ISSN (O) : 2582-3271
PubMed-National Library of Medicine - ID: 101766917

International Journal of Dental Science and Clinical Research (IJDSCR)

Dr. Angel Vaidic Publication

Available Online at: http://www.ijdscr.org

Volume – 2, Issue – 4, July – August - 2020, Page No. : 10 - 16

Efficacy of Three Different Disinfectants on Alginate Impression Material by Spray Method: An In Vitro Study

[1]Dr. Abhyutthan Sinha, Post Graduate Student, Department of Prosthodontics, Crown and Bridge, New Horizon Dental College and Research Institute, Bilaspur, Chhattisgarh, India

[2]Dr. Arunachalam Sudheer, H.O.D. and professor, Department of Prosthodontics, Crown and Bridge, New Horizon Dental College and Research Institute, Bilaspur, Chhattisgarh, India

[3]Dr. Anupam Purwar, Reader, Department of Prosthodontics, Crown and Bridge, New Horizon Dental College and Research Institute, Bilaspur, Chhattisgarh, India

[4]Dr. Sudeepti Soni, Senior lecture, Department of Prosthodontics, Crown and Bridge, New Horizon Dental College and Research Institute, Bilaspur, Chhattisgarh, India

[5]Dr. Prajakta Barapatre, Post Graduate Student Department of Prosthodontics, Crown and Bridge, New Horizon Dental College and Research Institute, Bilaspur, Chhattisgarh, India

[6]Dr. Megha Sahu, Post Graduate Student, Department of Prosthodontics, Crown and Bridge, New Horizon Dental College and Research Institute, Bilaspur, Chhattisgarh, India

Citation Of This Article: Dr. Abhyutthan Sinha, Dr. Arunachalam Sudheer, Dr. Anupam Purwar, Dr. Sudeepti Soni, Dr. Prajakta Barapatre, Dr. Megha Sahu, "Efficacy of Three Different Disinfectants on Alginate Impression Material by Spray Method: An In Vitro Study", IJDSCR July – August - 2020, Vo2. – 2, Issue -4, P. No. 10-16.

Corresponding Author: Dr. Abhyutthan Sinha, Post Graduate Student, Department of Prosthodontics, Crown and Bridge, New Horizon Dental College and Research Institute, Bilaspur, Chhattisgarh.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

According to a study conducted by Egusa et al, he observed that impressions removed from patients' mouth, contained microorganisms like *Streptococci, Staphylococcus aureus, Methicillin resistant* Staphyloccocus and *Candida* .[3]

Staphylococcus aureus is associated with a number of distinct oral infections like angular cheilitis, parotitis, and staphylococcal mucositis.[4] Pawel J. Zawarzki et al identified *Escherichia coli* as a pathologic constituent of the oral cavity associated with hand-to-mouth cross-infections from infected individuals and may cause respiratory and urinary tract infections, severe diarrhoea in children and adults, particularly in individuals with weakened immune systems.[1]*Candida* causes common

opportunistic infections known as oral candidiasis, found in patients with immune deficiency.[3]

As impressions form the first contact of dental materials with the patient, knowledge of their disinfection is of utmost importance. The Federation Dentaire International requires, as a standard precaution, that all patients' impressions should be rinsed under running water to remove saliva and visible blood. Then, they should be disinfected and afterwards placed and sealed in proper containers and labelled as disinfected.[5]

The American Dental Association (ADA), The Centre for Diseases Control and Prevention (CDC) with the Australian Dental Association, have all listed a similar standard procedure for all dental impressions.[6,7]

Alginate is an elastic irreversible hydrocolloid impression material, which form an inseparable part of indirect restorations and it is one of the most frequently used dental materials.[8,9]The fear of transmission of microbes to users of alginate impressions has led to the practice of either spraying (Kakigawa et al., 1989) or soaking alginate impressions in solutions of disinfectants (Setcoser al., 1984; Minagi et al., 1986; Thomasz et al., 1986; Marker et al., 1989). [6,7]The present study was conducted to investigate the disinfection efficacy of Glutaraldehyde solution (2%), Sodium hypochlorite solution (0.525%) and Chlorhexidine (0.2%) solution by spray method on alginate impression material.

Methodology

The present study was conducted at New Horizon Dental College and Research Institute, Chhattisgarh, Approval to conduct the study was obtained from the college authorities and Ethical Review Committee. The study was conducted during June to December 2017.

