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Research Article

A Comparative Evaluation of the Efficacy of Disinfectants on Patient Derived Irreversible Hydrocolloid Impressions and their Effect on the Dimensional Accuracy of the Impression Material

Verma K[1]*, Mahesh GE[2], Parag D[3], Rashima V[4] and Ashish K[5]

[1]Graded Specialist, Division of Prosthodontics, MDC, BEG, India

[2]Commandant, Division of Prosthodontics, CMDC (WC), India

[3]Classified Specialist, Division of Prosthodontics, 14 CDU, India

[4]Dental Surgeon, ECHS Polyclinic, Kirkee, India

[5]Graded Specialist, Division of Prosthodontics, CMDC (CC), India

***Corresponding author:** Kamal Verma, Graded Specialist, Division of Prosthodontics, MDC, BEG, India

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Abstract

Purpose: Assessing the efficacy of disinfectants on irreversible hydrocolloid impressions and their effect on the dimensional accuracy.

Materials and Methods: The study was carried out to compare the efficacy of three commercially available disinfectants (Cidex – 2% Glutaraldehyde (Johnson & Johnson), 1% Sodium Hypochlorite (I - Dent), MD 520 – 0.5% Glutaraldehyde and 0.25% Ammonium chloride (Durr)) in eliminating or reducing the microbial colonies on patient derived irreversible hydrocolloid impressions and the resultant effect on the dimensional accuracy of the impression material when exposed to these agents.

Results: MD 520 system resulted in maximum (95.6%) removal of the visible colonies for all samples investigated and 1% sodium hypochlorite was found to cause the least amount of dimensional changes in irreversible hydrocolloid impressions.

Conclusions: It is entirely the clinician's choice to select disinfecting agents to use considering all their advantages and disadvantages. It should be kept in mind that though MD 520 was shown to be the most effective disinfectant, sodium hypochlorite caused lesser dimensional changes in the alginate impressions.

Keywords: Disinfection; Irreversible Hydrocolloid; Dimensional stability

Introduction

Disinfection of dental impressions has drawn much attention and research interest in recent years [1]. To address cross contamination concerns, the American Dental Association has issued guidelines for disinfecting impressions while using spray or immersion disinfectants. Three important factors must be considered when dental impressions are disinfected - how are the impression material and resultant cast affected, how stable are the disinfectant solutions and how effective are the disinfection procedures [2]. Very few studies have been undertaken till date which reveals answers to all these aspects together. Meanwhile, manufacturers claim disinfectants are better for disinfection of irreversible hydrocolloid impressions, but they do not mention about the dimensional accuracy of the impression. The idea behind conceptualizing this study was the fulfillment of the following objectives: (1) To clinically examine the carriage of oral pathogens on the impression surface. (2) To clinically evaluate the disinfection efficacy of three commercially available agents in removing oral pathogens from patient derived impressions. (3) To evaluate the effect of these disinfecting agents on the dimensional stability of irreversible hydrocolloid impressions.

Materials and Methods

The study was carried out at our centre to compare the efficacy of three commercially available disinfectants (Cidex – 2% Glutaraldehyde (Johnson & Johnson), 1% Sodium Hypochlorite (I - Dent), MD 520 – 0.5% Glutaraldehyde and 0.25% Ammonium chloride (Durr)) (Figure 1) in eliminating or reducing the microbial colonies on patient derived irreversible hydrocolloid impressions and the resultant effect on the dimensional accuracy of the impression material when exposed to these agents.

The study was performed in two stages:

(A) Comparison of Antimicrobial Effect on Patient Derived Irreversible Hydrocolloid Impression Material Thirty dentulous

Figure 1: Three disinfectants used in the study.

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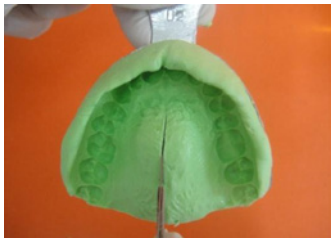


Figure 2: Maxillary impression split sagittally with sterile surgical blade.

