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RESEARCH ARTICLE

COMPARATIVE EVALUATION OF THE EFFICACY OF THREE COMMERCIALLY AVAILABLE DISINFECTANTS ON IRREVERSIBLE HYDROCOLLOID MATERIALS BY SPRAY METHOD – AN IN VITRO STUDY

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ABSTRACT

Objective: Present study was carried out to evaluate and compare the efficacy of three most commonly used disinfectants on irreversible hydrocolloid material.

Materials and Methods: A total of 60 alginate disks with 13mm diameter and 2mm height were prepared with irreversible hydrocolloid material. These specimens were divided into four groups (n=15), i.e, Group A Distilled Water, Group B Sodium Hypochlorite, Group C Glutaraldehyde and Group D Iodophor. All alginate specimens were contaminated with Staphylococcus aureus strain for 1 min. After contamination Group B, C and D specimens were disinfected by spray method, whereas, group A specimens were only washed with distilled water. All the specimens were placed into the test tubes containing nutrient broth media for 30 sec. 25um of nutrient broth solution was plated on soyabean casein digest agar plates for determination of minimum inhibitory concentration. The number of colonies were identified and colony forming units (CFU) were counted. Between and within group differences in antibacterial activity was analyzed using one way ANOVA test of significance and Post Hoc Test.

Result: 53.33% of the samples disinfected with Sodium Hypochlorite and 33.33% of the samples disinfected with Gluteraldehyde showed complete disinfection. All the samples disinfected with Iodophor showed bacterial growth but colonies were countable, whereas, all the samples washed with distilled water showed uncountable bacterial growth.

Conclusion: Sodium Hypochlorite is the most effective disinfectant followed by Glutaraldehyde and Iodophor.

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INTRODUCTION

Dentistry has always aimed at providing relief from pain, correcting unesthetic appearance and treating loss of function. In execution of a smooth treatment plan, unforeseen complications could arise due to infectious status of the patient (Samra and Bhide, 2010). With increase awareness of diseases like Hepatitis B and Acquired immune deficiency syndrome (AIDS), use of disinfectant has been accentuated (Crawford, 1985). Infection control is an indispensible part of dental practice and impression disinfection is an integral part to prevent cross infection among dentists, dental office staff, dental technicians and patients (Haralur *et al*., 2012). The American Dental Association (ADA), the Centers for Disease Control and Prevention (CDC), the Occupational Safety and Health Control and Health Administration states that, every impression should be disinfected after removing from the patient's mouth and before entering the dental laboratory in order to prevent cross infection (Ghahramanloo *et al*., 2009). Irreversible hydrocolloid is the most common dental impression material used in everyday practice for diagnostic impression procedures. However, these materials, because of their composition, texture and hydrophilic setting mechanism gets easily contaminated with microorganisms present in the oral cavity. Few studies have shown that irreversible hydrocolloid impression carries two to five times more micro organisms than elastomers. Because of greater awareness and concern about infection control, disinfection procedure has been suggested to reduce the transmission of infection (Ghahramanloo *et al*., 2009). A wide range of disinfectants are used like Glutaraldehyde, Sodium Hypochlorite, Iodophor and Phenolics for disinfection of the impression materials. However, the efficacy of various disinfectants in achieving the target is still questionable. Therefore, this study was planned to compare the efficacy of three most commonly used disinfectants i.e. Sodium Hypochlorite, Glutaraldehyde and Iodophor. These disinfectants can be used either in spray form

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or by immersing the impression in them (Ghahramanloo *et al*., 2009). Immersion disinfection of irreversible hydrocolloid impression deteriorates the dimensional accuracy of resultant stone models due to imbibitions of the impression during immersion. To overcome distortion issues of irreversible hydrocolloid, ADA recommended that these impressions be sprayed with ADA approved disinfectant and then sealed in plastic bag according to the recommended disinfection time (Hiraguchi *et al*., 2010). Matyas *et al* studied the effect of disinfectants on the dimensional accuracy of casts made from impressions and concluded that using the disinfectants with either the immersion or spray techniques did not significantly affect the dimensional accuracy (Matyas *et al*., 1990). The present study was carried out to evaluate and compare the efficacy of three most commonly used disinfectants i.e. Sodium Hypochlorite, Glutaraldehyde and Iodophor by spray technique on irreversible hydrocolloid material. The null hypothesis was that there would not be a significant defference in the efficacy of these disinfectants on irreversible hydrocolloid material.