Preparation of microbial broth

Three tests tubes were selected and sterilised by autoclaving. In each of the test tube 5 ml of brain heart infusion broth was poured aseptically. The first test tube was marked as "S", the second one as "E" and the third one as "C". To the test tube "S", 1-2 colonies of a 48 hours culture of *Staphylococcus aureus* was added with the help of a Nichrome wire which was heat sterilised in a Bunsen burner. To the test tube "E", 1-2 colonies of a 48 hours culture of *Escherichia coli* was added in the same way. To the test tube "C", 1-2 colonies of a 72 hours culture of *Candida albicans* was added in the same way.

The three test tubes were put into an incubator at 37^{o}C for 1 hour. After one hour the test tubes were taken out and turbidity of the broth in the tubes was checked. A turbidity equivalent to 0.5 Mcfarland solution was accepted. This meant that microbial density of the broth was equivalent to 1.5 X 10^{8} CFU/ml.

After the required turbidity was verified, the contents of all the three test tubes were poured into as sterile container.

Preparation of The Samples

A sterile 1 ml disposable insulin syringe was selected. A sharp B.P. knife was used to make an opening of the syringe larger (4 mm) and length of the barrel of the syringe 60 mm.

Alginate mixing spatula was autoclaved at 121^{o}C for 15 minutes at 15 lbs pressure and rubber bowl was disinfected with Sodium Hypochlorite (5%) for 1 hour. According to manufacturer's instruction alginate impression material and water was dispensed and mixed using the sterilised spatula without a handle.

The material was loaded into the syringe and allowed to set. The set alginate was syringed out onto a sterile petridish to make a string of alginate material of 4 mm diameter and 60 mm length. The string was cut using a

sterile B.P. knife at 15 mm length to produce 4 samples of alginate. Each sample was a small cylinder of 15 mm length and 4 mm diameter 10 such syringes were prepared and the same procedure was followed to make 40 samples of alginate.

Inoculation of samples with microbes

A pair of tweezers was selected and autoclaved. The tip of the Tweezers was sterilised in open flame till it became red hot. It was then cooled and used to transfer the samples into the sterile container containing the mixture of microbial broth. The sterile container was then screw-capped and placed into an incubator at 37°Cfor 1 hour. After 1 hour of incubation, the container was taken out of the incubator. All the samples were washed in running tap water for 30 seconds.

The samples were divided into 4 groups, 10 samples were allotted for each group. GROUP A – samples were not treated with any disinfectant. After they were washed with running water, each sample was placed into an airtight bag with moist cotton. GROUP B – 10 samples were sprayed with Glutaraldehyde 2% solution using a sprayer. Spraying of disinfectant was done at the rate of 10 puffs for 15 seconds. The samples were then individually placed into air tight plastic bags. GROUP C – 10 ml of Sodium Hypochlorite (5.25%) solution was mixed with 90 ml of distilled water to produce 100 ml of Sodium Hypochlorite (0.525%) solution. 10 samples were sprayed with Sodium Hypochlorite (0.525%) solution using a sprayer. Spraying of disinfectant was done at the rate of 10 puffs for 15 seconds. The samples were then individually placed into air tight plastic bags. GROUP D - 10 samples were sprayed with Chlorhexidine (0.2%) solution using a sprayer. Spraying of disinfectant was done at the rate of 10 puffs for 15 seconds. The samples were then individually placed into air tight plastic bags.

Making of microbial cultures

After 5 minutes, following the previous procedure, 5 samples of GROUP B, C and D were taken out of their respective air tight bags. One sample was picked up by sterilised Tweezers and slid directly on a Blood agar plate. This procedure was then carried out for every sample. Separate blood agar plates were used for each sample. After 10 minutes the remaining samples were taken out of their respective air tight bags and the same procedure was followed for transferring them on to the agar plates. All the plates were streaked in the same pattern by the help of sterilised Nichrome wire and were incubated at 37°Cfor 48 hours.

