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Comparing the Effect of Pouring Time and Disinfection Protocols using Autoclave and Ozone on Dimensional Stability of Polyvinyl Impression Material: *In vitro* study

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ABSTRACT

Background: Infection control in dental offices and laboratories is an essential concern due to the high risk of cross infection with diseases. The aim of this study is to assess and compare the effect of autoclave and ozone disinfection protocols on the dimensional stability of polyvinyl siloxane (VPS) impression material after immediate and delayed pouring. Standardized stainless steel master model was fabricated to simulate a fixed partial prosthesis with one pontic and two crowns at the posterior area. Special trays were also constructed to formulate the impressions. 66 impressions were taken and divided into 3 equal groups. Group A (GA): (n=22) impressions which were disinfected using steam autoclave, Group B (GB): (n=22) impressions which were disinfected by ozonated water and Group C (GC): (n=22) impressions which were considered the control group with no disinfection procedures. Each group was further subdivided into two equal subgroups n=11, subgroup (I) in which the impressions were immediately poured and the subgroup (II) in which the impressions were poured after 24 hours (delayed pouring). All impressions were poured with type IV extra hard dental stone. Different dimensions were measured on the casts. **Results:** there was a statistically significant difference in mean values between (GC and GB), and (GC and GA) while there was a high statistically significant difference in the mean values between (GB and GA). Ozone and autoclave are considered as effective disinfection methods not adversely affecting the impressions' dimensional stability if the impression was poured immediately in case of ozone disinfection or delayed after 24 hours in case of using autoclave. **Conclusion**: VPS impression materials are dimensionally stable and produce accurate impressions with minimal distortion with the ability to retain these properties even after the disinfection procedures either with autoclaving or ozone disinfection with no effect on its dimensional stability.

Keywords: Impressions, polyvinyl siloxane, dimensional stability, disinfection, ozone, autocalve.

1. Introduction

Controlling the infection in dental offices and laboratories has become an important disquiet in recent years due to the high risk of cross infection with diseases caused by various pathogenic microorganisms (Nejatidanesh *et al.,* 2010; Klevens and Moorman 2013; Savabi *et al.,* 2021). These micro-organisms can be the reason of infectious diseases such as common cold, pneumonia, tuberculosis, herpes, hepatitis B, C and acquired immunodeficiency syndrome (AIDS) (Redd *et al.,* 2007). Dental professionals are bare to saliva and blood of their patients which is considered a potential occupational hazard in dentistry (Radcliffe *et al.,* 2013; Grignani *et al.,* 2021). Infection control is thus mandatory through the use of common measures and precautions in the dental offices

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and laboratories. This control will prevent the cross contamination that could extend to the dentist, dental office staff members, dental technicians and the patients (CDC 2003).

The principal route of transmission between dental offices and laboratories is via contaminated impressions and other prosthetic materials. Items such as impressions, jaw relation records, casts, prosthetic restorations and devices that have been in the patient's mouth should be appropriately disinfected prior to their translation to the dental laboratory as these could be a potential source of cross infection. This hazard can be reduced by using various disinfection protocols (Elie 2010).

A number of methods have been proposed to disinfect dental impressions. Disinfection means the destruction or removal of all pathogenic organisms capable of giving rise to infection. The elimination of most pathogenic microorganisms on impressions can be achieved by either physical and /or chemical methods (Al-Abidi and Ellakwa 2006; Gheena and Ezhilarasan 2019; Sree lakshmi *et al.,* 2020). Also the dimensional changes that may arise due to the impressions being soaked in the disinfectants can cause distortion affecting the dimensions of the impressions which is of major concern when procedures for their disinfection are considered. Therefore, sterilizing the impressions would be preferable (Kumar and Gheena 2015; Kamran *et al.,* 2015).

Polyvinyl siloxane (VPS) putty and light-body impression materials are well suited for making good impressions. With good results obtained having less expenditure of time as well as less discomfort and inconvenience for the patient but still its' working time is more affected by temperature. Steam autoclaving is considered to be the most effective method of sterilization used by hospitals and dental professionals (Radcliffe *et al.,* 2013; Nespraydko *et al.,* 2015).