Figure 3: Impressions rinsed under running water and treated with disinfection treatment regimes.

subjects were randomly selected after getting their verbal consent and with following inclusion criteria: no denture on either jaw, more than 10 teeth present in the maxilla, age of 20 – 40 years with no systemic illness and the subjects should not have received oral hygiene or tooth brushing instructions.

Maxillary perforated metal stock trays were selected. Four impressions of the maxillary arch of each subject were obtained at an interval of 1 week. According to the manufacturer's recommendations irreversible hydrocolloid powder (Plastalgin, Septodont, France) and distilled water was mixed utilizing vacuum mixer. Distilled water was used in lieu of tap water because for required standardization and ion concentrations present in tap water may interfere with irreversible hydrocolloid chemical reactions. The mixed irreversible hydrocolloid was then loaded on the tray and impression was made of each patient's maxillary arch.

Figure 4: Brain Heart Infusion (BHI) Agar Medium poured onto the impression.

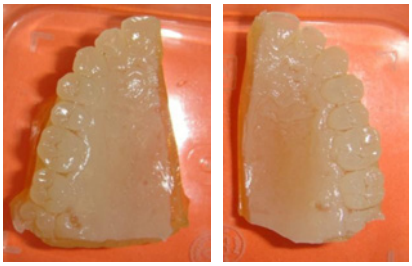


Figure 5: Separated Agar samples.

After setting of the impression, the impression was removed and washed under running tap water for 15–20 seconds. Impression was split sagittally down the middle with the help of sterile surgical blade (Figure 2). One half of separated impression was left untreated (Control) to evaluate the amount of microorganisms carried by the impression and the other half was subjected to one of the disinfectant treatment regimes (treated sample) (Figure 3, Table 1). The impressions were taken out of the disinfectant solution and rinsed with running water. Both the treated and the untreated samples were then carefully placed into a sterile plastic container partitioned into two compartments with boxing wax. Brain heart infusion (BHI) agar medium (M211- 500G, Himedia) was prepared at 50°C by mixing 52g of powder in 1000ml of distilled water. It was autoclaved (Runyer, Unicorn Denmart) at 121°C for 15 minutes at 15psi and poured onto the impression (Figure 4). The agar was allowed to cool for 1 hour at 4°C following which it was separated from the impression and was incubated at 37°C for 48 hours to facilitate adequate growth of the micro organisms (Figure 5).

The presence of colonies on the culture specimen was determined (Figure 6). The number of bacterial colonies appearing on each specimen was counted using a colony counter (Scope, India) and the results were tabulated. The procedure was repeated for each of the four groups included in the study for each patient. As a negative

Table 1: Disinfection treatment regimes used in the study.

Group No.	Treatment regime	Composition	Manufacturer	Procedure	No. of Impressions
1	Running tap water only	-	-	Rinsing under running water for 01 minute	30
2	Running tap water followed by Cidex	2% Glutaraldehyde	Johnson & Johnson	Rinsing followed by immersion for 05 minutes (as per manufacturer's instructions)	30
3	Running tap water followed by Sodium Hypochlorite	1% Sodium hypochlorite	I – Dent	Rinsing followed by immersion for 05 minutes (as per manufacturer's instructions)	30
4	Running tap water followed by MD 520	Glutaraldehyde, alkylbenzyl-dimethyl, Ammonium chloride	Durr	Rinsing followed by immersion for 05 minutes (as per manufacturer's instructions)	30

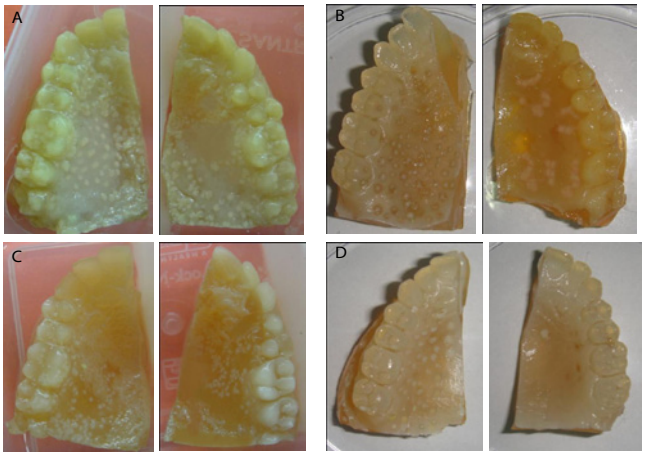


Figure 6: Colony growth on untreated and treated Agar samples after 48 hrs incubation at 37°C.
(A) Samples washed under tap water, **(B)** Samples disinfected with 2% glutaraldehyde**, (C)** Samples disinfected hypochlorite with MD 520, **(D)** Samples disinfected with 1% sodium**.**

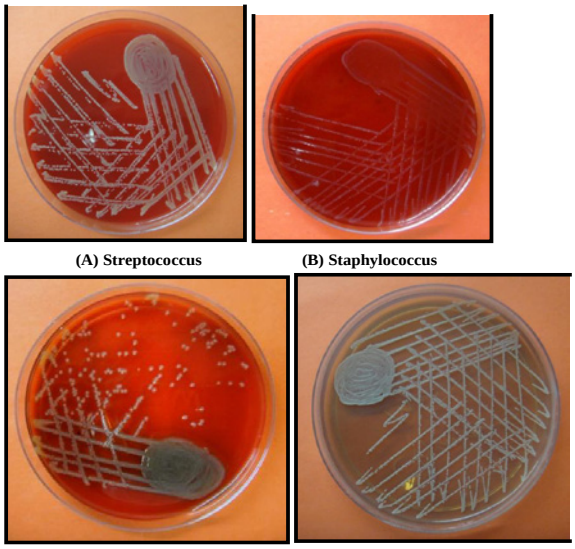


Figure 7: Showing different microbial colonies on selective medium.

control, an irreversible hydrocolloid impression was also made of the maxillary arch of a sterilized typhodont to see if any colony growth was observed after 48hrs of incubation.

Selective isolation of oral microorganisms: The colonies on the surface of the BHI impression culture for control and treated samples were collected and were then suspended in 1 ml of sterile phosphate-buffered saline. The colony suspension was placed on selective agar medium plates to detect the presence of Streptococci, Staphylococci, Pseudomonas aeruginosa and Candida. After 48 hours of incubation at 37°C, the existence of positive colonies for each selective medium was determined visually (Figure 7).

Data collection and statistical analysis: Descriptive statistics including mean, standard deviation and minimum and maximum values of microbial colonies were calculated for each group. A one-way analysis of variance (ANOVA) was used to determine whether significant differences in values existed among the various groups (Table 2). Non parametric test i.e. Kruskal-Wallis test was performed to determine significant differences in values of microbial colonies before and after disinfection (Table 3).

Table 3: Kruskal – Wallis test statistics of all microorganisms.

	Chi-Square	df	p value
Streptococcus Untreated	3.729	3	0.292 Non significant
Streptococcus Treated	40.14	3	0 Significant
Staphylococcus Untreated	3.701	3	0.296 Non significant
Staphylococcus Treated	38.722	3	0 Significant
P. aeruginosa Untreated	3.846	3	0.279 Non significant
P. aeruginosa Treated	25.173	3	0 Significant
Candida Untreated	0.321	3	0.956 Non significant
Candida Treated	6.051	3	0.109 Non significant

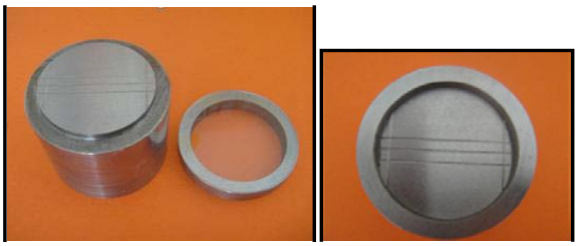


Figure 8: Round metal Test Die block (According to ADA specification 18).

(B) Comparison of effect of disinfectants on the dimensional stability of irreversible hydrocolloid impression material

Impression making protocol was followed as per American Dental Association Specification No. 18 (ADA 18) for irreversible hydrocolloid impression materials. 120 impressions were made on

Table 2: Analysis of variance (ANOVA) for comparison of mean number of colonies between treated and untreated samples.

		Sum of Squares	df	Mean Square	F value	p value
Mean Colonies Untreated	**Between Groups**	9.333	3	3.111	0.012	0.998 Non significant
	Within Groups	30910.533	116	266.47		
	Total	30919.867	119			
Mean Colonies Treated	**Between Groups**	15447.933	3	5149.311	92.321	0 Significant
	Within Groups	6470.067	116	55.776		
	Total	21918	119			

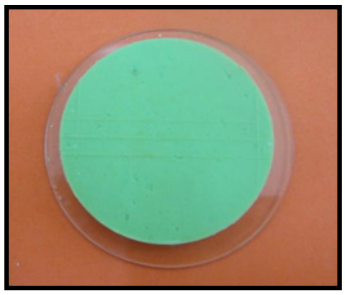


Figure 9: Irreversible Hydrocolloid impression removed from Die.

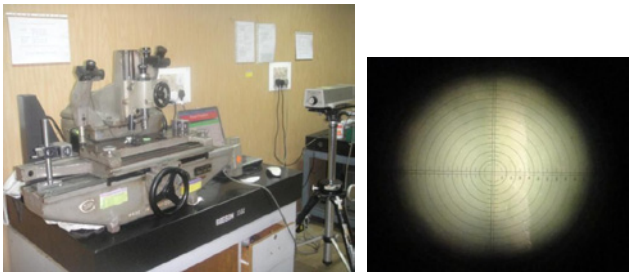
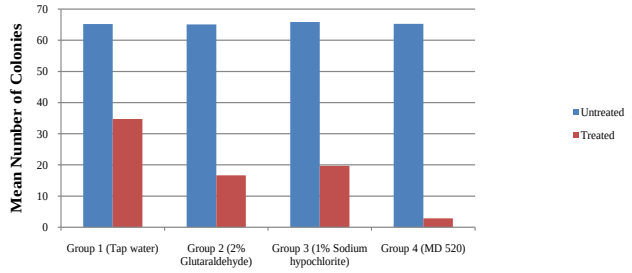
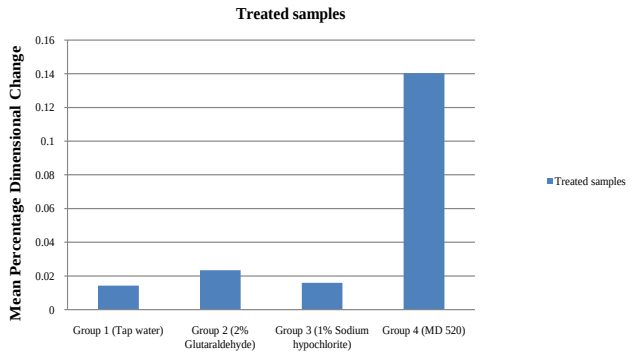


Figure 10: Measuring the linear scores between the reference lines obtained from the Test Die under a Universal Measuring Microscope.



Graph 1: The mean number of colonies before and after disinfection regimes.



Graph 2: The mean percentage dimensional change.

standardized metal die (similar to those described in the specification) scored with three equidistant horizontal and two vertical lines with the same irreversible hydrocolloid that was used to record impressions of the patients. A metal ring mold with glass lid was also fabricated to fit over the die (Figure 8).

To make the impressions, the ring mold was slightly overfilled with irreversible hydrocolloid and was centered and pressed over the test die for 3 minutes (as per manufacturer's instructions). The irreversible hydrocolloid flash from the sides of the mold was removed. The set irreversible hydrocolloid impression was separated from the metal die (Figure 9). An alcohol swab was used to clean the die and its assembly before reuse. After impression removal, impressions were rinsed with tap water to simulate rinsing following impression removal from the mouth and the excess water was shaken off. The impressions were divided into 4 groups and subjected to different disinfection regimes as per the manufacturer's instructions, as shown in Table 1. The impressions were taken out of the disinfectant solution and rinsed with running water.

Each impression was evaluated for dimensional change by measuring the linear scores (X-X') between the reference lines obtained from the test die under a Universal Measuring Microscope (Figure 10). Three readings were obtained for each impression and a mean value was calculated. The percentage dimensional change of the irreversible hydrocolloid impression from the metal die was computed using the following equation: [(D-A)/ D] x 100, where A = mean irreversible hydrocolloid impression measurement and D = die measurements.

Descriptive statistics including mean, standard deviation, and minimum and maximum values of irreversible hydrocolloid impression measurement and dimensional change were calculated for each group. A one-way analysis of variance (ANOVA) was carried out to determine whether significant differences in percentage dimensional change existed among the various groups (Table 4).

Results

The use of the BHI impression culture detection method produced a large number of obvious colonies on the samples of the irreversible hydrocolloid impression which were distributed predominantly over the areas of the palate and the dental arch. In contrast, no colonies

Table 4: Analysis of variance (ANOVA) for comparison of percentage dimensional change between and within groups.

		Sum of Squares	df	Mean Square	F value	p value
Mean alginate impression measurement	**Between Groups**	0.05961	3	0.01987	2.287	0.082 Non significant
	Within Groups	1.008	116	0.008687		
	Total	1.067	119			
Percentage dimensional change	**Between Groups**	0.416	3	0.139	35.755	0 Significant
	Within Groups	0.45	116	0.003878		
	Total	0.866	119			

were observed on the BHI impression cultures from the negative controls obtained from sterilized typhodonts, thus indicating the reliability of sterilization procedures. The occurrence of different microorganisms on impression surfaces has been summarized in Figure 10. The split-impression culture method demonstrated that all disinfection procedures investigated reduced the number of microbial colonies versus the untreated samples (Graph 1). The reduction in the number of colonies was greatest following disinfection with the MD 520 system followed by 2% glutaraldehyde, 1% sodium hypochlorite, and least while rinsing with tap water. Disinfection using the MD 520 system resulted in maximum (95.6%) removal of the visible colonies for all samples investigated. In contrast, treatment with rinsing under tap water resulted in 46.7% reduction in the number of colonies.

One-way ANOVA and Kruskal-Wallis Test were employed to statistically analyze the results.

A statistically significant difference was observed by ANOVA in the mean number of colonies between groups of treated samples. F values of 92.321 with 3 degrees of freedom were found to be significant ($p<0.05$), thus signifying a successful disinfection regime. The F values of 0.012 with 3 degrees of freedom between groups of mean number of colonies of untreated samples were found to be non significant ($p=0.998$) (Table 2).

A statistically significant difference was also observed by Kruskal Wallis test in the mean number of microorganisms between groups of untreated and treated samples. P values of treated microorganisms except Candida were significant (p value< 0.05) with 3 degrees of freedom (Table 3).

Effect of disinfectants on the dimensional accuracy of irreversible hydrocolloid impression material

A planned comparison approach with one way ANOVA (Table 4) was used to detect the differences in irreversible hydrocolloid impression measurements with each disinfectant. Irreversible hydrocolloid was found to be most stable when treated with 1% sodium hypochlorite followed by 2% glutaraldehyde (Graph 2). Impressions dipped in MD 520 exhibited greatest amount of dimensional change within groups. Few samples rinsed under tap water and treated with 1% sodium hypochlorite showed shrinkage as compared to expansion of samples treated with 2% glutaraldehyde and MD 520.

The results of one way ANOVA revealed that there were significant differences in percentage dimensional change between groups of treated samples ($F=35.755$, $P< 0.05$). The F values of 2.287 with 3 degrees of freedom between groups of mean irreversible hydrocolloid measurement were found to be non significant ($p=0.082$) (Table 4).

Discussion

Prosthodontics is the field of dentistry where prevention of cross contamination seems to be an insurmountable problem. During impression making procedure, the material comes in contact with saliva and blood, which are sources of contamination and carries a great number of microorganisms of oral flora upon removal from mouth [3,4].

The ADA Council on Dental Therapeutics has recognized 32 brands of commercial products as being effective disinfecting or sterilizing agents for use in dentistry and immersion is the method most recommended [3]. Immersion disinfection is based on the assumption that immersion is more likely to expose all surfaces of the impression to the disinfectant for the recommended time [5]. Spraying disinfectant onto the surface of an impression reduces the chance of distortion but may not adequately reach the areas of undercuts. Bergman and Thomasz [6,7] reported that spraying method was compatible with the irreversible hydrocolloid impression material and produced clinically acceptable results. Though microbial contamination of patient-derived impressions has been documented; however, few studies have characterized the pathogenic microorganisms on the impressions [8-10].

The present study examined a total of 120 specimens for detection of pathogens on the patient-derived impressions. The isolation frequency of Streptococci, Staphylococci, Candida, and P aeruginosa species on untreated impressions was 97.5%, 60%, 11.6%, and 35.8% respectively. This result confirmed the ability of patient-derived dental impressions to carry pathogenic microbial contamination and was in accordance with the results of Al- Jabrah, and Egusa H [11,12].

The disinfection with 2% glutaraldehyde or 1% sodium hypochlorite was only partially successful against oral pathogens, but more effective with the MD520 system which achieved 95.7% reduction in colony growth as compared to 74.3% reduction with 2% glutaraldehyde and 70% with 1% sodium hypochlorite. But at the same time, irreversible hydrocolloid impressions were not as dimensionally stable in a solution of MD 520 as compared to 1% sodium hypochlorite and 2% glutaraldehyde. Though the dimensional changes of irreversible hydrocolloid were within the acceptable limits (0 – 0.15%, according to ADA specification 18) when dipped in any of the three disinfectants, it was observed that the mean values of percentage dimensional changes were the least when 1% sodium hypochlorite was used.

Another interesting finding was the irreversible hydrocolloid impressions exhibited a small amount of shrinkage when dipped in 1% sodium hypochlorite or rinsed in tap water. The above observations raise the question of the reason for shrinkage. Although irreversible hydrocolloid contains water, it may be due to an initial expansion caused by the ions present in the irreversible hydrocolloid (e.g. $Na+$, $SO4^{2-}$, $PO4^{3-}$) creating an osmotic potential. However, subsequently the ions diffuse out into the surrounding water, reversing the osmotic potential, so that some water diffuses out again. When the external solution itself contains ions i.e. in the case of sodium hypochlorite, then there will be a two way transport of ions, until the set irreversible hydrocolloid is in equilibrium with the external solution. These results are similar to those noted by Martin and Nallamuthu [13,14].

Conclusion

Within the limitations of this study, the following conclusions were drawn:

1. The irreversible hydrocolloid impression act as potential carriers of oral microbes by way of blood and saliva. Cross contamination through irreversible hydrocolloid impressions in the dental laboratory may pose serious health hazard threats to dental personnel. Therefore, adequate disinfection of irreversible hydrocolloid impressions is an important concern for the dentist.

2. Streptococcus was most commonly found on the

impressions followed by Staphylococcus, P aeruginosa and Candida. Rinsing of the impressions with water alone is not effective in removing the microbial load adequately.

3. Distilled water should be used to mix alginate powder in order to prevent the reaction of ions that are present in tap water.

4. It should be kept in mind that though MD 520 was shown to be the most effective disinfectant, sodium hypochlorite caused lesser dimensional changes in the alginate impressions. It is entirely the clinician's choice to select disinfecting agents to use considering all their advantages and disadvantages.

5. Though none of the disinfectants used in this study caused changes in dimensional accuracy beyond the permissible limits (0–0.15%), 1% sodium hypochlorite was found to cause the least amount of dimensional changes in irreversible hydrocolloid impressions.

6. Further clinical studies are required to try out newer methods of disinfection like gaseous, where the factors like compromised dimensional stability never arise.

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