Identification and colony count

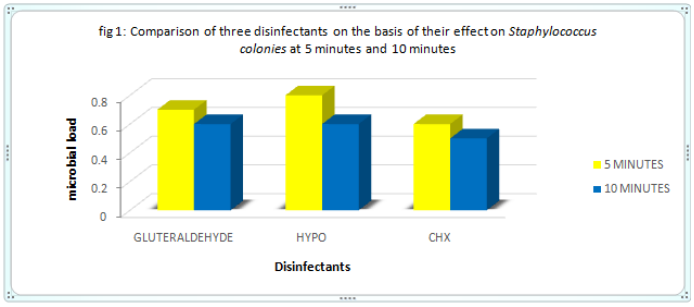
After the incubation period, the Blood agar plates were examined for microbial growth. Once identified, colonies on culture for differing morphologies were counted on the blood agar plates. According to colony characteristics, gram staining was performed following standard guidelines. The glass slides were viewed under an optical microscope for identifications of the microbes.

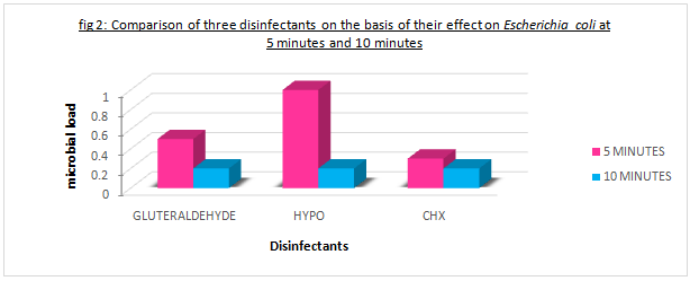
Results

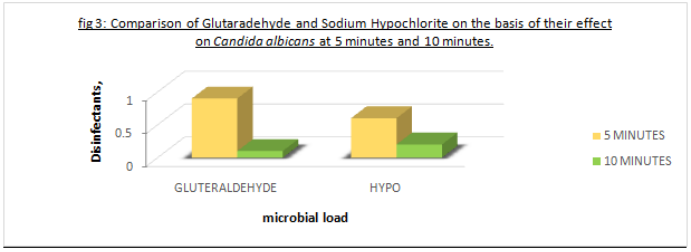
Data obtained were tabulated and analyzed using SPSS statistical software 23 was used. The present study was conducted to find efficacy of three different disinfectants on alginate impression material by spray method. Table 1 shows ANOVA comparison of the disinfection efficacy of the three disinfectants at 5 minutes and 10 minutes. A significant difference was observed **(P<0.05).**

Figure 1 & 2 illustrates that chlorohexidine has high effect on *Staphylococcus colonies* & *Escherichia coli* at 5minutes and when compared with Glutaraldehyde, Sodium Hypochlorite whereas at 10 minutes results showed equal effect on the microorganisms. Figure 4 shows Chlorhexidine effect on *Candida albicans* at 5

minutes and 10 minutes when compared. **Table 2** shows a comparison of the efficacy of the three disinfectants at 5 minutes on disinfection of *Staphylococcus aureus, Escherichia coli, Candida albicans.* There was a significant reduction of *Staphylococcus aureus, Escherichia coli,* Candida *albicans* colonies by Chlorhexidine 0.2% at 10 minutes for all samples **(P<0.05). Table 3** shows a comparison of the efficacy of the three disinfectants at 10 minutes on disinfection of *Staphylococcus aureus, Escherichia coli, Candida albicans.* There was a significant reduction of *Staphylococcus aureus, Escherichia coli ,Candida albicans* colonies by Chlorhexidine 0.2% at 10 minutes for all samples **(P<0.05).**







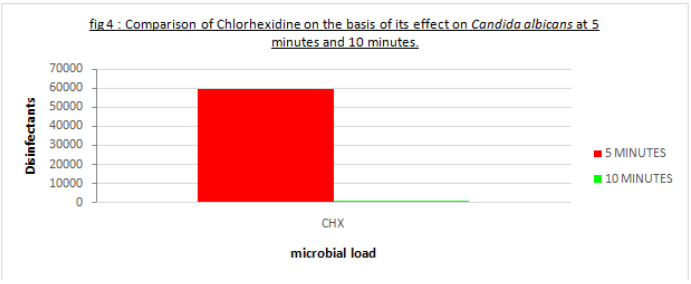


Table 1: Comparison of microbial load in samples washed with water only

Groups	N	Mean	Std.Deviation
Staphylococcus aureus	10	136300.00	13864.824
Escherichia coli	10	13090.00	1356.015
Candida albicans	10	1234.00	287.796

Table 2: Comparison of efficacy of three disinfectants at 5 minutes on Staphylococcus aureus, Escherichia coli, Candida albicans..