Ozone, as a potent oxidizing agent presenting strong antimicrobial action, recognized since the 19th century and is being used in wide range of applications as disinfecting agent. The inactivation mechanism of microorganisms with ozone is based on its effect on their cell membrane, on vital proteins, unsaturated lipids as well as on intracellular enzymes. In addition it is capable of affecting the microbial deoxyreibonucleic acid (DNA) structure and viral capsid protein which of great importance in disinfection. In dentistry accurate and dimensionally stable impressions are the first step toward fabrication of a successful prosthesis as any restoration fabricated in the laboratory cannot be clinically more accurate than the impression used for its fabrication (Rajiv, 2011; Kumar and Chandi 2021).

This study will evaluate and compare the effect of autoclaving and ozone disinfection protocols on the dimensional stability of VPS as one of the elastomeric impression materials after immediate and delayed pouring.

2. Materials and Methods

This in-vitro study was conducted at the Fixed Prosthodontics Department, Faculty of Oral and Dental Medicine, Cairo University and at the Material Testing Laboratory, Central Services Unit, National Research Centre, Cairo. According to the pre-clinical *in vitro* modified CONSORT guidelines, the intervention, outcomes and sample size were recorded. 66 impressions were taken using VPS impression material (Imprint^{TM} II Garant, 3M^{TM} ESPE^{TM}, Germany) and divided into 3 equal groups. Group A (GA): (n=22) impressions which were disinfected using steam autoclave, Group B (GB): (n=22) impressions which were disinfected by ozonated water and Group C (GC): (n=22) impressions which were considered the control group with no disinfection procedures. Each group was further subdivided into two equal subgroups n=11, subgroup (I) in which the impressions were immediately poured and the subgroup (II) in which the impressions were poured after 24 hours. All impressions were poured with type IV extra hard dental stone and different dimensions were measured on the casts.

1. Mold fabrication

A stainless steel master model was constructed following the American Dental Association (ADA) specifications no.19 to simulate a fixed partial prosthesis with one pontic and two crowns at the posterior area. The anterior abutment was 7 mm in height, 5 mm in diameter and 8 degree taper, while the posterior abutment was 7 mm in height, 7 mm in diameter and 8 degree taper. The abutments were placed 11 mm apart representing the diameter of missing lower first molar as shown in figure (1).

Fig. 1: Standardized Stainless Steel Master Model with Teflon base

2. Special Tray Fabrication

Rectangular perforated custom made aluminum trays were fabricated to hold the impression material in place. Uniformly spaced perforations were placed in the tray to retain the impression material.

3. Impression Making

The VPS impressions were taken from the master model using two-step/two viscosity technique. The first is performed with the putty phase of the impression material and the second with the light body phase impression material. A digital precision scale was used to weight and mix with a ratio: 1:1 of both base and catalyst masses of the VPS according to the manufacturer's instructions. The material was loaded into the perforated metallic custom made trays to make an impression of the dies and leave it to set for 4 minutes. After loading the impression material it was immediately covered by a thin sheet of polyethylene (plastic spacer) followed by application of sufficient force on a rigid flat metal plate to seat it firmly against the mold. In the second step, the resin sheet spacer was removed from the mold in order to create the space that would be occupied by the light body material. Dispense syringe-able material was introduced into the set putty impression in the tray until it was completely filled using a stirring motion and keeping the tip immersed in the material to avoid trapping air. Then the impression was taken as before, with the putty consistency phase to have the final impression as seen in figure 2.

