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To Compare and Evaluate The Efficacy and Effect on Dimensional Accuracy of the Different Disinfection Techniques on Polyvinyl Siloxane Impression Material When Exposed To Commonly Occurring Oral Micro Flora

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Abstract

Aim: The purpose of this in vitro study was to compare and evaluate the efficacy and effect on dimensional accuracy of the different disinfection techniques on polyvinyl siloxane impression material when exposed to commonly occurring oral micro flora.

Materials And Methods: The impressions were divided into five groups (fifteen samples per group) and subjected to a spray disinfection with 2% glutaraldehyde (Group I), UV light (Group II), freshly prepared electrolyzed oxidizing water (EOW) with different pH values - acidic (Group III a), alkali (Group III B) and neutral (Group III C).The samples were examined pre and post-immersion under 2x magnification for surface detail reproduction, stereovision microscope for measurement of dimensional stability and surface

profilometer for evaluation of surface texture. A standardized master die was fabricated and seventy-five PVS test samples were made. The samples were subjected to immersion disinfection and studied for surface detail reproduction, dimensional stability and surface texture. Post hoc test and ANOVA were used to analyze dimensional stability statistically both within and between the test groups.

Results: The surface detail reproduction was satisfactory with both pre and post-immersion test samples. A statistically significant difference was observed on inter group comparison in group II and group III C whereas group III, group III B and groupI showed non significant dimensional changes. There was a negligible change in surface roughness post-immersion in Groups I, III a, III b, III c test samples with a slight increase in surface roughness post-immersion in Group I samples. All disinfectants showed statistically significant values for efficacy with highest 4log10 reduction in gluteraldehyde.

Conclusion: In this study, all the test disinfectants produced satisfactory surface detail reproduction on PVS impressions. Except UV light and electrolysed water neutral which showed statistically significant dimensional changes others disinfectants showed non significant changes on polyvinyl silixone impression material surface. All the samples showed decrease in surface roughness except the surface disinfected with gluteraldehyde which increased the surface roughness values. Statistically significant values of efficacy was observed for all five disinfecting agent i.e gluteraldehyde, UV light and electrolysed water acidic, electrolysed water basic, electrolysed water neutral. Gluteraldehyde showed highest efficacy and electrolysed water neutral showed lowest amount on comparison within these 5 disinfecting agent.

Keywords: disinfection, polyvinyl siloxane, gluteraaldehyde, UV light, Electrolysed water.

Introduction

Oral cavity is one of the most common source for infectious microorganism that could be transferred or cause infection in another person. Failure to adequately clean, disinfect or sterilize dental instruments contaminated with pathogenic microorganisms from previous patients can endanger subsequent patients[1,2]. This route of pathogenic microorganisms transfer is known as cross-contamination and the resulting infection is referred to as cross-infection. The highest potential for cross-infection is between dentists, surgery assistants, and patients because blood, saliva, and other infected soft tissue present on contaminated instruments can cause cross contamination. Some of these microorganisms can survive outside the oral cavity and when not in contact with oral fluids for a long period and can transfer onto the dental models further exposing dental personnel[3]. Studies have reported that both oral and non-oral pathogens associated with local and systemic diseases are present on both contaminated prosthesis and dental laboratory equipments[4-6]. The most prevalent contaminants were found to be bacteria such as Bacillus species, Streptococcus species, Micrococcus species, Coagulase - Negative Staphylococci, Candida species, E. coli, Klebsiella, Pseudomonas, and Acinetobacter.

Disinfection means eliminating infecting microorganisms excluding the spores on non-viable medical equipment. The method that is mostly used is employing chemical substances generally categorized as disinfectants. When selecting the type of disinfectants to be used at hospitals, criteria such as the effect spectrum of the disinfectants, the convenience of the disinfectants concerning the area of use, the period required to see the desired effect, any damages to occur potentially on any atmosphere or

equipment and the cost of the relevant disinfectants are taken into account[7]. Guidelines set by the ADA and CDC suggest that all surfaces that have been splashed or touched by human body fluids be disinfected with a hospital graded disinfectant registered with the Environment protection agency. Dental impression is one the most common source of cross infection between dentist and dental assistants and other dental auxiliary staff. To address cross-contamination concerns, ADA has issued guidelines for disinfecting impressions using spray or immersion disinfectants. Three important factors that must be considered when dental impressions are disinfected were that how are impression materials and resultant cast affected, stability of disinfection solutions and effectiveness of disinfection procedures.

