

A Comparative Evaluation of Antifungal Efficacy of Clove Oil, 2% Glutaraldehyde, and 5% Sodium Hypochlorite Disinfectants and its Effect on Dimensional Accuracy of Addition Silicone Impression Material

Harsh Patel, Sonal Pamecha*, Manish Chadha, J. Muthuvignesh, Vaibhavi Hathi, Aditi Joshi

Department of Prosthodontics, Crown and Bridge and Implantology, Pacific Dental College and Hospital, Debari, Rajasthan, India.

Abstract

Background: The present study was conducted with the purpose of comparative evaluation of the efficacy of clove oil and glutaraldehyde on addition silicon contaminated with *Candida albicans*. On intercomparison of all four groups (distilled water, glutaraldehyde, hypochlorite, and clove oil) dimensionally, no difference was found, whereas disinfectant property of 100% clove oil is almost same as that of 2% glutaraldehyde. **Materials and Methods:** Addition silicone impression material, Clove oil, Digital Vernier Caliper, Die stone (type IV), and *C. albicans* strain were taken. The test conducted: I) For disinfection – After incubation, the grown fungal colonies of *C. albicans* on Sabouraud Dextrose Agar were counted using colony counter and II) for dimensional accuracy – 15 sample to measure the distance between buccal cusp tip of mandibular 1st molar to opposite arch buccal cusp of mandibular 1st molar and measure and mandibular 1st molar to mesial tip of the central incisor with Digital Vernier Caliper. The data were subjected to statistical analysis using independent *t*-test and one-way Analysis of Variance (ANOVA) test. **Results:** Significant difference was found between 2% glutaraldehyde and 100 % clove oil and disinfectant property of 2% glutaraldehyde was same as that of 100% clove oil extract. On comparison microbiological mean value, it was observed that mean value of Group – I, distilled water was 204.67, Group – II, glutaraldehyde was 0.20, group – III, hypochlorite was 0.33, and Group – IV, clove oil was 0.27. Thus, there are very negligible changes observed when compared with glutaraldehyde, hypochlorite, and clove oil. Significantly, the ANOVA test shows $P = 0.000$. **Conclusion:** The present study was carried out with the purpose of comparative evaluation of the efficacy of clove oil and glutaraldehyde on addition silicone impression material contaminated with *C. albicans*. Furthermore we conclude that, disinfectant property of 2% glutaraldehyde was same as that of 100% clove oil extract.

Keywords: Addition silicon impression material, *Candida albicans*, Dental impressions, Dimensional accuracy, Disinfection.

INTRODUCTION

The aim of this study was to evaluate and compare antifungal property and dimensional accuracy of clove oil, 2% glutaraldehyde, and 5% sodium hypochlorite when incorporated in addition silicone impression material. Clove oil is highly dimensionally accurate with chemical disinfectant, but we use 100% clove oil, which is having highly antimicrobial property, so this study refers to check dimensional accuracy and antifungal property with addition silicone.

Clove oil additionally has organic activities, consisting of antibacterial, antifungal, insecticidal, and antioxidant properties and is used historically as a savoring and antimicrobial cloth in food.

Clove oil is a natural product that is cheap, and easy to find and apply by the patient, with an approved antifungal effect. Utilizing clove oil to treat and even prevent adherence of *Candida albicans* to addition silicon is a valuable study to establish and to compare the effectiveness of disinfectant solutions in disinfection of prosthesis with commercially available disinfectant solutions (2% glutaraldehyde).^[1]

Address for correspondence: Dr. Sonal Pamecha, Department of Prosthodontics, Crown and Bridge and Implantology, Pacific Dental College and Hospital, Debari, Rajasthan, India. Email: spamecha@gmail.com

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Furthermore, various findings and studies have pointed to the superiority of the addition silicone compared to the condensation silicone.^[2]

However, it has been shown that washing impression materials with water alone remove as little as 40% of bacteria, viruses, and fungi and, therefore, are totally inadequate as a means to reduce potential infection risks.^[3]

C. albicans are one of the components of normal oral microflora and around 30–50% people carry this organism. *C. albicans* is a dimorphic fungus which exists in both the blastospore phase and the hyphal or mycelial phase.^[4]

It has been studied before that dimensional accuracy changes with different disinfectants.

A study is performed further to check the effect of dimensional accuracy on clove oil, glutaraldehyde, and sodium hypochlorite disinfectants changes on addition of addition silicone impression material. It is, generally, believed that HOCl is the active species in the germicidal action, whereas the concentration of -OCl is a key factor determining the cleaning efficiency. This implies that the optimal pH region of the germicidal activity of sodium hypochlorite differs from that of its cleaning activity.^[5]

Several studies^[4,6-8] also have focused on the methods and materials for disinfecting dental prostheses before submission to the dental laboratory such as sodium hypochlorite solutions, chlorhexidine, and clove oil^[8] and may be used as a new resource for hygiene.

