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Comparative evaluation of anticandidal activity of pre-incorporated quaternary ammonium compound disinfectant alginate with 5.25% sodium hypochlorite spray disinfectant on the conventional alginate: An In Vivo study

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ABSTRACT

Context: A lot of research and literature exists regarding the methods of disinfecting alginates; however, there is still need for a infallible, quick, and standardized method, which can be used as chair side disinfectant for alginate impressions in patients with candida related denture stomatitis.

Aim: An In Vivo study was undertaken to evaluate the anticandidal activity of pre-incorporated quaternary ammonium compound disinfectant in the alginate with conventional alginate impression material disinfected with 5.25% sodium hypochlorite spray (1 min) in primary impressions of completely edentulous patients with denture stomatitis.

Methods and material: Group 1 consisted of 10 conventional alginate (Imprint) impressions disinfected by 5.25% sodium hypochlorite spray for 1 min. Group 2 consisted of 10 pre-incorporated quaternary ammonium compound alginates (Jeltrate Plus). Totally, 20 swabs (10 per group) were collected from palatal concavities of primary impressions and inoculated on Sabouraud GC Agar media at 37 °C for 24–96 h. Digital colony counter was used for counting colony-forming units (CFU).

Statistical analysis used: The data analysis was done using descriptive statistics and independent *t*-test.

Results: According to *p* value ($p < 0.01$), there was a statistical significant difference in mean values of the two groups. Group 1 showed significant anticandidal activity in comparison to Group 2.

Conclusions: According to limitations of this In Vivo study, sodium hypochlorite (5.25% for 1 min) spray disinfectant is more clinically effective as compared to the pre-incorporated quaternary ammonium compound alginate for patients with denture stomatitis.

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Key messages

Sodium hypochlorite (5.25% for 1 min) spray disinfectant is a clinically effective disinfectant against Candida and saves clinical chairside time.

1. Introduction

Denture stomatitis is a very common condition found among the elderly edentulous population. Human oral cavity is a reservoir of approximately 700 species of microorganisms including 20 species of Candida.[1,2] Candida is not considered harmful in healthy hosts but may cause opportunistic infections resulting in candidiasis.[3] Candida colonization increases with increasing age irrespective of the denture-wearing status.[4] Aging causes a progressive increase in Candida counts in the oral cavity.[5] This condition is very common in denture-wearing patients, but 52 per cent of nondenture-wearing patients are also affected.[6]

Alginates have become one of the most indispensable dental materials for Prosthodontic usage. Any impression made in the oral cavity must be disinfected as soon as possible in order to minimize the risk of cross infection.

It was also observed that pre-incorporated disinfectant materials imparted antimicrobial activity to the impression materials with reduced overall quantity of the bacteria on the impression surface eliminating the need for conventional disinfection.[7] Flanagan et al.[8] have proved in their In Vitro study that pre-incorporated quaternary ammonium compound disinfectant in the hydrocolloid material is effective in reducing the microbial counts. That is why quaternary ammonium compound pre-incorporated irreversible hydrocolloid was tested as one of the methods to disinfect alginate in patients with denture stomatitis.

5.25% sodium hypochlorite spray disinfectant for one minute was tested in the undertaken study as recommended by Doddamani et al.[9] This method sounded a very promising way to disinfect alginate, as one-minute spray is a very convenient procedure, which was never evaluated before in studies against *Candida albicans*.

The suggested null hypothesis was that there existed no difference between anticandidal activity of pre-incorporated quaternary ammonium compound disinfectant in alginate with conventional alginate material disinfected by routine spray disinfectant of 5.25% sodium hypochlorite for 1 min.

2. Subjects and methods

Patients who reported to the Department of Prosthodontics and Crown & Bridge, K. M. Shah Dental College and Hospital (KMSDCH), Sumandeep Vidyapeeth having complete edentulism with clinical diagnosis of denture stomatitis were selected as participants (Fig. A1). Ethical approval for the study was granted by the Institutional Ethics Committee, Sumandeep Vidyapeeth with Approval No. SVIEC/ON/DENT/SRP/15197. Basic information about the study was given to the patient in the form of patient information sheet. A signed informed consent form was obtained.