Microorganisms	Disinfects	Df	T-test	p-value
Staphylococcus aureus	GLUTERALDEHYDE	18	31.087	.000*
	SODIUM HYPOCHLORITE			
	CHLORHEXIDINE			
Escherichia coli	GLUTERALDEHYDE	18	30.526	.000*
	SODIUM HYPOCHLORITE			
	CHLORHEXIDINE			
Candida albicans	GLUTERALDEHYDE	18	13.558	.000*
	SODIUM HYPOCHLORITE		13.557	
	CHLORHEXIDINE		3.102	.006*

* STATISTICALLY SIGNIFICANT DIFFRENCES EXIST BETWEEN THE GROUPS (P<0.05)

Table 3: Comparison of efficacy of three disinfectants at 10 minutes on Staphylococcus aureus, Escherichia coli, and Candida albicans

Microorganisms	Disinfects	Df	T-test	p-value
Staphylococcus aureus	GLUTERALDEHYDE	18	31.087	.000*
	SODIUM HYPOCHLORITE			
	CHLORHEXIDINE			
Escherichia coli	GLUTERALDEHYDE	18	30.525	.000*
	SODIUM HYPOCHLORITE			
	CHLORHEXIDINE			
Candida albicans	GLUTERALDEHYDE	18	13.549	.000*
	SODIUM HYPOCHLORITE		13.552	
	CHLORHEXIDINE		30.204	

* STATISTICALLY SIGNIFICANT DIFFRENCES EXIST BETWEEN THE GROUPS (P<0.05)

Table 4: ANOVA test to compare 5 minute values of three disinfectants

Time factor	Test	Df	Mean	P value
5 minutes	Between Groups	8	3.52	.000*
	Total	89		
10 minutes	Between Groups	8	1.2	.000*
	Total	89		

ANOVA comparison of the disinfection efficacy of the three disinfectants at 5 minutes & 10minutes . A significant difference was observed **(P<0.05).**

Images

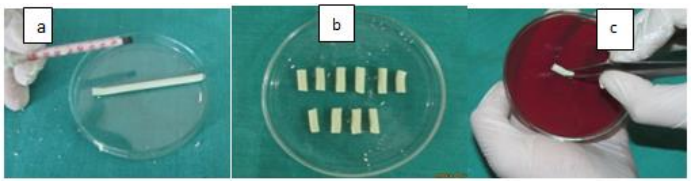
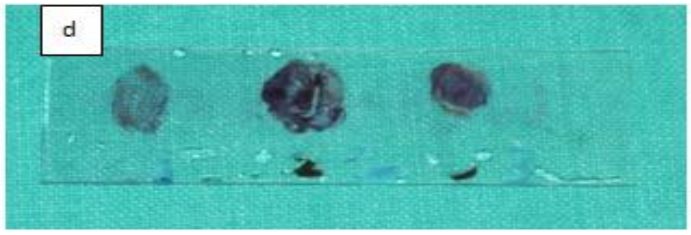


Figure 9: (a) 60 mm string of alginate (b) 15 mm alginate samples Prepared (c) Alginate sample slid on Blood agar plates after storage period



(d) Microbes smeared on glass slide and gram stained

Discussion

In the present study, alginate samples were infected with three micro-organisms and the efficacy of three disinfectants (Glutaraldehyde 2%, Sodium Hypochlorite 0.525% and Chlorhexidine 0.2%) to reduce or remove the pathogens from the alginate samples by spray method was investigated.

All samples of GROUP A which were only washed with water showed heavy growth of all three micro-organisms. These finding were in tandem with the findings of Samarnayake L. P. and Jennings K. J. (1992) and Sukhija U. et al (2009).[10,11]It was observed that Glutaraldehyde 2% as a spray disinfectant at 5 minutes and 10 minutes on *Staphylococcus aureus, Escherichia coli* and *Candida albicans* respectively was highly effective. There was no significant difference in the effect of Glutaraldehyde 2% on the three micro-organisms at 5 minutes and 10 minutes. There was an effective reduction in the microbial load at both time intervals.

These finding were in tandem with the findings of Sukhija U. et al (2009) and Doddamani S., Patil R. A., and Gangadhar S. A. (2011).[11,12]It was observed that Sodium Hypochlorite 0.525% as a spray disinfectant at 5 minutes and 10 minutes on *Staphyloccous aureus, Escherichia coli* and *Candida albicans* respectively was effective. There was no significant difference in the effect of Sodium Hypochlorite 0.525% on the three micro-organisms at 5 minutes and 10 minutes. There was an effective reduction in the microbial load at both time intervals.

These finding were in tandem with the findings of Badrian H., Ghansemi E., Khalinghinejad N. and Hosseini N. (2012) and Hemmati MD et al (2017). [13,14]It was observed that Chlorhexidine 0.2% as a spray

disinfectant at 5 minutes and 10 minutes on *Staphyloccous aureus, Escherichia coli* and *Candida albicans* respectively. There was no significant difference in the effect of Chlorhexidine 0.2% on *Staphyloccousaureu* and *Escherichia coli* at 5 minutes and 10 minutes. But there was a significant difference in the effect of Chlorhexidine 0.2% on *Candida albicans* at 5 minutes and 10 minutes.

These finding were in tandem with the findings of Toyuz L.Z.G. and Rosen M. (1991) and Gupta R., Aggrawal R., Tiwari S. and Bharat A. (2016).At 10 minutes period, Both Glutaraldehyde 2% and Sodium Hypochlorite 0.525% were equally efficacious in reduction of *Staphylococcal* and *Escherichia* colonies. In case of *Candidal* colonies, Glutaraldehyde 2% was found to be more efficacious than the other two disinfectants at the 10 minutes period.

Conclusion

Samples of alginate without any disinfection procedure showed heavy growth of all three micro-organisms on blood agar plates. Alginate samples that underwent disinfection procedures showed significant reduction of microbial colonies. The disinfectants were found to be efficacious in their antimicrobial activity on alginate by spray method. Glutaradehyde 2% was found to be most the effective spray disinfectant on alginate impression material both at 5 minutes and 10 minutes.

References

1. Patil S, Rao R S, Sanketh DS, Amrutha N. Microflora in oral diseases. The Journal of Contemporary Dental Practice, November-December 2013;14(6):1202-1208.
2. Powell GL, Runnells RD, Saxon BA, Whisenant BK. The presence and identification of organisms transmitted to dental laboratories. J Prosthet Dent 1990;64:235-7.
3. Egusa H, Watamoto T, Matsumoto T, Abe K, Kobayashi M, Akashi Y, et al. Clinical evaluation of the efficacy of removing microorganisms to disinfect patient-derived dental impressions. Int J Prosthodont 2008;21:531-8.
4. McCormack M G etal.Staphylococcus aureus and the oral cavity: An overlooked source of carriage and infection? American Journal of Infection Control 2015;43;35-7.
5. Recommendations for hygiene in dental practice, including treatment for the infectious patient. Fédération Dentaire Internationale. A revision of technical report no 10. Int Dent J 1987;37:142-5.
6. Infection control recommendations for the dental office and the dental laboratory. ADA Council on Scientific Affairs and ADA Council on Dental Practice. J Am Dent Assoc 1996;127:672-80.
7. Recommended guideline for infection control in dental health care settings. CDC 2003.
8. Anusavice K, Rawls Rh, Shen C. Phillips' Science of Dental Materials 12th edition.
9. Rudd k, Morrow R, Strunt R. Accurate alginate impressions. J Prosthet dent 1969;22;3.
10. Samaranayake LP, Hanjan M, Jennings KJ. Carriage of oral flora on irreversible hydrocolloid and elastomeric impression materials. J Prosthet Dent. 1991; 65 (2): 244-9.
11. Sukhija U., Rathee M., Kukreja N., Khindria S., Singh V., Palaskar J.. Efficacy of various disinfectants on dental impression materials. Internet J Dent Sci. 2009;9(1):1–6.
12. Doddamani S, Patil RA, Gangadhar SA. Efficacy of various spray disinfectants on irreversible

hydrocolloid impression materials: An in vitro study. Indian J Dent Res 2011;22:764-9.

13. Badrian H, Ghasemi E, Khalighinejad N, Hosseini N. The effect of three different disinfection materials on alginate impression by spray method. ISRN Dent 2012 Jul;2012:695151.
14. