl siloxane (PVS) impression material (AQUASIL, DENTSPLY) medium body consistency were used in this study. The samples were prepared using the standardized stainless steel die, as described in ADA specification no 19 (FIG 1), scored with three horizontal parallel lines X, Y, Z (25,50,75 μm wide and 25mm length respectively) spaced 2.5 mm apart were inscribed between two vertical lines D1 and D2. Die was ultrasonically cleaned and air dried to ensure that it was free of any surface contaminants before each impression was made. Pre-packaged cartridges of PVS Impression material with an auto mixing gun were used for impression making. A total of 108 samples were prepared, 54 each for group 1 (2% glutaraldehyde) and group 2(ozone water) (FIG 2).

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2% Glutaraldehyde (CIDEX, Johnson & Johnson private limited) and ozone water were used as disinfectants. Ozone water is produced using an Ozone generator (Dent ozone India) with a high voltage electrical discharge at a constant flow rate by the apparatus and was ejected into the diffuser through the output tube. The concentration of ozone used was 2 ppm, and it was generated for 5 minutes from the apparatus for 100 ml water. The samples were disinfected with the solution for 15 minutes.

Evaluation of disinfectant efficacy

Streptococcus mutans (ATCC 25175), Staphylococcus aureus (ATCC 25923), Candida albicans (ATCC 10231) were used for this study.

Inoculation procedure

The strains were supplied by HI media Pvt Ltd. The inoculating swab which contains Streptococcus mutans and staphylococcus aureus were seeded on to blood agar and of Candida albicans were seeded on to Sabouraud dextrose agar (SDA). Quadrant streaking was done and the plates were incubated at 370 c for 24 hours in case of bacteria and for 48 hrs for Candida albicans. The microorganisms were harvested after confirming their presence with the help of a microscope.

S.mutans and S.aureus were then diluted to a suspension of 105 cfu/ml and C. albicans were diluted to 107 cfu/ml respectively. Before the inoculation procedure, the samples were made sterile by autoclaving in order to avoid other microbial contamination. 200 ml of the diluted suspensions of each organism were taken in a separate glass bottle with tight lid and the samples were added to the suspension. Then the bottles were incubated at 370 c for 24 hrs for S. mutans and S.aureus and 48 hrs for C.albicans. After the incubation period, samples were removed with sterile forceps and washed with 15ml of sterile phosphate buffer solution to remove the non-adherent organisms.

Disinfection Procedure

Samples (9 each) containing the microorganism were disinfected with 2% glutaraldehyde and with 2 ppm ozone water respectively for 15 minutes. After disinfection procedure the samples contaminated with S.mutans and S.aureus, were transferred to the sterile BHI broth using a sterile forceps, and the samples contaminated with C.albicans were transferred to sterile SDA broth and incubated for 24 hrs and 48 hrs respectively. In order to measure the viable count of organisms after disinfection, quadrant streaking was again performed by taking 1 microliter from the broth using a sterile wire loop and incubated at 37 0 C for 24 hours in case of bacteria and 48 hrs for fungi. After incubation period, colonies of microorganisms were observed and the numbers of colony forming units (cfu) were calculated.

Evaluation of wettability

Wettability was evaluated by measuring the contact angle on the impression surface. Contact angles were measured for each sample before and after disinfection procedure, using contact angle measuring instrument- OCA 15EC (Data physics). The samples were placed on the platform of the testing apparatus, a water droplet (3μl) dispensed onto the surface of the sample and the contact angle was measured at 20 seconds. The mean contact angles were analysed by statistical methods. (Fig 3)

Evaluation of dimensional accuracy

The length of horizontal line between the cross lines D1 and D2 were measured using stereomicroscope (Magnus) of 4x magnification with the help of image analysis software before and after disinfection with 2% glutaraldehyde (group 1) and ozone water (group 2). Dimensional accuracy

expressed as percentage dimensional change (L), was calculated in accordance with ISO 4823 using the equation:

$$L = (L1\text{-}L2)/L1 \times 100$$

Where, L1 is the length of horizontal line between D1 and D2 on the samples before disinfection and L2 is the length of horizontal line after disinfection.

Evaluation of surface detail reproduction

Surface qualities of the samples obtained were examined using stereomicroscope with 4 x magnification and rating values were assigned.

Samples were then subjected to disinfection by immersing in 2% glutaraldehyde solution (group 1) and ozonated water (group 2) for 15 minutes. After disinfection procedure, the two groups were again examined.

- Rating 1 – well defined sharp detail and continuous line.
- Rating 2 – continuous line but with some loss of sharpness.
- Rating 3 – poor detail or loss of continuity of line.
- Rating 4 – marginally or completely not discernible line.

Table -1 Intergroup Comparison of Colony Forming Units of Microorganisms after Disinfection using Paired t-Test.

Microorganisms	Groups	N	Min	Max	MEAN CFU	SD	P value
Streptococcus mutans	Group 1	9	2600	8900	5611.11	1966.24	23.00
	Group 2	9	2600	11200	6811.11	1301.39	
Staphylococcus aureus	Group 1	9	3600	7200	5477.78	1247.78	12.00
	Group 2	9	4800	10200	7322.22	475.45	
Candida albicans	Group 1	9	3200	8900	5566.67	2041.45	0.114
	Group 2	9	5500	8200	6888.89	775.31	

Table -2 Intergroup comparison of mean contact angle using chi-square test.

GROUPS	N	MINIMUM	MAXIMUM	MEAN	SD	P Value
CONTROL	9	60.83	70.20	64.5244	3.00079	0.114
GROUP 1 2% GLUTARALDEHYDE	9	49.23	52.30	51.0867	1.01426	
GROUP 2 OZONE WATER	9	46.20	52.13	48.4856	2.30366	

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Statistical analysis

The results of the present study were analyzed using the SPSS Version 21 software (Statistical Package for Social Sciences). The observations were statistically analyzed. The values were subjected to student t- test and Chi-square test. The comparisons were made through the p value derived from the tests with a significance level of $p<0.05$.

Results:

Table (1) and figure (7-12) show the mean CFU of the studied microorganisms before and after disinfection with 2% glutaraldehyde and ozonated water for 15 minutes. Disinfection of the samples with 2% glutaraldehyde reduced the number of S.mutans, S.aureus, and C. albicans to 5611.11, 5477.78, and 5566.67 respectively. Disinfection of the samples with ozonated water reduced the number of S.mutans, S.aureus, and C. albicans to 6811.11, 7322.22, and 6888.89 respectively. All the $P>0.05$, suggest that there was no statistically significant difference between the groups in inhibiting the growth of tested microorganisms.

The statistical comparison of resulting contact angles, presented in Table (2), showed that the contact angle reduced after disinfection. The mean contact angle was reduced to 51.080 and 48.480 after disinfection with 2% glutaraldehyde and ozone water respectively. Both the disinfectants reduced the contact angle thereby improving the wettability of the material.

The present study evaluated the dimensional accuracy in terms of mean percentage dimensional change before and after disinfection. The result showed significant dimensional changes in samples disinfected with 2% glutaraldehyde, p

Table -3 Intra group comparison of mean % dimensional change of control and group 1 (2% glutaraldehyde) using paired student - t test.

GROUPS	N	MEAN	SD	p Value
CONTROL	9	0.2933	0.15492	0.018
GROUP 1	9	0.4844	0.08819	

Table -4 Intra group comparison of mean % dimensional change of control and group 2 (ozone water) using paired student t –test

GROUPS	N	MEAN	SD	p Value
CONTROL	9	0.2933	0.15492	0.133
GROUP2	9	0.3911	0.05207	

Table -5 Inter group comparison of surface detail reproduction of x line (25 μm) using chi-square test

VARIABLE		GROUPS			p value
		CONTROL	GROUP 1	GROUP 2	
X LINE	1.00	6	1	6	0.061
	2.00	3	4	3	
	3.00	0	3	0	
	4.00	0	1	0	

Table -6 Inter group comparison of surface detail reproduction of y line (50 μm) using chi-square test

VARIABLE		GROUPS			p value
		CONTROL	GROUP 1	GROUP 2	
Y LINE	1.00	6	0	4	0.038
	2.00	3	6	4	
	3.00	0	3	1	
	4.00	0	1	0	

value less than 0.05, whereas samples disinfected with ozone water showed statistically insignificant dimensional changes when compared with control group samples that is samples before disinfection, p value obtained was 0.133 (>0.05)(Table 3-4) (Graph 5)

The surface detail reproduction was evaluated in rate scoring system. Hence, the proportions were compared using chi-square test, and the p value was calculated. Table 5–8 shows the intergroup comparison of surface detail reproduction of PVS medium body material samples before and after disinfection. The samples before disinfection and samples disinfected with ozone water showed more rating value 1 and rating value 2 in X line (25 μm), Y line (50 μm), and in Z line (75 μm). Whereas the samples disinfected with 2% glutaraldehyde (group 1) showed more of rating value 2 and rating 3in X, Y, and Z lines. (Table 5-8)

The statistical analysis showed that the significant value p for samples disinfected with 2% glutaraldehyde in x line was 0.06, and y line and in z line was 0.007. The significant $p < 0.05$ in y and z lines, hence there were significant change in surface details in samples disinfected with 2% glutaraldehyde. Whereas the significant value p for samples disinfected with ozone water in x line was 0.06, and in y and z line was 0.462 and 0.447 respectively. All the p values were greater than 0.05 ($p > 0.05$), hence there were insignificant surface quality changes in samples disinfected with ozone water. (Graph 6)

Table -7 Inter group comparison of surface detail reproduction of z line (75 μm) using chi-square test

VARIABLE		GROUPS			p value
		CONTROL	GROUP 1	GROUP 2	
Z LINE	1.00	5	0	3	0.018
	2.00	4	4	5	
	3.00	0	5	1	
	4.00	0	1	0	

Discussion:

Infection control is an indispensable and imperative concern in the dental practice to prevent the spread of infections[5]. Dental impressions are categorized under semi critical objects in dental practice and require high level disinfection[5]. American dental association (ADA) and Centre for disease control (CDC) suggested disinfection of impressions to prevent cross infection and thiscanbeaccomplished by either immersion or sprayingwithdisinfectants or other methods[1].

The disinfecting process is to eliminate the microorganisms from the surface of the impression without affecting the quality of the impression. In the present study, the disinfectant efficacy of ozone water on the medium body PVS impression samples was compared with that of 2% glutaraldehyde; one of the most commonly used disinfectants in dentistry. Also evaluated and compare their effect on wettability, Dimensional accuracy and Surface detail reproduction of the material samples.

The results of the present study confirm those obtained by Bustos J et al[6] and Samra R K et al[7] who reported that impression materials

Table -8 Intra group comparison of surface detail reproduction of y and z lines.

GROUPS	VARIABLE	p value
GROUP 1 2% GLUTARALDEHYDE	Y LINE	0.007
	Z LINE	0.007
GROUP 2 OZONE WATER	Y LINE	0.462
	Z LINE	0..447

retain bacteria even after disinfection. However, irreversible hydrocolloid and silicone impressions can successfully be disinfected with 0.5% NaOCl solution or 2% glutaraldehyde for 5 minutes. According to Egusa H e t al[8] 2% glutaraldehyde and 1 ppm ozone water can effectively eliminate the microorganisms from the dental impressions. Faria I etal[9] studied the effects of ozonated water on Candida albicans oral isolates. He observed that Ozone is a powerful oxidative agent with greater bactericidal properties when compared to chloride. Arita et al[10] also stated that application of ozonated water may be useful in reducing the number of C. albicans on denture plates. The results of the present study are in agreement with these results.

Reduction in CFU of the microorganisms was noted when the impressions were disinfected with 2% Glutaraldehyde and Ozone water. For Streptococcus mutans the mean CFU was reduced from 105 to 5.61 x 103 when using 2% glutraldehyde as disinfectant and 6.81 x 103 when ozone water was used. For Staphylococcus aureus, after disinfection the mean CFU was reduced from 105 to 5.47 x 103 and 7.28 x 103 respectively for 2% glutaraldehyde and ozone water. After disinfection the mean candidal colony was reduced from 107 to 5.57 x 103 and 6.89 x 103 respectively for 2% glutaraldehyde and ozone water.

There was no statistically significant difference between 2% glutaraldehyde and ozone water in eliminating the tested microorganisms. These results indicate that disinfecting PVS impression samples with ozone water could lead to reductions in viable microorganisms comparable to those achieved with the 2% glutaraldehyde.

The wettability of a surface can be determined by measuring the magnitude of the contact angle, which indicates the degree of wetting when the surface and liquid interact. In the present study, disinfection with 2% glutaraldehyde reduces the contact angle of the impression samples, which was in accordance with the results obtained by the study done by Alzain S, who[11] reported that disinfection with 0.5% glutaraldehyde improved wettability of polyvinyl siloxane material samples. Glutaraldehyde acted like a surfactant that improved wetting potential of the impression materials[16].

Disinfection with ozonated water could render material more hydrophilic. The mean contact angle of the control samples was 64.52^0, while the mean contact angle after disinfection with 2% glutaraldehyde was 51.08^0 and that of ozone water was 48.48^0. The reduction in contact angle was more in samples disinfected with ozone water. The results are in agreement with the study done by Celebi H et al[12], who stated that gaseous ozone treatment was a promising method of disinfecting PVS impression materials because of its positive effect on the wettability of the material.

Dimensional accuracy and accurate reproduction of surface details of the oral structures are inherent qualities of an impression material. So it is very important to confirm the dimensional accuracy and surface quality of the impression even after disinfection. In the present study disinfection with 2% glutaraldehyde showed significant change in dimensional accuracy when compared with the samples before disinfection, p value obtained was 0.018 ($p < 0.05$).

There is a mean % dimensional change of 0.48 observed in samples disinfected with 2% glutaraldehyde, whereas samples disinfected with ozone water showed no significant dimensional changes when compared with samples before disinfection, p value obtained was 0.133 (> 0.05). The results are in agreement with the studies done by Langenwalter et al[13], Melilli D et al[14] and Johnson. G. H et al[15]. They reported that there were dimensional changes produced after disinfection with 2% glutaraldehyde but found to be clinically in significant.

There is paucity of data in the literature regarding dimensional stability after disinfection with

ozone water. In our study Ozone disinfection produced minor dimensional change but also found to be clinically in significant. The mean percentage dimensional change of PVS medium body material samples disinfected with ozone water was 0.39%. The PVS impression material samples disinfected with ozone water maintain the dimensional accuracy when compared to the samples disinfected with 2% glutaraldehyde.

However, the dimensional changes produced by both 2% Glutaraldehyde and ozone water was within the standards of ADA specification 19 that is less than 0.5%[13].

Abinaya k et al[3] in their study evaluated the surface quality of silicone impression materials after disinfection with ozone water. The authors concluded that the ozone water showed comparatively least changes and well defined lines when compared to 5.25% sodium hypochlorite, followed by 2% glutaraldehyde. These findings are in concur with the results of present study.

The results of the present study showed that the samples disinfected with ozone water showed more of rating 1 (well defined sharp detail and continuous line) and rating 2 (continuous line but with some loss of sharpness) whereas the samples disinfected with 2% glutaraldehyde showed more of rating 2 (continuous line but with some loss of sharpness) and rating 3 (poor detail or loss of

FIGURE 1: Stainless steel die with ADA specification no: 19

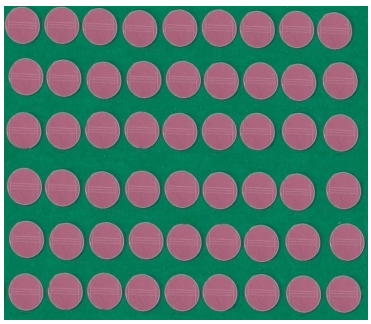

FIGURE 2: Samples of polyvinyl siloxane impression material prepared using the die for Group-1

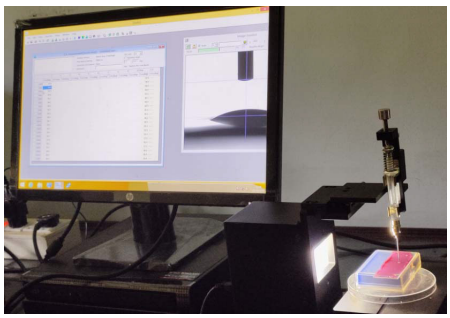

FIGURE 3: Contact angle measuring instrument - OCA15EC (DATA PHYSICS)

FIGURE 4: Stereo microscope (MAGNUS) used to evaluate the dimensional accuracy and surface detail reproduction of sample

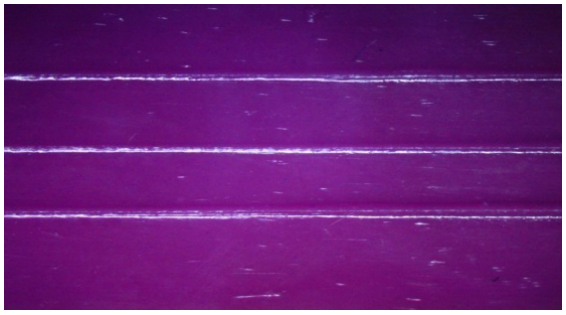

FIGURE 5: Stereomicroscopic image of surface detail reproduction of PVS impression material sample after disinfection with 2%

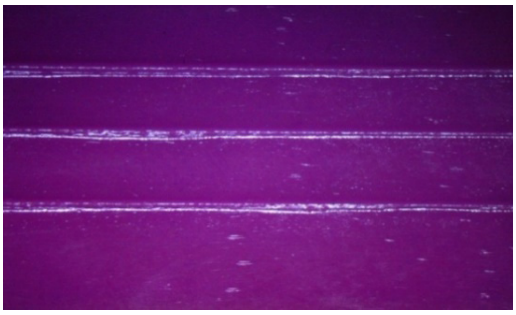

FIGURE 6: Stereomicroscopic image of surface detail reproduction of PVS impression material sample after disinfection with ozone water

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continuity of line) in X, Y, and Z lines respectively.

The statistical analysis showed that the value p for group 1 and group 2 in X line (25 μm) was 0.061. The p value for Y and Z lines in samples disinfected with 2% glutaraldehyde was 0.007 and that of ozone water was 0.462 and 0.447 respectively. The results showed there is significant change in surface detail reproduction of PVS impression material samples after disinfection with 2% glutaraldehyde, whereas there is no significant change in Y and Z lines after disinfection with ozone water.

According to Al Kheraif[16] and Abinaya et al[14] it has been advised to use ozone water as an alternative to 5.25% NaOCl, 0.525% NaOCl, and 2% glutaraldehyde for the disinfection of polyvinyl siloxane impression material. According to Poulis et al[17,18], clinically ozone disinfection is a new method, which needs no consumables, is time saving, and requires limited space in the dental office. This minimizes liquid waste generation resulting in superior environmental protection.

FIGURE 7: Colony forming units of streptococcus mutans in blood agar after disinfection with 2% Glutaraldehyde

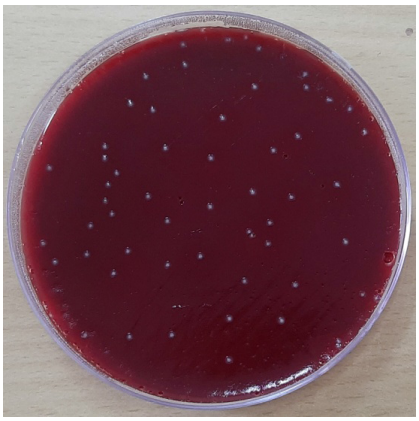

FIGURE 8: Colony forming units of streptococcus mutans in blood agar after disinfection with Ozone water

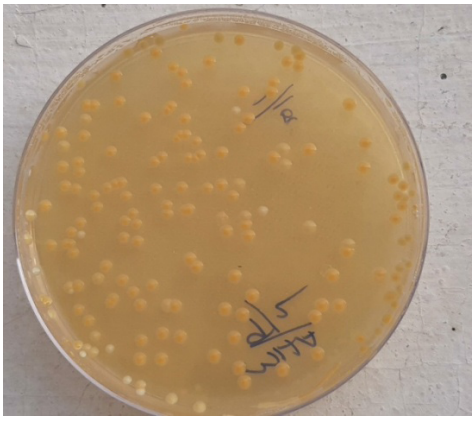

FIGURE 9: Colony forming units of Staphylococcus aureus in nutrient agar after disinfection with 2%

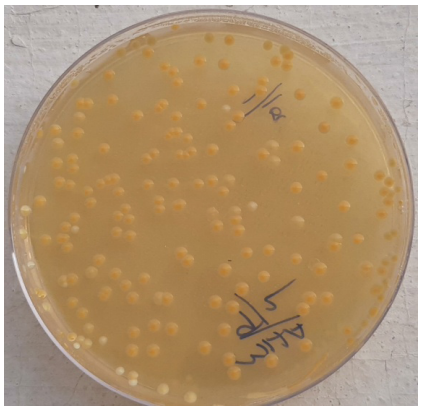

FIGURE 10: Colony forming units of Staphylococcus aureus in nutrient agar after disinfection with Ozone water.

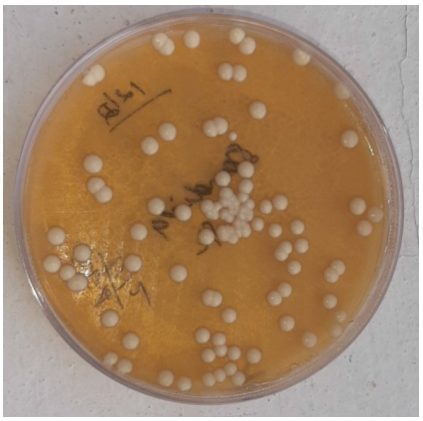

FIGURE 11: Colony forming units of Candida albicans in Sabouraud Dextrose Agar after disinfection with 2% Glutaraldehyde

FIGURE 12: Colony forming units of candida albicans in Sabouraud Dextrose Agar after disinfection with Ozone water

Therefore, ozone is considered as an environment friendly dental impression disinfection method.

Conclusion

Within the limitations of this study, it can be concluded that

- Immersion in ozonated water can reduce the load of microorganisms on the surface of polyvinyl siloxane impression, which is comparable to that of 2% glutaraldehyde. Hence Ozone can be used as an alternative to 2% glutaraldehyde.
- Both the disinfectants showed a significant increase in the hydrophilicity of the polyvinyl siloxane impression material.
- Ozone water disinfection showed least dimensional changes and preserved the surface details more accurately when compared to disinfection with 2% glutaraldehyde. However, the dimensional changes produced by both 2% Glutaraldehyde and ozone water were within the standards of ADA specification no.19, which is less than 0.5%.
- Hence concluded that Ozone water treatment could be considered as a potential method of disinfecting polyvinyl siloxane impressions.

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List of Abbreviations:

Abbreviation	Definition
ADA	American Dental Association
ATCC	American Type Culture Collection
CDC	Centre for Disease Control
0	Degree
0 c	Degree Celsius
hrs	Hours
min	Minute
O3	Ozone
ppm	Parts per million
%	Percentage
p value	Probability of obtaining result
PVS	Polyvinyl siloxane
SD	Standard deviation
Sec	Seconds
SPSS	Statistical Package for Social Sciences
SDA	Sabouraud dextrose agar