Minimum samples required was 20 according to the study done by Cubas et al.[10] (10 per group) to detect mean difference of microorganisms saliva by 3 with standard deviation (SD) of 1.7 and effect size 2.45 at 1% risk and 90% power.

Completely edentulous patients, requiring prosthetic rehabilitation with clinical diagnosis of denture stomatitis, and ready to sign the informed consent were considered as the inclusion criteria of the study. Medically compromised, with mental or physical disabilities, psychological disorders, palatal defect, refusal to give consent for the study were considered as exclusion criteria of the study. Patients on heavy steroids or antibiotics were also excluded from the study.

Impression procedure (Fig. A2):

According to the patient's upper arch, suitable stock metal perforated tray was selected. Any kind of mouthwash was not used in both groups before making impressions.

Group 1: Maxillary preliminary impression was made using conventional alginate material (Imprint), and afterward disinfection was done with sodium hypochlorite (5.25% for 1 min) spray disinfectant.

Group 2: Maxillary preliminary impression was made using pre-incorporated quaternary ammonium compound disinfectant alginate material (Jeltrate plus – Dentsply).

5.25% sodium hypochlorite was formulated in the pharmacy department of Sumandeep Vidyapeeth.

The selection of each group impression was decided by flip coin randomization technique, and second impression in the same patient was taken after one day as relief period. Powder and water were measured according to manufacturer's instructions, and a clean flexible bowl and stiff-bladed spatula were used for mixing. Vigorous figure-of-eight motion was used. The material was loaded on a sterilized, maxillary perforated stock metal tray, and impression was made by the blinded operator. After taking each impression, the impression was washed under running tap water as a routine protocol.

For Group 1, Irreversible hydrocolloid impression material (Imprint, DPI, Mumbai) was used, and primary impression was made with suitable metal stock perforated tray. Impression was rinsed under running tap water as a routine protocol. The impression was then disinfected with sodium hypochlorite (5.25% for 1 min) as a spray disinfectant. The impression was later sealed in a ''Zip Lock'' polythene bag and stored at room temperature for the contact time of 1 min (Fig. A3). Alginate impression was then taken out from the plastic bag and rinsed under running tap water to rinse off excess sodium hypochlorite as disinfectant. Sterile swab was taken on the palatal concavity of the impression. Minimum exposure to light both before and during incubation was maintained. Each sample was processed and inoculated on Sabouraud GC Agar media at 37 °C for 24–96 h. Digital colony counter was used for counting growth of the microorganisms (Fig. A4). Scores obtained were entered into Performa sheet by blinded observer and later tabulated for statistical analysis.

For Group 2, Maxillary impression was made with pre-incorporated quaternary ammonium compound disinfectant

in irreversible hydrocolloid (Jeltrate Plus – Dentsply). Then, the impression was washed with running tap water as a routine protocol. Sterile swab was taken on the palatal concavity of the impression. Same culturing procedure as with Group 1 was followed. Scores obtained were entered into Performa sheet by blinded observer and later tabulated for statistical analysis.

Baseline swabs have not been taken in the study, because when using pre-incorporated quaternary ammonium compound, it was not possible to take the baseline swab for the reason that the disinfectant might immediately start working as soon as the alginate is placed in the mouth, and if another alginate material is used, there can be a dilution in the Candida counts. A gap of 24 h was purposely maintained between making impressions for the two different materials. The clinical examination of participants with denture stomatitis was done by a standardized clinical protocol by the Department of Oral Medicine and Prosthodontics.

3. Results

This study was conducted on 10 patients with denture stomatitis, where anticandidal activity of conventional alginate disinfected with 5.25% sodium hypochlorite spray disinfectant for 1 min (Group-1) (Fig. A5) and pre-incorporated quaternary ammonium compound alginate (Group-2) (Fig. A6) were evaluated. Individual colony-forming unit count of each patient in both impression materials was recorded (Table A1).

The data analysis was done using descriptive statistics and independent *t*-test. The descriptive analysis is illustrated in Table A2.

The mean value in Group 1 was 10.70 ± 2.21, whereas in Group 2, it was 40.30 ± 14.37 (Graph A1). Therefore, mean values demonstrated that anticandidal activity of 5.25% sodium hypochlorite spray disinfectant for 1 min on alginate impression material (CFU) was 10.70 ± 2.21, and for pre-incorporated quaternary ammonium disinfectant in alginate impression material (CFU), it was 40.30 ± 14.37. The standard deviation for Group 1 was 2.21, and for Group 2, it was 14.37. To check difference in means of these two groups, independent *t*-test was applied (Table A3).