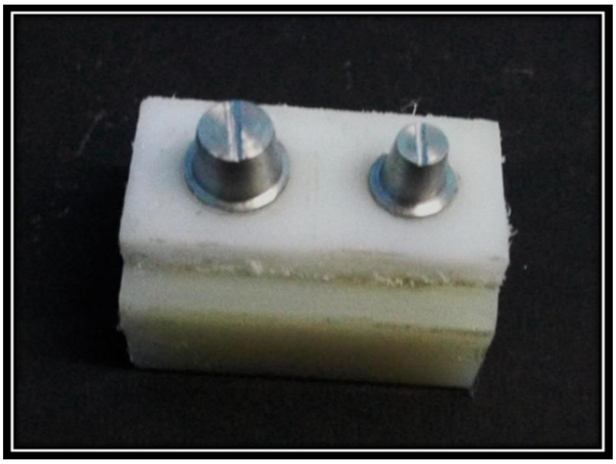

Fig. 2: Final Impression

4. Disinfection procedures

I. Steam Autoclave

Impressions were disinfected by steam autoclave (MELAG Medizin techniko HG, Berlin, Germany) at 121^0 C for 15 minutes by a standard cycle in the autoclave to reflect the autoclaving methods employed by dental practices to disinfect different dental equipments. After completing the autoclave cycle impressions were left for 30 minutes to reach the room temperature before pouring them in case of immediate pouring subgroup but in case of delayed pouring subgroup impressions they were stored in special container for 24 hours under the same conditions on the laboratory bench before pouring them.

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II. Ozone

A disinfection procedure was carried out using ozone. Ozone concentration in water was attained by adjusting the flow rate of gaseous ozone in the double distilled water for a specific time. Ozone generator (type N 1888A, China) was used with an ozone rate of 500 mg/hour. After adjusting the ozonated water concentration (125mg/L) the impressions were completely immersed in the ozonated water for 15 minutes (Hikal *et al.,* 2015). After completing the cycle impressions were left for 30 minutes before pouring them in case of immediately poured subgroup and in case of delayed pouring subgroup impressions they were stored in special containers for 24 hours under the same conditions on the laboratory bench before pouring them.

5. Construction of stone model

The manipulation of die material was done following the manufacturer's instructions using type IV extra hard dental stone. A water /powder ratio of 22 ml water to 100 gm powder was used for each mix. The impressions were filled with the mix over a vibrator to avoid bubbles entrapment. The stone casts were allowed to set for the recommended setting time of the die material. Then the casts were separated. The models were checked for any defects and left at room temperature for a minimum of 24 hours before testing.

6. Measuring procedures

Dimensional accuracy of the impressions was assessed by using the universal travelling microscope (Carl Zeiss, Germany) at 10 times magnification. The distance between the two abutments and the abutments height in the master models were measured and compared with the same dimensions on the stone casts and dimensional changes (µm) between the disinfected and non-disinfected working casts will be compared as seen in Figures 3, 4. Abutment 1 height (H1), abutment 2 height (H2) and inter-abutment distance (IAD) were measured in mm.

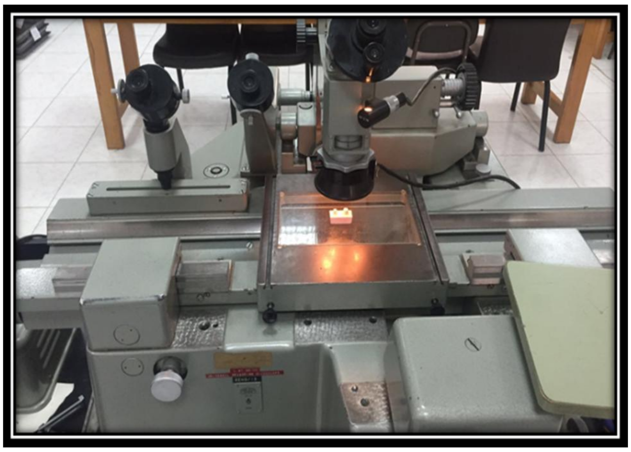

Fig. 3: Model measured by Travelling Microscope.

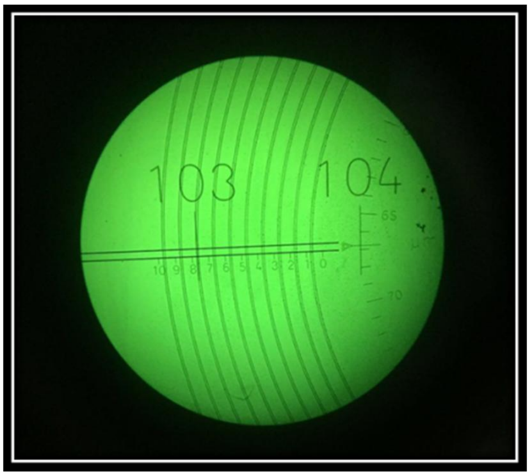

Fig. 4: Scale of measuring the model under Travelling Microscope.

