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Efficacy of microwave disinfection and its effect on dimensional stability of polyether impression material

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Abstract
Statement of Problem: Immersion in chemical disinfectant solutions have been recommended for impression disinfection. However, a recent approach involving microwave radiation disinfection of impression material has been suggested by some authors.
Purpose: The purpose of this study was to evaluate the efficacy of microwave disinfection and its effect on dimensional stability of polyether impression material.
Materials and Method: Polyether impression material was selected for study. Efficacy of microwave irradiation for 7 minutes at 720 watts and 2% glutaraldehyde for 30 minutes as disinfection procedures was evaluated against test organisms Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans. Dimensional changes due to microwave and 2% glutaraldehyde disinfection were evaluated according to American Dental Association Specification no. 19. Data were analyzed by performing t test.
Result: Microwave irradiation and 2% glutaraldehyde were effective in reducing the number of colony forming units (CFU) per unit volume (mL) for all the tested microorganisms compared with the control. Dimensional changes due to microwave and glutaraldehyde disinfection were 0.299% and 0.072% respectively.
Conclusion: Both disinfectant methods i.e. microwave irradiation and 2% glutaraldehyde solution were effective against microorganisms tested when compared to control. Dimensional changes seen in microwave radiation and 2% glutaraldehyde disinfection were well within the maximum dimensional change permitted according to ADA specification number 19 (0.5%).

Keywords: polyether, microwave, 2% glutaraldehyde, disinfection, dimensional stability

1. Introduction

A growing concern regarding the control of cross-infection in dentistry can be seen in the literature [1]. Dental professionals are normally exposed either directly or indirectly to a wide variety of microorganisms during routine practice [2]. One of the major concern for a Prosthodontist is that dental impressions along with trays are often contaminated by exposure to saliva, blood, or both with the chance of infection from communicable diseases, such as acquired immune deficiency syndrome (AIDS) and hepatitis B. To address these cross-contamination concerns, the American Dental Association (ADA) issued guidelines for disinfecting impressions. These guidelines recommend using an ADA-accepted spray or immersion disinfectant, depending on the material, for the duration suggested by the product manufacturer [1]. Four chief categories of disinfectants are accepted by the Council on Dental Therapeutics i.e. chlorine solutions, formaldehyde, glutaraldehyde and iodophors. Among these glutaraldehyde is the material of choice [3].

In Fixed Prosthodontics elastomeric impressions materials are widely used because of their various advantages. Among these materials polyvinyl siloxane and polyether are the most popular material. They generally contact saliva and blood, allowing transfer of microorganisms to the stone cast [4]. Many authors have reported the possible ways of disinfecting impressions, particularly rubber materials, as well as about their effect on precision and stability [5]. Current practice regarding handling and disinfection of dental impressions before sending them to laboratory varies from washing the impressions in water to immersing it in a 2 % glutaraldehyde for up to 12 hours for sterilization and 30 minutes for disinfection [6].

Polyether is hydrophilic in nature. The disinfecting process should be adequate, but should not adversely affect the dimensional stability or the surface detail of the impression. Some studies have shown that the immersion disinfectant has no clinically relevant effect on polyethers; however, other studies have indicated that the dimensional stability of these hydrophilic materials was adversely affected by immersion. Merchant also warned that polyether should be disinfected for short periods with the disinfectants. ADA in its guidelines recommends immersion not exceeding 30 minutes for polyether impression materials [1].

Recently, the use of domestic microwave oven, to disinfect complete dentures, nitrous oxide nasal hoods, contact lenses, dental casts, hard chairside reline resins, for resilient liner are proved to causes total sterilization. Microwave ovens are simple to use, low in cost, and can provide disinfection effect. An important concept in the microwave process is that microwave heating is energy conversion and not heating as in a conventional

oven. Microwave absorbent material exposed to a microwave field converts this energy into heat within itself [6].
Literature reveals that polyether impression material is a hydrophilic material. Various studies have been reported on draw backs of chemical disinfection and its effect on dimensional stability of hydrophilic impression materials. Studies are being conducted worldwide to promote microwave irradiation as an alternative to chemical immersion.

2. Materials and Method

Methodology were divided into two parts:

2.1 Determining the efficacy of disinfection

Preparation of thermoplastic mold and test specimens: Thermoplastic sheets (Avec S) of 2 mm thickness were used. The sheets were punched with 5 mm leather belt hole punched tool. The Polyether (Soft Monophase, 3M ESPE, Germany) cartridges were loaded into the Pentamix Dynamic Mechanical mixing Unit (3M ESPE, Germany). The punched sheet was placed over the glass slab. Impression materials loaded in the holes in the sheet and another glass slab was kept on top until the material set. (Figure 1)
A total of 180 specimens were fabricated and distributed into 3 groups with 60 specimens each as follows:

- **Group A**: Microwave disinfection
- **Group B:** 2% Glutaraldehyde Disinfection
- **Group C**: Control

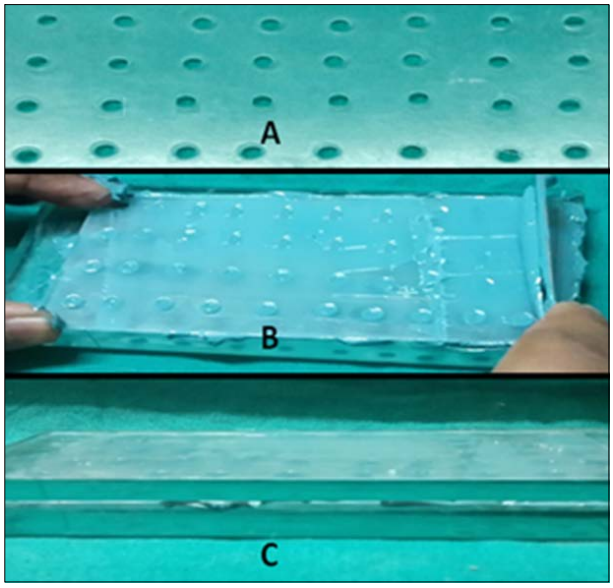

Fig 1: Specimen fabrication for efficacy of disinfection. **a)** Thermoforming mold with punched holes. **b)** Loading of impression material. **c)** Loaded thermoforming sheet kept in between two glass slabs

Preparation of microorganisms: Organisms used in this study were American type culture collection strains of S.aureus (ATCC Strain No. 25923) (KWIK STIK, Microbiologics Inc.), P. aeruginosa (ATCC Strain No. 27853) (KWIK STIK, Microbiologics Inc.), C. albicans (ATCC Strain No. 10231) (KWIK STIK, Microbiologics Inc.) and E.coli (ATCC Strain No. 25922) (KWIK STIK, Microbiologics Inc.). Standard cultures of bacteria i.e. S. aureus, P. aeruginosa and E.coli were individually inoculated in brain–heart infusion broth (HiMedia Lab). Standard cultures of C. albicans were inoculated in Sabouraud's broth (HiMedia Lab). The turbidity of the broths were adjusted corresponding to 10^6 organism/ml in 10 ml of broth. (Figure 2)

Fig 2: A) Microorganisms and **B)** Culture medias

Inoculation of the microorganisms over the test specimens and incubation: All the specimens prepared from Polyether impression material were autoclaved. Sterile forceps was used to immerse the test specimens into test tubes. Out of 60 specimens of each group 15 were inoculated with each type of microorganisms used. One specimen was immersed in one test tube with 1ml of appropriate broth and inoculums and incubated at 37 ºC for 24 hours for bacteria and 48 hours for fungus.

Disinfection of test specimens: After completion of incubation period the specimens were cleaned with distilled water.(Figure 3)The specimens were then subjected to disinfection cycles as follows:

- **Group A:** All test specimens of each subgroup of Group A were added in separate beakers with distill water (London workshop) and exposed for 7 mins at 720 watts microwave radiation in a household microwave (Videocon (2450 MHz), India).
- **Group B:** All test specimens of each subgroup of Group B were added in separate beakers and were immersed in 2% glutaraldehyde solution (Gigasept, Schulke) for 30 mins.
- **Group C:** All test specimens of each subgroup of Group C were left untreated

Fig 3: Disinfection of test specimens

Incubation of test specimens in selective media: After disinfection cycle each specimens of each subgroup was added to test tubes with 1 ml of respective broth and kept for 30 mins. Petri Dishes were prepared containing culture media. Each Petri Dishes was divided into 6-7 sections and one specimen was transferred to one section of Petri Dishes and labeled properly. All Petri Dishes inoculated and incubated. (Figure 4)

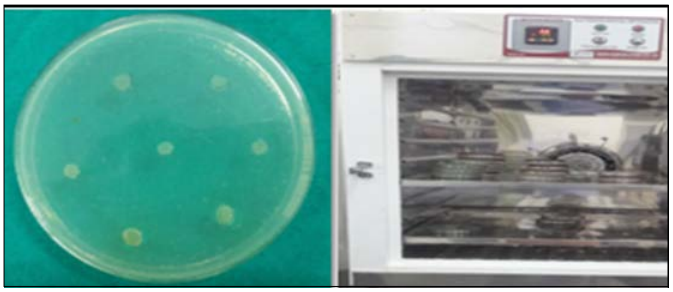

Fig 4: Incubation of test specimens

Determining the efficacy of disinfection by colony count: After incubation colonies on each labeled section of the plates were counted by using a magnifying lens. Each count was multiplied with the dilution factor (10^6) and expressed as CFU/ml.

2.2 Measurement of dimensional stability

Preparation of metal mold: A stainless steel die was prepared according to ADA specification No. 19 for measuring the dimensional stability. The die consisted of a ruled block, mold and riser. Ruled block had height of 31 mm. The diameter of inner ring was 29.97 mm and that of outer ring was 38 mm. Three vertical lines were made of the ruled block labeled X, Y and Z with the lines widths 50 µm, 20 µm and 70 µm respectively. The distance between the two consecutive vertical lines was 2.5 mm. Two horizontal lines were scored intersecting the vertical lines on either side. The distance between two horizontal lines was 25 mm. The intersection of the horizontal with the vertical line X was marked as C and C' and with vertical line Z was marked as D and D'. (Figure 5)

Fig 5: Metal mold. **a)** Ruled block. **b)** Impression material mold. **c)** Riser

Fabrication of test specimens: The test material was polyether impression material medium body in the form of pre-packed cartridges consisting of a base and catalyst. This was to be used with Pentamix Dynamic Mechanical mixing Unit. A new mixing tip was used for every mix. Mold was placed on the beveled edges on the ruled block to contain the material and ensure a consistent thickness of 3 mm. The cartridges were bled in compliance with manufacturer's recommendation to ensure proper dispensing ratio in controlled temperature (22 ± 1^0 C) and humidity ± 5% conditions. The material was loaded with a fine tipped nozzle and applied to the lined areas of the dies. A glass plate was placed on the top of the mold to contain the material and weight of 500 grams was kept on the glass plate to standardize the pressure on the impression material. (Figure 6) Impression material was allowed to polymerize for 3 min more than the time recommended by the manufacturer as indicated in ADA specification no. 19 for laboratory testing. The impression was separated from the die using riser. Following removal of the impression, the die was cleaned using fresh cotton roll moistened with isopropyl alcohol. Excess material was removed with a bard parker blade no. 15 and specimens were stored at room temperature. 20 specimens were prepared and divided into two groups; Group A and Group B.

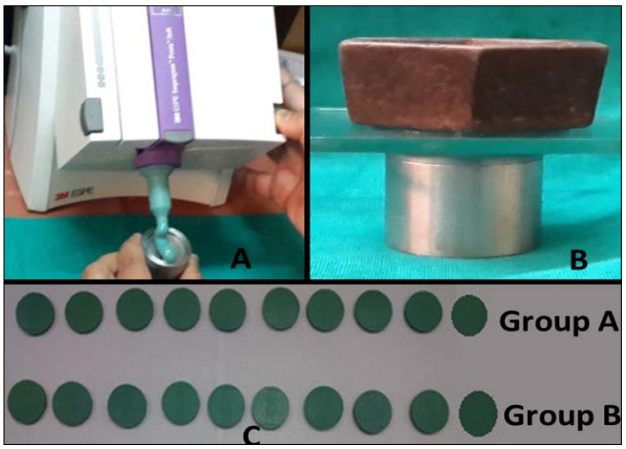

Fig 6: Fabrication of test specimens for dimensional changes. **a)** Loading of impression material on metal mold. **b)** 500gms load kept over the glass plate. **c)** Specimens of polyether impression material used to measure dimensional change

Initial measurement of test specimens: Digital picture of all the specimens were obtained by using a scanner. The resolution 600 dpi were used. Images were analyzed by using Autocad software. Distances between the cross lines (CD and C'D') were measured with accuracy of 0.01 mm. This gave the Reading A. (Figure 7)