Hence, the three materials, that is, clove oil, glutaraldehyde, and sodium hypochlorite are being compared with each other, further according to our research, we will conclude the efficacy of clove oil, glutaraldehyde, and sodium hypochlorite on addition silicone impression material.

Aims and objectives

The study aimed to evaluate and compare antifungal property and dimensional accuracy of 100% clove oil, 2% glutaraldehyde, and 5% sodium hypochlorite when incorporated in addition silicone impression material.

MATERIALS AND METHODS

Methodology: (According to Modified CONSORT guidelines for *in vitro* studies).

Test for disinfection

- “*Candida albicans*” in lyophilized form obtained from Pacific Medical College and Hospital (Umarda), which were activated by culturing in sterile trypticase soy broth (HI MEDIA, U.S.A) and incubated at 30°C for 24 h. Then, contaminated swab was applied to the Sabouraud Dextrose Agar (SDA) culture media and was incubated in incubator at 35°C for 48 h. After that contaminated Petri dish, inoculation loop was heated for disinfection. Then, the colonies were collected from Petri dish.

A suspension was prepared to be made in distilled water. The turbidity of the solution was checked in densiCHECK plus device. Glucose broth culture media were added to this suspension.

- The specimens were first sterilized under autoclave and then were individually kept in sterile test tubes with 2 cc inoculums of culture and incubated at 25°C for 24 h. Distilled water specimen was kept in 2 ml of candida suspension and incubated at 25°C for 24 h for disinfection of samples. After that, hypochlorite was kept in 2 ml of candida suspension and incubated at 25°C for 24 h for disinfection of samples. These were later treated with various solution. After that, glutaraldehyde was kept in 2 ml of candida suspension and incubated at 25°C for 24 h for disinfection of samples.
- And then, after clove oil was kept in 2 ml of candida suspension and incubated at 25°C for 24-h for disinfection of samples. After 24 h of contamination, the resin specimens were removed from incubator and were rinsed with sterile distilled water for 30 s. Except the control group, the other two group samples were immersed in 20 ml of each disinfectant for 10 min. After this, the disinfectants of all groups these specimens were rinsed in sterile distilled water for 2 s to remove excess disinfectant solution. Now, 100 ml solution were spreaded over the SDA culture media.

Microbiological survey

- From the solutions, 100 ml were plated on SDA media on Petri dish and were spread uniformly or drying the Petri dishes were placed in an incubator at 25°C for 24 h. After incubation, the grown fungal colonies of *C. albicans* on SDA were counted using colony counter.

Test for dimensional accuracy

- To evaluate the dimensional accuracy, from Group I (distilled water), Group II (hypochlorite), Group III (glutaraldehyde), and Group IV (clove oil), 15 sample from each group were taken to measure the distance between buccal cusp tip of mandibular 1st molar to opposite arch buccal cusp of mandibular 1st molar, that is, a-c and measure and then mandibular 1st molar to mesial tip of the central incisor, that is, a-b and, that is, b-c with help of Digital Vernier Caliper (Generic LCD) (Figure 1).

Sample size

- One hundred and twenty samples were selected using a power analysis that was established by G*Power, version 3.0.1 (Franz Faul university, Kiel, Germany).

Fabrication of cast

- An impression was fabricated using addition silicone impression material and a diagnostic cast was fabricated using die stone (Type-IV).

Fabrication of addition silicone blocks

- Addition Silicone block was made in square shaped using BP-blade having dimensions of 10 mm × 10 mm × 10 mm.

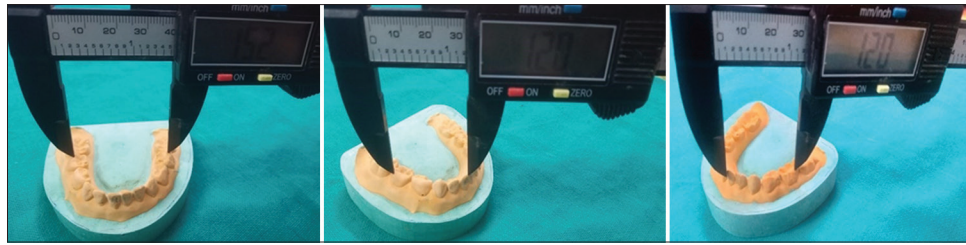


Figure 1: Dimensional Accuracy of a-c, a-b and b-c

Statistical evaluation

- The data obtained were coded and entered Microsoft Excel spreadsheet and analysis was done using SPSS version 16 windows software program (IBM SPSS Statistics Inc., Chicago, Illinois, USA). Descriptive analysis was done for each variable of the four groups for computation of means and standard deviations. Statistical tests used are as follows:
- Group I - Distilled water with addition silicone (Independent *t*-test)
- Group II - With 2% glutaraldehyde (Analysis of Variance [ANOVA] test)
- Group III – With Hypochlorite (ANOVA test)
- Group IV – With Clove oil (ANOVA test).
- Group – I shows independent *t*-test for statistically significant difference between the means in two unrelated groups
- Group – II–Group – IV show one-way-ANOVA test for quantitative data within three groups
- For comparison of all clinical indicators, the level of significance was set at $P \leq 0.05$
- Mean and standard deviation was calculated for three systems separately.

RESULTS

In the present study, 60 samples were tested. These 60 samples were divided into four groups,

- Group I – Washed with distilled water,
- Group II – Disinfected with 2% glutaraldehyde,
- Group III – Disinfected with clove oil extract and
- Group IV – With hypochlorite. Each group had 15 samples. The purpose of the study was to compare and evaluate the efficacy of clove oil, hypochlorite, and glutaraldehyde for disinfection of addition silicone blocks contaminated with *C. albicans* and checking for dimensional accuracy (Figure 2).

Mean and standard deviation values were calculated. Independent *t*-test and one-way ANOVA test were used (Table 1).

DISCUSSION

- According to the studies obtained by Lin JJ *et al.*, the disinfection of impressions is most effective means of minimizing infection risks. There are several factors

Table 1: ANOVA test of dimensional accuracy and CFU

	Sum of Squares	df	Mean Square	F	Sig.
a-b					
Between Groups	4.924	3	1.641	8.271	0.000
Within Groups	11.113	56	0.198		
Total	16.037	59			
a-c					
Between Groups	5.330	3	1.777	9.284	0.000
Within Groups	10.716	56	0.191		
Total	16.046	59			
b-c					
Between Groups	1.396	3	0.465	1.762	0.165
Within Groups	14.793	56	0.264		
Total	16.190	59			
Microbio					
Between Groups	470017.933	3	156672.644	1098.906	0.000
Within Groups	7984.000	56	142.571		
Total	478001.933	59			

to considered when developing a disinfection protocol for dental impressions, including the efficacy of the disinfection procedure, the chemical stability of the disinfecting solution, and surface reproduction of the impression materials and resultant casts.^[7]

- This study aims to evaluate the effectiveness of disinfectant solutions (distilled water, 5% sodium hypochlorite, 100% clove oil, and 2% glutaraldehyde) in the disinfection of addition silicone specimens contaminated *in vitro* by *C. albicans*, as measured by residual CFU. Among these four disinfectants, we found that distilled water, 5% sodium hypochlorite, 100% clove oil, and 2% glutaraldehyde are valid alternatives for the disinfectants.^[9]
- From this, an *in vitro* investigation was conducted in the Department of Prosthodontics at Pacific Dental College and Hospital, Udaipur to compare and evaluate the efficacy of 100% clove oil, 5% hypochlorite, and 2% glutaraldehyde disinfectants on addition silicon impression material contaminated with *C. albicans*.
- On comparing the effectiveness of glutaraldehyde, sodium hypochlorite and clove oil for the disinfection of *C. albicans* on addition silicon, data were analyzed using ANOVA test.

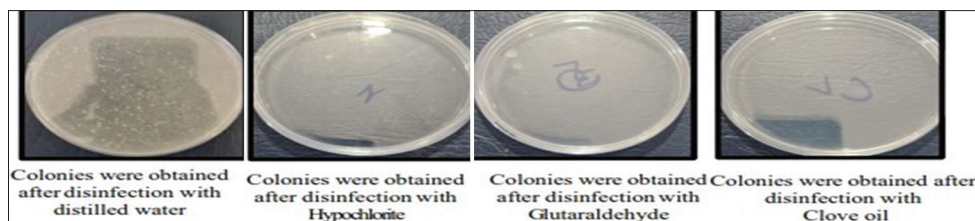


Figure 2: Obtained colonies

- *C. albicans* were collected. Addition silicon impression material was fabricated, 100% clove oil extract was fabricated, and microbiological study was performed at microbiology department of pacific medical college and hospital.
- The null hypothesis formulated was rejected as the 100% clove oil did show not antifungal properties like 2% glutaraldehyde.
- Thus, we found that clove oil (100%) and, (2%) glutaraldehyde removed 100% of the *C. albicans* cells, followed by, sodium hypochlorite (5%) which removed 99% of the adhered *C. albicans* from the addition silicone plates.

CONCLUSION

- The present study was carried out with the purpose of comparative evaluation of the efficacy of clove oil and glutaraldehyde disinfectants on addition silicone contaminated with *C. albicans*. Within limitations of this study, following conclusion was drawn.
1. Significant difference was found between 2% glutaraldehyde and 100% clove oil
 2. Disinfectant property of 2% glutaraldehyde was same as that of 100% clove oil extract.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this article were reported.

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