Statistical analysis

Numerical data were offered as mean, standard deviation (SD) values. Data were analyzed for normality using Kolmogorov-Smimov and Shapiro-Wilk tests. Abutment 1 height (H1), abutment 2 height (H2) and inter-abutment distance (IAD) data showed parametric distribution. For parametric data, one-way ANOVA test was utilized to compare between the three groups. Tukey's test was used for pair-wise comparison between the groups when ANOVA test is significant. Unpaired t-test was used to compare between the means and SD of master die and the three groups. For previous tests a probability value (p value) ≤ 0.05 was regarded statistically significant. Statistical analysis was performed using IBM® (IBM Corporation, NY, USA) SPSS® (SPSS, Inc., an IBM Company) statistical version 20 for windows.

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3. Results

The dimensions of each specimens were measured and the results showed that there was statistically significant difference in dimensional changes and % of dimensional changes between the three groups as shown in tables (1, 2 and 3) and figures (5, 6 and 7). The least dimensional % of change was in the delayed autoclave subgroup followed by the immediate poured ozone subgroup and lastly the control group. The highest % of dimensional change was in the immediate autoclave subgroup. From the statistical analysis of the results it is advisable when using VPS impression material the impression should be poured within 30 minutes. If the impressions will be disinfected, in case of using ozone pour the impressions immediately within 30 minutes but in case of autoclave disinfection it is preferred to delay the impression pouring for 24 hours to maintain the dimensional stability of the impression and obtain the most accurate indirect prosthesis.

Table 1: Mean ± standard deviation (SD) values and results of one-way ANOVA test for comparisons between height of abutment 1 (H1) in the 3 groups and subgroups in mm.

Groups / **Subgroups (pouring time)**	**GC**	**GA**	**GB**	***p*-value**
Immediate	7.170 ± 0.03153	7.090 ± 0.02513	7.117 ± 0.01274	*<0.0001**
Delay	7.092 ± 0.06783	7.158 ± 0.009869	7.127 ± 0.02091	*0.0056**

* Significant *at p ≤ 0.05*

Table 2: Mean ± SD values and results of one-way ANOVA test for comparisons between height of abutment 2 (H2) in the 3 groups and subgroups in mm.

Groups / **Subgroups (pouring time)**	**GC**	**GA**	**GO**	***p*-value**
Immediate	7.053±0.01599	7.020±0.02251	7.079±0.06653	0.0078*
Delay	7.008±0.003033	7.009±0,003814	6.997±0.001059	< 0.0001*

Table 3: Mean ± SD values and results of one-way ANOVA test for comparisons between inter-abutment distance in the 3 groups and subgroups in mm.

Groups / **Subgroups (pouring time)**	**GC**	**GA**	**GO**	***p*-value**
Immediate	11.02±0.06885	11.04±0.02268	10.98±0.01228	0.0089*
Delay	11.05±0.04185	11.04±0.005252	11.01±0.01088	0.0060*

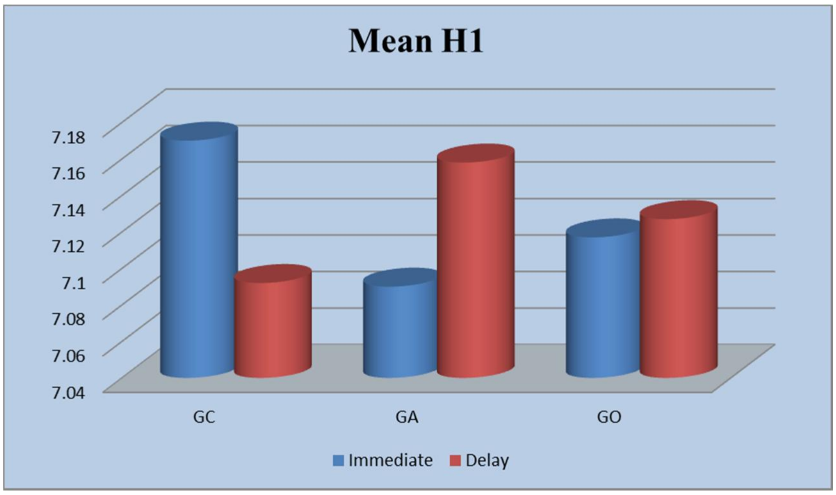

Fig. 5: mean height of abutment 1 (H1) values in mm in each group

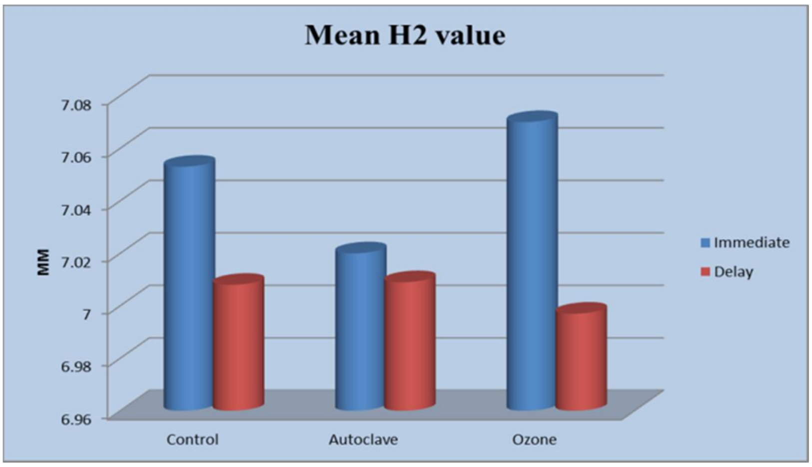

Fig. 6: Mean height of abutment 2 H2 values in mm in each group

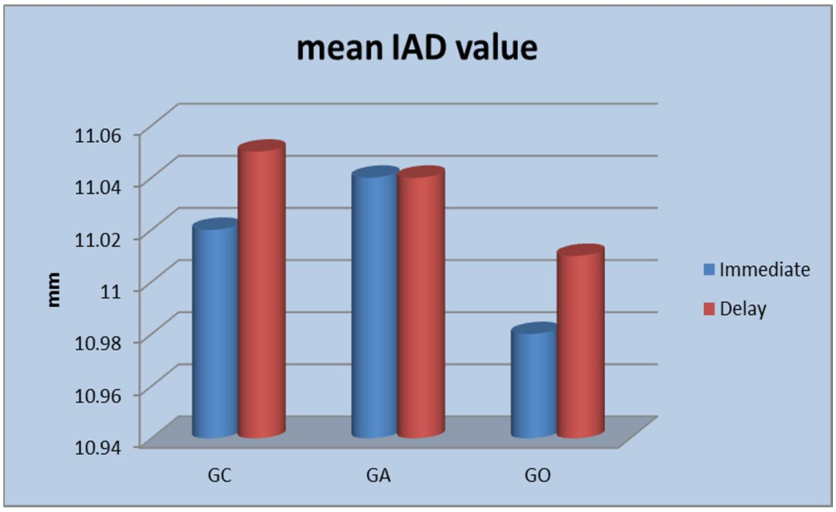

Fig. 7: Mean inter abutment distance (IAD) values in mm in each group

Table 8: Percentage of dimensional change of H1 in the 3 groups

Subgroups (pouring time) / Groups	GC	GA	GB	*p*-value
Immediate	0.41 %	0.70 %	0.32 %	*<0.0001**
Delay	0.68 %	0.25 %	0.18 %	*0.0056**

Table 9: Percentage of dimensional change of H2 in the 3 groups

Subgroups (pouring time) / Groups	GC	GA	GB	*p*-value
Immediate	0.61 %	0.14 %	0.97 %	*0.0078**
Delay	0.029 %	0.014 %	0.18 %	*<0.0001**

Table 10: Percentage of dimensional change of IAD in the 3 groups

Subgroups (pouring time) / Groups	GC	GA	GB	*p*-value
Immediate	0.42 %	0.61 %	0.06 %	*0.0089**
Delay	0.69 %	0.61 %	0.34 %	*0.0060**

4. Discussion

In recent years, the achievement of disinfection protocols for impressions has made substantial difference in dental offices and laboratories (Kambhampati, 2014; Savabi *et al.,* 2018; Mahalakshmi *et al.,* 2019; Sahar AlZain, 2020).

There is still no universally recognized impression disinfection protocol but recent recommendations advocated the use of impression sterilization (Thota, 2014). There is a difference between sterilization and disinfection; sterilization result in demolition of all forms of microbial life (bacteria, viruses and fungi) where disinfection result in destruction of specific pathogenic microorganism (Valente *et al.,* 2012). The present study was done to evaluate the effect of autoclave and ozone disinfection protocols of VPS impression material on its dimensional stability after immediate and delayed pouring with no documented previous studies discussing the same aim.

Disinfection of dental impressions affects the physical properties of the impression and the resultant cast, particularly as regards dimensional accuracy and surface characteristics. Many studies have evaluated the physical properties of impressions and dental models after different disinfection protocols. The results were varied and controversial (Kim and Yousef 2000).

The dimensional accuracy of any material is time dependent and many studies investigated the consequence of storage time on the accuracy of various impression materials and found that impression storage time, type of material and brand used would significantly affect linear dimensional stability of impression elastomers over time (Daou, 2010; Surendra *et al.,* 2011; Khalaf and Mahmood 2013). Stainless steel master model was fabricated in this study following the American Dental Association (ADA) specification no.19 providing a dental replica of two prepared teeth with an edentulous space interposed representing the clinical situation of two abutment teeth to receive fixed partial prosthesis to replace missing lower first molar. The abutments were placed 11mm apart representing the mesio-distal diameter of the first molar (Garrofé *et al.,* 2011).

Custom made metal tray was fabricated for the study to provide a uniform thickness of impression material thus improving the accuracy of the working cast. The tray was constructed from metal which allowed dimensional stability over time with no permanent deformation during the impression making or removal from the dental model (Walker *et al.,* 2010).

The tray fabricated with holes on the upper and lateral faces of the tray for impression retention, creating a way out and reduction of internal pressures of the impression material (Heidari *et al.,* 2013). The tray had a 2 mm space for the impression material to be equally distributed on all the sides of the abutment (Khalaf and Mahmood, 2013). Addition of silicone based impression material having the least amount of shrinkage on setting making them the most accurate class of elastomeric impression materials (Saber 2011). The two step VPS putty wash impression is dimensionally most accurate when the light body material thickness is not more than 2 mm (Amin *et al.,* 2009).

In this study two step putty wash impression technique was utilized as previously mentioned that the two step putty wash impression technique to be dimensionally more accurate, as uniform thickness of light body VPS material is achieved by providing a relief space (Words 2014, Kumari and Nandeeshwar 2015). Since the thickness of light body material in the two step putty wash impression technique is a decisive aspect that influences the dimensional accuracy when using VPS (Amin *et al.,* 2009; Kumari and Nandeeshwar, 2015; Mubashir *et al.,* 2015). Some authors believed that the amount of putty material shrinkage in the final putty-wash impression is lower in the two-step than one-step technique based on the fact that the wash material in the two-step technique is added to already set putty material which has exhibited most of its shrinkage, thus shrinkage of putty material may be compensated by the wash material and may not be included in the total shrinkage of the final impression (Basapogu, 2016).

Different methods for disinfection of impression materials have been suggested, autoclave and ozone are the most effective that's why these two methods were used in the present investigation. An autoclave uses standard settings that can kill most bacteria, spores, viruses and fungi (Sivakumar *et al.,* 2012; Mehta *et al.,* 2014; Ajeti *et al.,* 2018). Previous studies evaluated the effect of autoclave on dimensional stability of elastomeric impression materials and showed that autoclaving of impressions is an effective method of sterilization without any deleterious change in dimensions (Kamran *et al.,* 2015; Mumatz-ul-islam and Khan 2015; Sahar AlZain, 2020) proving that the vacuum cycle or heat does not adversely affect the dimensional accuracy of the cured impression material.

On the other hand ozone disrupts microbial cell walls in seconds, leading to immediate cell lysis. An ozone application of 10–20 second has been reported to eliminate more than 99% of the microorganisms found in the dental caries and associated bio-films and a 40 second treatment time covers all eventualities. It was reported that ozone at low concentration of 0.1 ppm, is sufficient to inactivate bacterial cells including their spores (Bikash *et al.,* 2011; Gopalakrishnan and Parthiban 2012; Daif, 2012). Other studies showed that ozone disinfection provides a quick, proficient, fully automated different method, limiting liquid waste generation (Kazaancioglu *et al.,* 2014; Poulis *et al.,* 2014; Smith *et al.,* 2017; Sharma *et al.,* 2020; Kumar and Chandi, 2021).

Type IV extra hard stone was selected in this study due to its stability and low expansion rate (achieves 0.08% expansion rate) in addition to its high strength and excellent aesthetic superiority from the high surface gloss of the model (Ahila and Subramaniam, 2012). During casting of the impressions water/powder (w/p) ratio was kept constant to control the setting expansion of gypsum; spaces between the nuclei of crystallization, growth interaction of the dehydrate crystals and outward thrust was constant (Chee and Donovan, 1992). Dimensional accuracy was assessed by comparing the measurements of stone casts to those of the master models. Standardization was maintained throughout the impression making till the production of stone casts and during the measuring procedures to reduce the effect of any undesirable variables in the investigation which in line with many studies (Ahila and Subramaniam 2012; Poulis *et al.,* 2014; Derchi *et al.,* 2015; Mahalakshmi *et al.,* 2019). In the present study dimensional stability was measured by the travelling microscope which is very precise for scientific measurements because of its extreme accuracy and ability to measure very small measurements, also it is easily managed and readable. Each measurement was repeated three times to monitor the operator error (Pandey *et al.,* 2014). The results of this study partially support the null hypothesis that there was no statistically significant difference in the dimensional stability of VPS impression material after disinfection with autoclave and ozone in immediate pouring time while there was significant difference between immediate and delayed pouring time in case of autoclave disinfection in both vertical (height) and horizontal (distance) dimensions. The results of the ozone disinfection protocol with immediate pour showed the least dimensional changes in measurements. This may be attributed to the fact that the VPS impression material is considered one of the materials that are resistant to ozone gas like other type of silicon, teflon or glass. Double distilled water was used to avoid any contamination or any confounders that can affect the results. Also ozone provides a quick and efficient disinfection in short time and its gaseous form is safe and passive which does not react with the materials. The only reported reaction was between the ozonated water and (Au-Cu-Ag-Pd) alloy which may be components of the tray used in the study so this may be the cause of changes which occurred with the samples disinfected with ozone and when the impressions were poured after 24 hours.

The results of the autoclave disinfection with immediate pouring showed more dimensional changes, these results were in agreement with previous studies (Kamran *et al.,* 2015; Mumtaz-ul-islam and Khan 2015) and can be attributed to that the VPS impression material needed time after autoclaving to reach room temperature (Chee and Donovan 1992). The results are also to be considered with previous results obtained (Pandey *et al.,* 2014; Azarpazhooh and Limeback, 2008) which stated that the effect of autoclaving on dimensional stability of elastomeric impression material (VPS) revealed that a higher mean dimensional change was obtained immediately after autoclaving when compared to the other 2 time intervals (24, 72 hours) thus it is desirable to delay the casting of an autoclavable elastomeric impression material by about 24 hours (Khalaf and Mahmood 2013).

4. Conclusion

Within the limitations of this in-vitro study, the following could be concluded: VPS impression material is dimensionally stable and capable of producing accurate impressions with minimal distortion with the ability to retain these properties even after the disinfection procedures. Autoclaving is considered to be the most effective method of sterilization with no affect on the impression's dimensional stability with its delay pouring. Ozone is considered a fast, simple and effective method of impression disinfection with no effect on the dimensional stability when the impression was poured immediately within 30 minutes.

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