But very few studies are conducted which reveal answers to all these aspects together[8]. Polyvinyl siloxane impression materials have been widely used in a variety of indirect procedures in prostheses. Favorable handling properties, good patient acceptance, and excellent physical properties make them materials of choice in today's practice[9,10]. Several studies have reported that the disinfection of elastomeric impression by immersion demonstrates no detrimental or clinically significant result regarding the accuracy of the impression or subsequent stone casts. The surface of elastomeric impressions routinely made in restorative dentistry come in contact with saliva and blood allowing the oral micro-flora like bacteria and viruses to transfer on stone casts. Therefore, the use of hospital-grade disinfectants intended to be applied by spray atomization makes it possible to disinfect elastomeric impressions quickly without effecting the accuracy of impression[3,12].

With the evolution of newer and more potentially infectious viruses and bacteria, research is going on for a sterilization technique that is easy to use and effective against disease control without affecting the accuracy of the impressions.

Materials and Method

The proposed in vitro was conducted in the department of Prosthodontics and Crown & Bridge, Bhojia Dental College And Hospital (Bhud), Baddi, with the assistance from Department of Microbiology, Bhojia Dental College And Hospital (Bhud), Baddi and TBRL, DRDO, Ramgarh, distt. Panchkula, Haryana. The purpose of this study was to compare and evaluate the efficacy and effect on dimensional accuracy of different disinfection techniques on polyvinyl siloxane impression material when exposed to commonly occurring oral microflora. The study was approved by the Institutional Ethical Committee of Bhojia Dental College & Hospital (Bhud) Baddi, District Solan (H.P.) BDCH/BHUD/3085.

Fabrication of Master Die

A standardized stainless steel master die was fabricated according to the ADA Specification No. 19 [Figure 1]. The die consists of three components: Ruled block, mold and metal riser. Circular Test Block consisted of 29.97mm of inner diameter consisting of three horizontal lines A, B, and C of 50 ± 8µm, 20 ± 4µm and 75 ± 8µm respectively and two vertical lines DE and D'E' of 75 ± 8µm. all lines have 90˚ included angles. The point of intersection of the vertical lines on line A was named X and X', on line B was named Y and Y' and on line C was named Z and Z'. Impression Material Mold was a hollow metallic ring of an inner diameter of 30 mm was fabricated which served as a tray for holding the impression material while making specimens. A circular riser was fabricated which acts as a plunger for retrieval of set impression material from the metal ring.

Preparations of Specimens

Specimens of polyvinyl siloxane (Flexceed GC India Dental Pvt. Ltd) were prepared by using the metallic die.

Before preparing each specimen, the impression surface of the ruled block was cleaned with a piece of cotton soaked in spirit for the removal of residue. Vaseline was applied to the mold for lubrication. After lubrication impression material mould (metallic ring) was placed on top of the ruled block. For making the impression with Polyvinyl siloxane, a single-stage technique was used. Nitrile gloves were used while mixing. For mixing the putty, an equal amount of base and catalyst were taken by measuring with scoops. One scoop of base and one scoop of catalyst were taken and mixed for 20 seconds by kneading the material with fingers until a uniform colour was obtained. For mixing the light-body automixing tip and dispensing gun was used. Light-body material was then expelled through a mixing tip with the help of a gun. Light-body material was directly spread on the ruled surface of the die. Precautions were taken not to incorporate air bubbles. The mixed putty material was loaded in the metallic ring fitted over the test block. After the impression material mold was filled with material, it was covered with a cellophane sheet and a glass plate was placed on top of it. A cellophane sheet was placed so that the material doesn't stick to the glass slab. Hand pressure was applied for 10 seconds initially to the glass plate to expel the excess material, followed by 1000gms weight on top of the glass plate. The assembly remained in place for 3 minutes for the setting of Polyvinyl siloxane impression material and extra 3 minutes' time was considered to ensure the complete setting of the material. After that impression material mould was separated from the die. The specimen was recovered from the impression material mould. Excess material was trimmed using bard parker knife no. 23 and all the specimens were evaluated for any gross discrepancy.

Grouping of the Study Samples

The 75 test samples were grouped randomly into five groups of 15 samples each, out of which 15 samples per test group were employed for evaluating the surface detail reproduction and dimensional stability. Five representative samples per test group were employed for evaluating the surface detail reproduction.

Group I samples to be disinfected with Glutaraldehyde, Group II samples to be disinfected in a UV chamber, Group IIIa samples to be disinfected with freshly prepared Electrolysed Water Acidic, Group IIIb samples to be disinfected with freshly prepared Electrolysed Water Basic and Group IIIc samples to be disinfected with freshly prepared Electrolysed Water Neutral .

Pre-Operative Recordings

All the samples were checked pre-operative for their dimensional stability, surface roughness and surface detail reproduction. Dimensions of all specimens were evaluated before immersion disinfection of all the groups. For obtaining the dimensions, the linear distance between the X-Y; Y-Z; X'-Y'; Y'-Z'; X- X' and Y-Y' was measured at the point of intersection of horizontal lines X, Y and Z with the two vertical lines i.e. CD and C'D'. The dimensions observed on the specimen were measured by using a Stereovision microscope with 20x magnification. The surface roughness of each test sample was measured at three points (P, Q, R). of these, the first points were at a distance of 5mm from line C-D and further points were located 5mm ahead respectively using Zeist surf tester. The surface detail reproduction of each test sample was evaluated immediately after removal from the die. The continuity of the appropriate horizontal cross line XX'; YY'; ZZ' reproduced on the test sample surface was evaluated under low-angle illumination at 5X magnification according to ADA Specification No. 19.

Values obtained before disinfection served as control. Glutaraldehyde used in the present study was commercially obtained. UV light chamber was used according to manufacturer instruction. EOW was freshly prepared using table salt and tap water according to the manufacturer's instructions.

Microbiological Study

Inoculum Preparation

The stock bacterial culture was received in Bhojia Dental College & Hospital, the streaking of the bacteria was done on freshly prepared nutrient agar plate from the frozen stock, and incubated for 24 hrs. after that 1 bacterial colony from each bacterial culture was picked and innoculated into the nutrient broth which was aerobically incubated for 24 hrs in a shaker incubator at 250rpm and 37^0 C. Using plain nutrient broth as a control in spectrophotometer, nutrient broth inoculated with bacterial culture was tested to match the turbidity of 0.5 McFarland standard dilution value. Serial dilutions were done and the final 600 OD = 1×10^6 cells/ml value corresponded to 1.0 x 10 cells/100 µl for each bacterium. From where 3ml pool of was made. O.d value was again checked at 600 nm.

Specimen contamination

The prepared PVS samples were then coated with artificial saliva for 20 minutes and dried further for 20 minutes to mimic the oral cavity. These samples were then coated with the cocktail of bacteria and then dried for 30 min so that bacteria may adhere to the sample. After that, the sample was plated for control. Again the samples were disinfected using 10 puffs of disinfectant or UV light and were kept for 30 minutes in a sterile perti – dish, washed with distilled water for 3sec., vortexed for 2 minutes and post plating was done. At the end of 24hrs, plates that exhibited no growth were subjected to further incubation for 24 hrs and at the end of 48 hrs, the plates were removed from the incubator. After incubation, all the plates were examined for growth and colonies were counted using a colony counter.

Post Disinfection

After all the samples have been evaluated for efficacy, their dimensional records, surface roughness and surface detail reproduction were taken at same points as in pre disinfection and the data collected was recorded as post-disinfection measurements.

Data Tabulation Analysis

The data obtained from the pre and post-immersion evaluation of all the test samples of all the test groups for surface detail reproduction, dimensional stability and surface texture were tabulated. Surface detail reproduction was assessed descriptively based on the scores obtained. Dimensional stability was analyzed statistically both within and between the test groups using post hoc test, paired t-test and ANOVA. All statistical analysis was performed using SPSS Statistics for Windows.

Results

Dimensional Stability

On comparison lines ABC the mean % change observed in gluteraldehyde, UV light , Electroysed water acidic, electrolysed water basic, electrolysed water neutral was +0.75, , -1.24, -6.29, -2.08, -4.25 respectively. Whereas mean % change observed on lines AC in gluteraldehyde, UV light , Electroysed water acidic, electrolysed water basic, electrolysed water neutral was +0.07, -0.27,+0.15, -0.30 respectively. The samples disinfected with Gluteraldehyde showed shrinkage and others shows slight expansion. Except UV light and electrolysed water which showed statistically significant results others disinfectants showed non significant changes on polyvinyl silixone impression material surface.

Surface Roughness

On comparison it was seen that all the samples showed decrease in surface roughness except the surface disinfected with 2% gluteraldehyde which showed slight increase in surface roughness with pre and post disinfection values of 1.2060 µ m and 1.4139µm respectively which was non significant with p=.098. A statically non significant difference was found on inter and intra group comparison which was in accord with ADA specification 19.

Surface Detail Reproduction

A statistically non significant results were found as there were no discripencies found on pre and post immersion samples which was in accord with ADA specification 19.

Discussion

Disinfection entails the elimination of pathogenic bacteria, based on several variables including the length of exposure to the disinfectant solution, its concentration, etc. However, an undesirable side-effect of the disinfection process is the potential for a change in the dimensions of the impression, or surface texture that may be associated with a chemical or physicochemical interaction between the set material and the disinfecting solution. The change of dimension of impression materials following the setting reaction or the immersion in disinfectant solutions has been the subject of several studies[13].

At least 67% of dental materials received by dental laboratories, including dental impressions, were indicated to be contaminated by various microorganisms. The most common microbes identified on the impressions are Streptococcus species, Staphylococcus species, Escherichia coli species, Actinomyces species, Antitratus species, Pseudomonas species, Enterobacter species, Klebsiella pneumonia, and Candida species therefore in this study, the bacteria Staphylococcus aureus, klebsiella pneumonia, and E. coli. were chosen. Staphylococcus aureus is a typical gram-positive bacteria with a thick outer cell wall gram-negative infections that become systemic are of particular concern because they possess lipopolysaccharides (endotoxin), which may initiate cascades of harmful cytokines such as tumor necrosis factor. The chemotherapy treatment of these microorganisms has been further complicated in recent years by the well-documented overall increase in antimicrobial resistance. Klebsiella pneumonia and E. coli are typical gram-negative bacteria, K. pneumonia causes severe bronchopneumonia; stomatitis[14]. Szymańska studied groups of microorganisms, such as prions, viruses, bacteria, fungi, and protozoa, to which a dentist is, or may be exposed. He found that the carriership rate for S. pneumonia is 20-32%, and it is 30% for S. aureus in nasopharanx[15].

In our present study, the changes in the vertical (ABC) and horizontal line(AC) observed in the study impressions might be explained based on the pattern of polymerization shrinkage of the impression material described by Mehta R, Wadhwa S, Duggal N et al, according to which the impression material tends to shrink towards the center of the mass or towards the greatest bulk of the material. Polymerization shrinkage occurred towards the restrained surface (tray) and away from the unrestrained (tooth) surface[16].

Lepe et al. determined the disinfection capability of 2% glutaraldehyde on the dimensional stability of elastomeric impression materials and found that the accuracy of the impression was extremely affected. It shows that chemical disinfectants induce surface roughness and alter the dimensions of the impression material[17] which was in line with our present study where glutaraldehyde showed an increase in surface roughness and contraction of the specimen which was clinically

insignificant as they were < 0.5% as suggested by ADA specification 19.

Studies evaluating the dimensional stability of PVS impressions post-immersion in different pH values of EOW are lacking. A recent study by Mahalakshmi et al evaluated the effect of chemical disinfectants on the surface detail reproduction, dimensional stability, and surface texture of polyvinyl siloxane (PVS) impressions. It was concluded that all the test disinfectants produced satisfactory surface detail reproduction on Polyvinyl siloxane impressions. 2% glutaraldehyde and electrolyzed oxidizing water (alkali) have resulted in statistically insignificant dimensional changes, while 1% sodium hypochlorite, electrolyzed oxidizing water (acidic), and electrolyzed oxidizing water (neutral) have resulted in statistically significant dimensional changes. All the test disinfectants except 1% sodium hypochlorite showed a reduction in surface roughness (Ra) values which were in line with the present study except that the glutaraldehyde showed an increase in surface roughness values rather than a decrease[18].

Wu et al. studied the dimensional stability of irreversible hydrocolloids by immersion in 1% SH and freshly prepared EOW (acidic) and concluded that immersion in 1% SH and EOW (acidic) caused significant dimensional changes and ultrasonically nebulized EOW (acidic) showed 4log10 reduction after 30-45 mins whereas 3 log10 reduction was found in our present study after spraying PVS impression with electrolyzed water acidic[19].

Yuki Nagamatsu et al in their study examined the bactericidal effects of the neutral electrolyzed water on disinfection of the alginate impression of a dental arch model contaminated by bacteria. The results showed that only 1-min immersion in neutral electrolyzed water could sufficiently disinfect the alginate impression including the metallic tray under ultrasonic with no significant differences from acid electrolyzed water. He concluded that neutral electrolyzed water may be the most appropriate for the disinfection of alginate impression[81]. Although the efficacy of neutral electrolyzed water used in the present study showed $3\log_{10}$ reduction it cannot be considered the best disinfecting agent amongst the other disinfectants used in this study.

K R Jnanadev et al in a study compared the efficacy of 2% glutaraldehyde spraying with Electrolysed water acidic on an acrylic resin plate and concluded that the disinfection potential of 2% glutaraldehyde was better than EAW when the specimens were disinfected for 1 and 3 mins. On the contrary, when the disinfection time was increased to 5 mins there was no difference between EAW and 2% glutaraldehyde[20].

Vidhya Jeyapalan et al comparatively evaluated the antimicrobial efficacy of freshly prepared electrolyzed oxidizing water (EOW) with that of 2.4% glutaraldehyde (GA) and 1% sodium hypochlorite (SH) on clinically derived polyvinyl siloxane (PVS) impressions. The result showed that Streptococci, Staphylococci, Pseudomonas, Candida, Proteus, Klebsiella, and E. coli were isolated from all impressions including the controls, except those disinfected by EOW. All three disinfectants showed significant reduction in CFU and log10 reduction values as compared to the controls. EOW showed a significantly higher reduction in log10 values compared to GA and SH, whereas GA and SH showed similar reductions. EOW, GA, and SH showed kill rates of 100%, 99.60%, and 99.82%, respectively[21]. Hence it was concluded from the study that EOW shows high antimicrobial efficacy when used as an immersion disinfectant as compared to GA and SH for clinically derived PVS impressions.

The main purpose of the study was to evaluate the most effective disinfectant, less time-consuming, least

technique-sensitive disinfectant, easily available, and does not cause any dimensional changes in the impression material.

There were some limitations to this study as no viral or fungal species were tested in this study. Only one impression material was used in the present study and the study was done in vitro, the results may vary when in vivo studies may be performed.

Conclusion

In this study, all the test disinfectants produced satisfactory surface detail reproduction on PVS impressions. Except UV light and electrolysed water neutral which showed statistically significant dimensional changes others disinfectants showed non significant changes on polyvinyl silixone impression material surface. All the samples showed decrease in surface roughness except the surface disinfected with gluteraldehyde which increased the surface roughness values. Statistically significant values of efficacy was observed for all five disinfecting agent i.e gluteraldehyde, UV light and electrolysed water acidic, electrolysed water basic, electrolysed water neutral. Gluteraldehyde showed highest efficacy and electrolysed water neutral showed lowest amount on comparison within these 5 disinfecting agent.