MATERIALS AND METHODS

For the purpose of this in vitro study, irreversible hydrocolloid impression material (Neocolloid, Zhermack clinical, Badia Polesine, Rovigo, Italy), 0.525% Sodium Hypochlorite (Dento Sure, Hy-po, Smile Distributor, Nashik, India), 2% Glutaraldehyde (Korsolex Rapid, Raman & Weil Pvt. Ltd., Daman, India) and (1:213) Iodophor (Otto Chemie Pvt. Ltd., Popatwadi, Maharashtra, India) were used. A total of 60 specimens were prepared with irreversible hydrocolloid material for evaluating the efficacy of three different disinfectants. These specimens were divided into four groups with 15 specimens in each group. Group A specimens served as the control specimens and did not receive any disinfection and were washed with distilled water only. Whereas, Group B, C and D specimens were disinfected with 0.525% Sodium Hypochlorite, 2% Glutaraldehyde and (1: 213) Iodophor respectively.

GROUP A:- Control Group: Distilled Water (n=15)
GROUP B:- 0.525% Sodium Hypochlorite (n= 15)
GROUP C:- 2% Glutaraldehyde (n=15)
GROUP D:- (1:213) Iodophor (n=15)

To evaluate the efficacy of disinfectants, precision machined metal rings with internal diameter of 13mm and height of 2mm were made for preparation of alginate disks (Ghahramanloo *et al*., 2009) (Fig. 1).

Preparation of Alginate Specimens

ADA specification no. 18 for alginate impression material was followed for preparation of alginate specimens (Council adopts American Dental Association Specification, 1968). Powder and water (2 spoons of powder with 20 ml water, according to the manufacturer's instruction) was mixed for one minute using a clean flexible rubber bowl and curve spatula. Rapid spatulation of the material was done. The mix was swiped and stropped against the side of the bowl with intermittent rotations of the spatula to press out air bubbles. Vigorous figure eight motion was used. The metal disk was placed on a sterile glass slab and the impression material was loaded into the ring when it appeared to be creamy in consistency. Alginate was pressed against the glass slab and pressed firmly against another glass slab. Flat metal weighing 1kg was placed on the glass slab to simulate finger pressure. The material was allowed to set for about 2 minutes according to the manufacturer's instruction. The specimen was carefully removed from the ring without damaging the surface of the specimen. A total of 60 specimens were prepared (Fig. 2).

Preparation of Staphylococcus Aureus Strain

Nutrient broth (2.60gm) was mixed with distilled water (120ml) in a flask and was autoclaved (Equitron Autoclave, Hyderabad, Telangana, India) to avoid growth of any other micro organism. The content was allowed to cool at room temperature and then staphylococcus aureus (BRC 128) was added to the nutrient broth. Staphylococcus aureus strain was cultured on nutrient broth using shaking unit (REMI shaker, Goregaon, Mumbai, India) for 24hrs (Fig. 3). Bacterial suspension of 10^8 CFU/ml of Staphylococcus Aureus strain was taken into 60 sterilized test tubes.

Contamination of Alginate Specimens

Alginate specimens were immersed into the test tube containing 10^8 CFU/ml of Staphylococcus Aureus strain for 1 minute for contamination. Individual test tubes were used for each alginate specimen to avoid decrease in suspension concentration, so 60 test tubes were used for 60 alginate specimens.