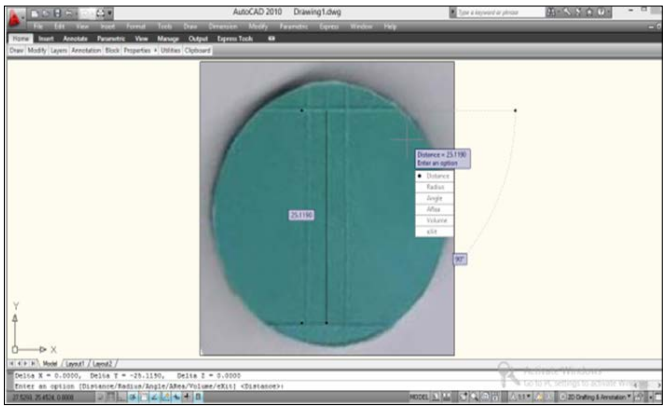

Fig 7: Measurement of test specimens

Disinfection of specimens

Group A: Test specimens were added in beakers with distill water and exposed for 7 mins at 720 watts in a household microwave. *Group B:* Test specimens were immersed in 2% glutaraldehyde solution for 30 mins. (Figure 8)

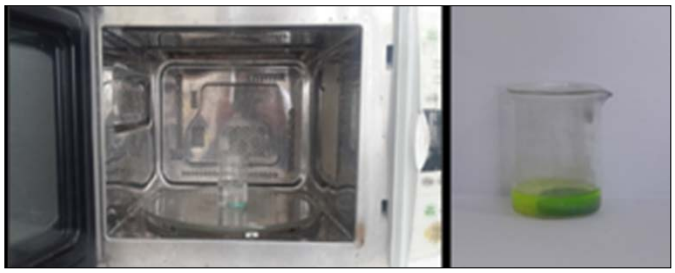

Fig 8: Disinfection of test specimens. **a)** Microwave irradiation. **b)** 2% glutaraldehyde disinfection

Final measurement and calculation of dimensional change: After disinfection digital picture of all the specimens were obtained similar to initial measurement. Distances between the cross lines CD and C'D' were measured with accuracy of 0.01 mm. This gave the Reading B. Then the change in dimension in mm and percentage were calculated as follows:

Dimensional Change (in mm) = A – B

$$\text{Dimensional Change (\%)} = \frac{(A - B) \times 100}{A}$$

Where,

A = distance between crosslines CD and C'D' before disinfection

B = distance between crosslines CD and C'D' after disinfection

3. Results

Table 1 shows mean viable colony count of four microorganisms on Polyether impression material in control group and after disinfection with microwave and 2% glutaraldehyde. Table 2 shows dimensional change (in mm and percentage) of Polyether impression material in Group A and Group B specimens. The data shows that there was shrinkage of the test specimen in Group A due to microwave disinfection. In Group B there was an expansion of dimension due to glutaraldehyde disinfection. The dimensional change in percentage for Group A was 0.299% and Group B was 0.072%. Table 3 shows the descriptive statistics and paired t test for dimensional change of Polyether impression material specimens of Group A and B. On subjecting the values of dimensional changes of test specimens to paired t test, the value of p for dimensional change was 0.126 indicating that the difference was statistically non-significant. (p>0.05)

Table 1

Group	Mean colony forming units per milliliter for each microorganisms.			
	Subgroup 1 S. aureus	**Subgroup 2 E.coli**	**Subgroup 3 P.aeruginosa**	**Subgroup 4 C.albicans**
Group A (Microwave disinfection)	0 (No growth)	0 (No growth)	0 (No growth)	0 (No growth)
Group B (Glutaraldehyde disinfection)	0 (No growth)	0 (No growth)	0 (No growth)	0 (No growth)
Group C	$101x10^6$	84×10^6	79.3×10^6	80×10^6
(Control)	$\pm 85.6x10^6$	$\pm 38 \times 10^6$	$\pm 38.1 \times 10^6$	$\pm 39x10^6$

Table 2

Specimen No.	Dimensional Change			
	Group A		Group B	
	mm	%	mm	%
1	0	0	-0.09	0.38
2	0.14	0.56	0	0
3	0	0	-0.09	0.38
4	0.12	0.56	0	0
5	0.13	0.56	0	0
6	0	0	0	0
7	0.14	0.56	0	0
8	0.19	0.75	0	0
9	0	0	0	0
10	0	0	0	0
Mean ± SD	0.072 ± 0.078	0.299	- 0.018± 0.038	0.072

Table 3

Paired Groups	Paired differences					t	dF	p
	Mean	Std. Deviation	Std. Error Mean	95% Confidence interval for mean				
				Lower bound	Upper bound			
A - B	0.09	0.069	0.022	-0.139	- 0.041	4.176	9	0.08

Level of significance was set at the probability level p<0.05.

4. Discussion

An undesirable side-effect of the disinfection process is the potential for a change in the dimensions of the impression that may be associated with a chemical interaction between the set material and the disinfecting solution. The change of dimension of impression materials following the setting reaction or the immersion in disinfection solutions has been the subject of a number of studies [7].

Some studies have indicated that the dimensional stability of

hydrophilic impression materials like polyether was adversely affected by immersion [8]. In a 1997 study, Lepe and Johnson found that overnight immersion (18 hours) of polyether or addition silicone impressions in 2% glutaraldehyde significantly affected their occlusogingival dimensions, as well as the mesiodistal dimensions of the addition silicone. Owen and Goolan recommended that polyether not be immersed for periods exceeding 5 hours, because it may expand. Polyether should be disinfected for short periods with the disinfectants accepted by the ADA, which in turn recommends immersion not exceeding 30 minutes [9].

Recently use of microwave radiation has been advocated for disinfection of impression materials [6]. Studies have found that metal instruments, including air turbine hand pieces and burs, and acrylic dentures can be sterilized in short periods. There are also studies concluding that microwave is efficient in disinfecting impression materials without any major change in dimensions.

In a previous study on disinfection with a microwave oven, microwaves not only changed the biologic state of bacteria and killed them but also influenced the charge distribution of the cell membrane because of the electric field generated and thus damaged the semi permeability of the cell membranes. This effect influenced the function of the Na-K pump and disabled the cell membrane. Microwave irradiation either alters or destroys the normal metabolism of cells and therefore suppresses and prevents the growth of bacteria, which has proven excellent for disinfection [10].

The results of the study revealed that efficacy of disinfection of polyether impression material by microwave irradiation were as effective as 2% glutaraldehyde compared to control specimens. Microwave and glutaraldehyde completely eradicated the tested microorganisms. This result was similar to Suresh et al. [11] and Ankur et al. [12] Effectiveness of microwave in disinfection of impression materials has also been reported by Abhilasha et al [6], Yu-Ri Choi et al [13]. Dimensional changes seen in microwave disinfection were 0.072 mm (0.299%) and in 2% glutaraldehyde immersion was -0.018 mm (0.072%). These results were in consensus with Cintia et al [14] who stated that the chemical disinfection caused minimal linear dimensional changes compared with the other disinfection techniques. In this study there was shrinkage of specimens in microwave irradiation and expansion in specimens disinfected with 2% glutaraldehyde. This result is in consensus with Ravikumar et al [15] and K.M. Abdelaziz et al [2] who found that specimens disinfected with microwave radiations showed mild contractions. The differences between dimensional changes for two disinfectant procedures were statistically insignificant. This result is in agreement with the results of Adabo et al [16], Ravikumar et al [15] and Handan Yilmaz et al [1].

According to ADA specification 19 criteria state that elastomeric impression materials should not be more than 0.5% dimensional change after 24 hours of polymerization of material [17]. Both disinfectant procedures i.e. microwave radiation at 720 watts for 7 minutes and 2% glutaraldehyde immersion for 30 minutes for polyether impression material used in this study were well within these standards similar to Poonam et al [18] and Suresh et al [11]. Thus both disinfection procedures can be recommended for use for disinfection of polyether impression materials. So, use of domestic microwave for disinfection and sterilization of elastomeric impressions can be considered as an effective, convenient and quick option.

There were few limitations of the study i.e. dies were calibrated for precise comparison, they do not resemble anatomy of oral tissues, microwaves must be used with caution when metal equipments are being disinfected and the present study was conducted *in vitro* which does not simulate intraoral conditions.

5. Conclusion

Within the limitations of the study, it can be concluded that microwave disinfection and 2% glutaraldehyde immersion were effective against all tested microorganisms in disinfecting polyether impression material specimens when compared to control. Dimension change in Microwave irradiation was due to shrinkage of specimens whereas in 2% glutaraldehyde there was expansion of specimens. The dimensional change of Polyether impression material for both the groups was well within the acceptable criteria of ADA specification 19 (0.5%) and use of domestic microwave for disinfection and sterilization of elastomeric impressions can be considered as an effective, convenient and quick option.

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