Legend Tables

Table 1: Intra Group Percentage Change Pre and Post Values of Linear Distance

Group	ABC Pre		ABC Post		P-value	Percentage change
	Mean	S.D.	Mean	S.D.		
Uv Light	2.70	.18	2.73	.17	<0.001	-1.24
Electrolysed Water Acidic	2.87	.23	3.05	.26	0.005	-6.29
Electrolysed Water Basic	2.60	.14	2.65	.13	<0.001	-2.08
Electrolysed Water Neutral	2.91	.15	3.03	.18	<0.001	-4.25
Glutaraldehyde	2.60	.08	2.58	.08	0.014	+0.75

Table 2: Intra Group Percentage Change Pre and Post Values of Linear Distance

	AC Pre		AC Post		P-value	Percentage change
	Mean	S.D.	Mean	S.D.		
Uv light	20.00	.18	20.05	.14	0.013	-.27
Electrolysed water acidic	20.47	.42	20.57	.43	0.283	-.54
Electrolysed water basic	20.07	.21	20.03	.04	0.604	.15
Electrolysed water neutral	20.63	.24	20.69	.23	<0.001	-.30
Glutaraldehyde	20.04	.20	20.03	.20	0.205	.07

Table 3: Intergroup Comparison of Surface Roughness

Group		N	Mean	Std. Deviation	F	P-value	Sig
Pre	Glutaraldehyde	15	1.2060	.46678	2.165	.082	NS
	UV Light	15	1.3316	.42613			
	Electrolysed Water Acidic	15	1.4900	.31778			

	Electrolysed Water Basic	15	1.5513	.30917			
	Electrolysed Water Neutral	15	1.2007	.54680			
	Total	75	1.3559	.43621			
Post	Glutaraldehyde	15	1.4139	.72179	2.439	.055	NS
	UV Light	15	1.2521	.40819			
	Electrolysed Water Acidic	15	1.3347	.38352			
	Electrolysed Water Basic	15	1.4287	.44614			
	Electrolysed Water Neutral	15	.9193	.54708			
	Total	75	1.2697	.53604			

Table 3 shows no statistically significant difference was observed among different groups in pre values and post values. (P-values>0.05).

Table 4: Intragroup Comparisons of Surface Roughness

Group	Comparison	Mean diff	S.D.	t-value	P-value	Sig
Glutaraldehyde	Pre-Post	-.20793	.45377	-1.775	.098	NS
UV light	Pre-Post	.07947	.18953	1.624	.127	NS
Electrolyzed Water Acidic	Pre-Post	.15533	.34920	1.723	.107	NS
Electrolyzed Water Basic	Pre-Post	.12267	.42831	1.109	.286	NS
Electrolyzed Water Neutral	Pre-Post	.28140	.19732	5.523	.000	S

Table 5: Surface Detail Reproduction of Polyvinyl Siloxane Pre and Post Disinfection

	Gluteraldehyde		UV Light		Electrolysed Water (Acidic)		Electrolysed Water (Basic)		Electrolysed Water (Neutral)	
SR.NO.	Pre-Sample	Post Sample	Pre-Sample	Post Sample	Pre-Sample	Post Sample	Pre-Sample	Post Sample	Pre-Sample	Post Sample
1	S	S	S	S	S	S	S	S	S	S
2	S	S	S	S	S	S	S	S	S	S
3	S	S	S	S	S	S	S	S	S	S
4	S	S	S	S	S	S	S	S	S	S
5	S	S	S	S	S	S	S	S	S	S
6	S	S	S	S	S	S	S	S	S	S
7	S	S	S	S	S	S	S	S	S	S
8	S	S	S	S	S	S	S	S	S	S
9	S	S	S	S	S	S	S	S	S	S
10	S	S	S	S	S	S	S	S	S	S
11	S	S	S	S	S	S	S	S	S	S
12	S	S	S	S	S	S	S	S	S	S

13	S	S	S	S	S	S	S	S	S	S
14	S	S	S	S	S	S	S	S	S	S
15	S	S	S	S	S	S	S	S	S	S

Table 6: Intergroup Comparison of Percentage Microbial Reduction

Group	N	Mean	Std. Deviation	F	P-value	Sig
glutaraldehyde	15	99.99840	.000507	3.050	.022	S
uv light	15	99.98900	.023955			
electrolysed water acidic	15	99.98893	.001486			
electrolysed water basic	15	99.98713	.002264			
electrolysed water neutral	15	99.98627	.001280			
Total	75	99.98995	.011381			

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