Disinfection of Alginate Specimens

After contamination, all the alginate specimens were washed with distilled water for 15seconds and excess water was removed by a gentle shake. According to the defined groups (Group B:- Sodium Hypochlorite, Group C:- Glutaraldehyde, Group D:- Iodophor), the disinfectant was sprayed on the alginate specimens for 30seconds (10sprays). For control group i.e, Group A (Distilled water), the alginate specimens after contamination were washed with distilled water only as mentioned above. No disinfectant was sprayed on the specimen for control group. After disinfection of the alginate specimens according to the defined groups, each alginate specimen was kept in a separate sterile plastic bag for ten minutes to avoid evaporation of the disinfectant. Afterwards, the specimens were placed into the test tubes containing nutrient broth media for 30seconds (Fig. 4). The test tubes were sterilized in an autoclave before using and were coded as per the defined groups. The test tubes were incubated for 24hours at 37degrees Celsius in the incubation machine (REMI cooling incubator, Goregaon East, Mumbai, India) (Fig. 5).

Preparation of Soyabean casein digest agar plate

Soyabean casein digest agar (150gm) was mixed with distilled water (5000ml) in a jar and was sterilized in an autoclave (Equitron Autoclave, Hyderabad, Telangana, India) to avoid growth of any other micro organism. When the mix was hot, it was poured into 60 sterile plastic petri dishes and was allowed to cool over night till it formed gel consistency (Fig. 6). The plates were also coded as per the defined groups like test tubes containing the nutrient broth. 25um of nutrient broth solution which was incubated for 24hours at 37degrees Celsius was plated on soyabean casein digest agar plates using pipette and plastic tip. These plates were again incubated (REMI cooling incubator, Goregaon East, Mumbai, India) for 24hours at 37 degrees celsius for determination of minimum inhibitory

concentration. The number of colonies were identified in each Petri Plate for all the four groups. Staphylococcus aureus was seen as golden yellow colonies, 1–3mm in size. Colony forming units (CFU) were counted. Descriptive statistics were expressed as mean ± standard deviation (SD) for each group. Between and within group differences in antibacterial activity was analyzed using one way ANOVA test of significance and Post Hoc Test. In the above test, p value less than or equal to 0.05 ($p \leq 0.05$) was taken to be statistically significant.

RESULTS

53.33% (8 out of 15) of the samples disinfected with 0.525% Sodium Hypochlorite (Group B) showed no growth of bacterial strain, whereas, 33.33% (5 out of 15) of the samples disinfected with 2% Gluteraldehyde (Group C) showed complete disinfection. All the samples disinfected with (1:213) Iodophor (Group D) showed bacterial growth but colonies were countable. Bacterial colonization with the use of (1:213) Iodophor (Group D) was greater (27 x 10^3 CFU/ ml) as compared to 0.525% sodium hypochlorite (1.5 x 10^3 CFU/ml) and 2% Gluteraldehyde (6.4 x 10^3 CFU/ ml). However, all the samples washed with distilled water showed uncountable bacterial growth (1 x 10^8 CFU/ ml). All the disinfectants i.e, 0.525% Sodium Hypochlorite (Group B), 2% Glutaraldehyde (Group C) and (1:213) Iodophor (Group D) showed statistically significant difference as compared to Distilled water ($p < 0.001$). Among the disinfectants, 0.525% Sodium Hypochlorite (Group B) showed statistically significant difference with Group D, whereas, statistically insignificant difference ($p = 0.140$) with Group C. Group D showed statistically significant difference with Distilled water (Group A) and with all the disinfectant ($p < 0.001$) (Table 1 and 2).

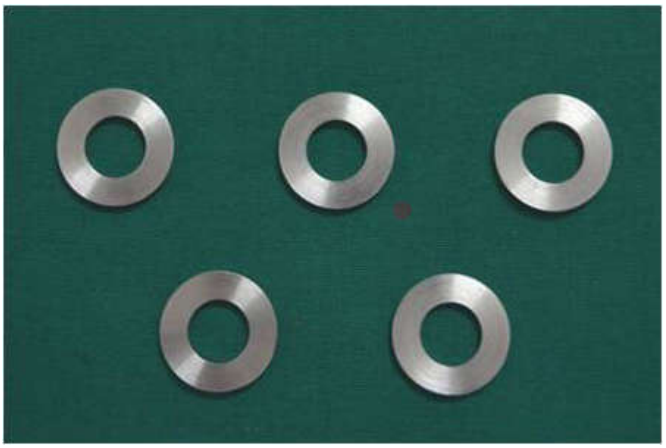

Figure 1. Precision machined metal rings

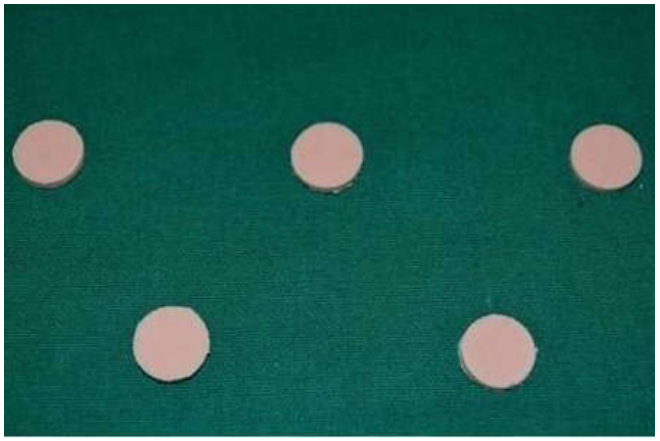

Figure 2. Alginate specimens

Table 1. Comparative effectiveness of various disinfecting agents and Distilled water on the surface of alginate for bacterial reduction ANOVA TEST

	Distilled Water Control, (Group A)	0.525% Sodium Hypochlorite (Group B)	2% Gluteraldehyde (Group C)	(1:213) Iodophor (Group D)	F Value	P Value (One Way ANOVA)
Mean CFU	10^8	1.5×10^3	6.4×10^3	27×10^3		
SD	0.00	2.6×10^3	5.8×10^3	9.6×10^3	$1.1X10^9$	< 0.001*

Within group comparison

CFU with

- 0.525% sodium hypochlorite < 2% gluteraldehyde, p = 0.140
- 0.525% sodium hypochlorite and 2% gluteraldehyde< (1:213) iodophor, p <**0.001***
- 0.525% sodium hypochlorite, 2% gluteraldehyde and (1:213) iodophor< distilled water (control group), p <**0.001***

*p≤0.05 is statistically significant

Table 2. Post Hoc Test

Groups		Mean Difference (I-J)	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
1	2	99998426.6667*	.000	99992669.745	100004183.588
	3	99993520.0000*	.000	99987763.078	99999276.922
	4	99972666.6667*	.000	99966909.745	99978423.588
2	1	-99998426.6667*	.000	-100004183.588	-99992669.745
	3	-4906.6667	.140	-10663.588	850.255
	4	-25760.0000*	.000	-31516.922	-20003.078
3	1	-99993520.0000*	.000	-99999276.922	-99987763.078
	2	4906.6667	.140	-850.255	10663.588
	4	-20853.3333*	.000	-26610.255	-15096.412
4	1	-99972666.6667*	.000	-99978423.588	-99966909.745
	2	25760.0000*	.000	20003.078	31516.922
	3	20853.3333*	.000	15096.412	26610.255

Figure 3. Staphylococcus aureus growth in nutrient broth after 24 hours

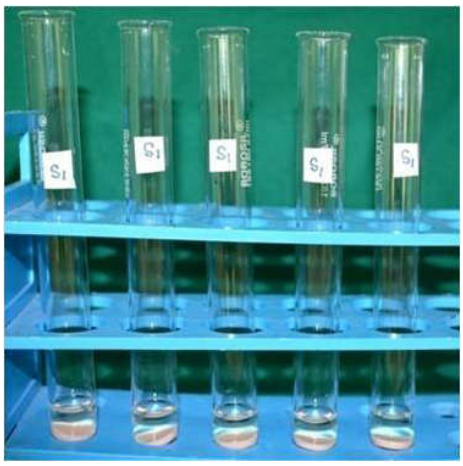

Figure 4. Alginate Disk in nutrient broth for 30 seconds

Figure 5. Nutrient Broth incubated for 24 hrs at 37 degree Celsius

DISCUSSION

Dentists and dental technicians are exposed either directly or indirectly to a wide variety of microorganisms in the blood and saliva of patients. These microorganisms cause infectious diseases such as common cold, pneumonia, tuberculosis, herpes, viral hepatitis and AIDS (Matyas *et al*., 1990). Study carried out by Leung and Schonfeld demonstrated that casts made of dental stone poured against contaminated impressions are medium for cross – contamination among patients and dental personnel (Leung and Schonfeld, 1983). Use of effective disinfection procedures in dental clinic and dental laboratory are necessary to prevent such cross contamination among dentists, dental assistants, dental laboratory technicians and patients (Shillingburg *et al*., 1997). Irreversible hydrocolloid is the most common impression material used in daily practice. However, because of its hydrophilic mechanism, it gets easily contaminated with microorganisms present in the oral cavity. Irreversible hydrocolloid carries significantly higher number of bacteria than elastomeric materials (Ghahramanloo *et al*., 2009). Disinfection of impressions is a challenging task (Ghahramanloo *et al*., 2009). Disinfectant must effectively kill the microorganisms that are transported on the impression from the mouth without damaging the impression or reducing its dimensional accuracy (Hiraguchi *et al*., 2010). It has been estimated that more than a million dental impressions are made by dentists for the fabrication of crowns, bridges, dentures, orthodontic appliances, and many other dental devices. If contaminated, beside the direct threat to the dentist, the transmission of microorganisms from impressions to dental laboratory technicians seems likely. The disinfection protocol is an essential precaution for preventing cross-infection and protecting laboratory personnel. ADA recommendation for disinfection indicates that impressions should be rinsed with water to remove blood, saliva and debris and then disinfected before being poured in stone or sent to dental laboratory. It states that irreversible hydrocolloid impressions should be rinsed with water and gently shaken to remove excess water. The impression should then be coated with disinfectant and sealed in plastic bag for 10 minutes to avoid evaporation of disinfectant and immediately cast in dental stone (Ghahramanloo *et al*., 2009). CDC recommends that any material that contact intact mucous membranes should be sterilized or receive high–level disinfection. According to ADA, disinfection should be used at room temperature and spray technique does not cause any significant change in dimensional stability and surface detail reproduction of irreversible hydrocolloid material (Connor C. Cross, 1991). Various disinfectants are available in the market with different mechanism of action. Controversy always arise which disinfectant is best for daily use in general practice. So the purpose of this microbiological study was to evaluate the efficacy of three commercially available disinfectants that is 0.525% Sodium Hypochlorite, 2% Glutaraldehyde and 1:213 Iodophor.

In this study, Staphylococcus Aureus strain was used to contaminate the alginate specimens because it is one of the most common bacteria found in the oral cavity. They are the most common cause of localized suppurative lesions in human beings. They may cause endocarditis and postoperative infections. They gain resistance to penicillin and other antibiotics by producing beta-lactamase. This enhances their importance as human pathogens, especially in the hospital environment (Ananthanarayan and Paniker, 2009). A total of 60 specimens were prepared with irreversible hydrocolloid material and were divided into four groups for evaluating the efficacy of three different disinfectants. Group A specimens served as the control specimens and did not receive any disinfection procedure and were washed with distilled water only. Group B, C and D specimens were disinfected after contamination with 0.525% Sodium Hypochlorite, 2%

Glutaraldehyde and (1: 213) Iodophor respectively. The results obtained from microbiological study showed that 53.33% (8 out of 15) of the samples disinfected with 0.525% Sodium Hypochlorite showed no growth of bacterial strain, whereas, 33.33% (5 out of 15) of the samples disinfected with 2% Gluteraldehyde showed complete disinfection. Bacterial colonization with the use of 0.525% sodium hypochlorite was 1.5 x 10^3 CFU/ml and of 2% Gluteraldehyde was 6.4 x 10^3 CFU/ml. Bacterial colonization with the use of (1:213) Iodophor was greater i.e. 27 x 10^3 CFU/ml as compared to 0.525% Sodium Hypochlorite and 2% Gluteraldehyde. However, the bacterial counts for samples disinfected with (1:213) Iodophor were much lesser as compared to the control group (Distilled water) which showed uncontrolled growth i.e. 1 x 10^8 CFU/ ml. Thus among the disinfectants used, 0.525% sodium hypochlorite showed the best results, whereas, (1:213) Iodophor was found to be least effective.