Results of independent *t*-test depicted that there was statistical significant difference in mean values of two groups. Mean value in Group 1 was significantly low as compared to that in Group 2. ($p < 0.01$) Graph A1 also shows mean values of Group 1 and Group 2 with standard deviation.

Result of this study showed that according to p value ($p < 0.01$), there was a statistical significant difference in mean values of two groups. Group 1 shows significant anticandidal activity in comparison to Group 2.

4. Discussion

As there is a continuous quest for some better method for easily disinfecting the impression materials, which reduces valuable chairside time and is also standardized, this study was undertaken to check the effectiveness of two methods against the very commonly found clinical condition of denture stomatitis.

Pathogenic microorganisms get easily incorporated in the impression materials while making any kind of dental impressions. The infection control protocol should be strictly followed keeping in mind that every patient can be carrier or can be suffering with some deadly communicable disease. Any standard protocol that we try to follow should be infallible and very convenient to use, which can save valuable chairside time and also eliminates a number of pathogenic microorganisms present. This in turn reduces the chance of transfer of cross infection to dentist or technicians.

The commonly recommended disinfection methods are to either immerse in the disinfectant solution or to use spray as disinfectant. More than 60 studies have observed the effect of disinfectant on the impression integrity or on the physical characteristics of the casts obtained.[8] It was proved that pathogens on/in the impression surface can be transferred to the stone casts and stay in the viable state.

Irreversible hydrocolloid has got its own disadvantages like porous nature and high water content. Alginate material has been reported to have an intrinsic retentive potential for microbes as compared to the impression compound material and thus poses higher challenge. It has been proved by Samaranayake[11] that alginate impressions carry three to four times more microorganisms than the impression compound. It has also been proved that alginate retains two or three times more bacteria than elastomeric impression material. Alginate is the most widely used economical impression material also. These considerations gave us the basis for testing alginate as the impression material in the undertaken study.

In the undertaken study, two different approaches to disinfect alginate impression materials were used. In Group 1, irreversible hydrocolloid impression material was disinfected with 5.25% sodium hypochlorite spray disinfectant for 1 min. In routine clinical practice, irreversible hydrocolloid material can also be disinfected by 0.525% sodium hypochlorite for a contact period of 10 min. However, the undertaken study has intentionally used 5.25% sodium hypochlorite for 1 min, as it could be a very convenient chairside time-saving method. According to Doddamani et al.[9] sodium hypochlorite is less expensive, readily available through many retail sources, and a fast-acting broad-spectrum disinfectant; 5% sodium hypochlorite diluted with an equal amount of bioburden is reported to kill HIV in 2 min and 1 min without bioburden[12] and is an effective bactericidal, fungicidal, and virucidal disinfectant.

This pre-incorporated material sounded promising, as it reduced the chance of human errors and saved the time of dentist. Immersion disinfectants cannot be used on irreversible hydrocolloid as they lead to dimensional instability. Spray disinfectants are therefore better and recommended for usage. It has been proved by authors that the most accurate casts were associated with spray disinfection rather than immersion.[13–16]

The undertaken study has several limitations. Surface detail reproducibility, linear dimensional changes, abrasion resistance, and surface hardness of the gypsum casts were not evaluated and need to be addressed in future studies. The aim of this study was only to evaluate and compare the anticandidal activity of disinfectant-incorporated

irreversible hydrocolloid as compared to conventional disinfection method. Once proved, further studies could be designed to address the effect of both the irreversible hydrocolloids on the various other laboratory parameters and gypsum properties as independent studies.

Another limitation of this study was that pre-incorporated quaternary ammonium compound is a comparatively weaker disinfectant compared to 5.25% sodium hypochlorite spray disinfectant used for 1 min on conventional alginate against *Candida albicans*. However, as we wanted to determine the most convenient, easy, and time-saving chairside procedure, we could procure only quaternary ammonium compound pre-incorporated alginate material. Further studies can be done to compare sodium hypochlorite with some better pre-incorporated disinfectant materials.

Furthermore, a limitation of the study was that two different disinfectant materials, that is quaternary ammonium compound with sodium hypochlorite, were compared. Sodium hypochlorite in pre-incorporated form can also be evaluated with sodium hypochlorite spray disinfectant.

Doddamani et al.[9] proved that 5.25% sodium hypochlorite for 1 min is an effective spray disinfectant. However, he did not evaluate the effect of sodium hypochlorite spray disinfectant on Candida patients who are routinely associated with denture stomatitis. Therefore, we attempted to evaluate the effectiveness of pre-incorporated quaternary ammonium compound disinfectant in the irreversible hydrocolloid (Jeltrate Plus – Dentsply) with another most convenient and time-saving conventional spray disinfectant method (5.25% sodium hypochlorite for 1 min) in clinically common condition of denture stomatitis.

Results of this study showed that according to p value ($p < 0.01$), there was a statistical significant difference in mean values of the two groups. Group 1 shows significant anticandidal activity in comparison to Group 2.

Thus, the suggested Null hypothesis of the study was rejected due to statistical significant difference observed between the two groups.

5. Conclusion

Within the limitations of this In Vivo study, the following can be concluded:

1. 5.25% sodium hypochlorite spray disinfectant for 1 min on conventional irreversible hydrocolloid impression material has more anticandidal activity compared to pre-incorporated quaternary ammonium compound disinfectant present in irreversible hydrocolloid impression material.
2. Sodium hypochlorite spray disinfectant (5.25% for 1 min) can be considered as a convenient disinfectant method against *Candida albicans* for alginate as compared to pre-incorporated quaternary ammonium compound disinfectant in alginate. As it is quite effective in 1 min, it does save the chairside time of the dentist.

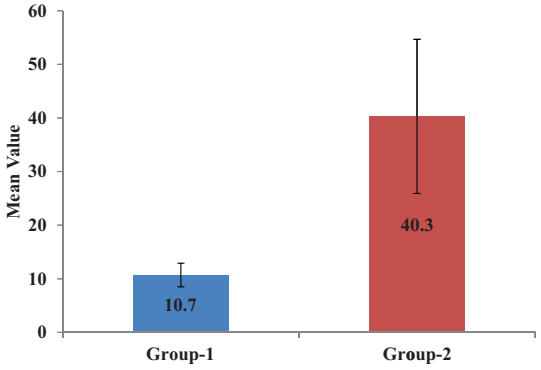
Conflicts of interest

The authors have none to declare.

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Appendices



Graph A1 – Mean values of Group 1 and Group 2 with standard deviation.



Fig. A1 – Armamentarium used for diagnosis of patient.



Fig. A2 – Materials and armamentarium used for making primary impression of the patient.



Fig. A3 – Conventional alginate impression stored in ''zip lock'' plastic bag for 1 min after spray disinfectant of sodium hypochlorite.



Fig. A4 – (1) Sterile swab, (2) culture media – Sabouraud GC Agar, (3) incubator, and (4) digital colony counter.

Table A1 – Candida colony-forming units (CFU).

Sr. No.	Group 1 (CFU) in Alginate disinfected with 5.25% sodium hypochlorite spray disinfectant for one minute	Group 2 (CFU) in Alginate with pre-incorporated quaternary ammonium compound
1	08	43
2	11	40
3	10	51
4	10	42
5	12	39
6	09	21
7	11	73
8	08	26
9	15	32
10	13	36

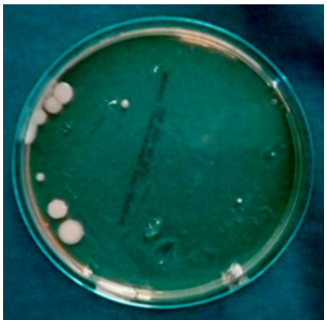


Fig. A5 – Representative colonies of Group 1.



Fig. A6 – Representative colonies of Group 2.

Table A2 – Descriptive statistics.

Group	N	Mean	Std. deviation	Std. error mean
Group 1	10	10.7000	2.21359	.70000
Group 2	10	40.3000	14.37629	4.54618

Table A3 – *t*-Test for equality of means.

t	df	*p*-Value	Mean difference	Std. error difference	95% confidence interval of the difference	
					Lower	Upper
−6.435	18	<0.01	−29.60	4.599	−39.263	−19.936

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