The result of this study is in accordance with the study carried out by Ahmad *et al* (2009). They studied the efficacy of three spray disinfectants i.e, 0.525% sodium hypochlorite, deconex and sanosil on contaminated alginate disks. Microbiological investigation was carried out following disinfection and they found that use of 0.525% Sodium Hypochlorite sprayed onto alginate surface effectively disinfected 96.6% of the samples (Ghahramanloo *et al*., 2009). Bustos *et al*. also studied the effectiveness of two disinfectants i.e, 0.5% Sodium Hypochlorite and 2% Glutaraldehyde on irreversible hydrocolloid and silicone impression material. They concluded that 5 minutes immersion in 0.5% NaOCl or 2% Glutaraldehyde is effective to reduce the level of bacterial contamination and hence the risk of cross infection (Bustos *et al*., 2010). Badrian H *et al* studied the effect of spraying three different disinfectants i.e. 0.525% sodium hypochlorite, Deconex and Epimax on condensational silicone impressions, Deconex showed the best results compared to the other agents (Badrian *et al*., 2015). The result of this study is similar with the study carried out by Hamid Badrian *et al*. They investigated the effect of three different types of disinfectants (0.525% Sodium Hypochlorite, Deconex, Epimax) on alginate impression material after 5 minutes and 10 minutes. They concluded that alginate can be effectively disinfected by all three types of disinfecting agents after spraying but Epimax showed the highest disinfectant action after 10 minutes (Badrian *et al*., 2012). 0.525% Sodium Hypochlorite is the least expensive and readily available disinfectant. It is bactericidal, virucidal and fungicidal disinfectant. It is 100% effective in decontaminating objects immersed in it for 10 min or longer (Gerhart and Williams, 1991). Hypochlorite is listed among the ADA's acceptable disinfectants for dental impressions (Council on Dental Materials, 1991). Glutaraldehyde is an irritant and some individuals develop acute sensitivities (Best practices for the safe use of glutaraldehyde in health care, 2006). These sensitivities may be displayed as itching of the skin with slight redness and swelling or yellowing of the skin with prolonged exposure, or irritation to eyes and nasal membranes, headache, coughing, sneezing and asthma-like symptoms. Glutaraldehyde can be absorbed by inhalation, ingestion and through the skin. In the present study, the samples were disinfected by the spraying method and this method was found to be effective in disinfecting 53.33% (8 out of 15) of the samples disinfected with 0.525% Sodium Hypochlorite. In this regard, studies by Ghahramanloo *et al*., Westerholm *et al*. and Rueggeberg *et al*. have also shown that impressions can be effectively disinfected by spraying hypochlorite and placing them in a sealed plastic bag (Ghahramanloo *et al*., 2009; Westerholm *et al*., 1992; Rueggeberg *et al*., 1992) The data from this study suggests that, 0.525% Sodium Hypochlorite is the most effective disinfecting agent. Due to its effective antimicrobial effect, impression spraying with Sodium Hypochlorite following removal from the patient's mouth is strongly advocated by the data from the present study. By disinfecting the impressions properly, the safety of both the dentists and the laboratory technicians would be guaranteed.

Conclusion

Within the limitations of this in vitro study, following conclusions can be drawn:

1. 53.33% (8 out of 15) of the samples disinfected with 0.525% Sodium Hypochlorite (GroupB) showed no growth of bacterial strain.
2. 33.33% (5 out of 15) of the samples disinfected with 2% Gluteraldehyde (Group C) showed no growth of bacterial strain.
3. All the samples disinfected with (1:213) Iodophor (Group D) showed bacterial growth but colonies were countable.
4. Bacterial colonization with the use of (1:213) Iodophor (Group D) was greater (27 x 10^3 CFU/ ml) as compared to 0.525% Sodium Hypochlorite (1.5 x 10^3 CFU/ml) and 2% Gluteraldehyde (6.4 x 10^3 CFU/ ml).
5. All the samples washed with distilled water showed uncontrolled bacterial growth (1 x 10^8 CFU/ ml) i.e. the colonies were not countable.
6. Sodium Hypochlorite is the most effective disinfectant followed by Glutaraldehyde and Iodophor.

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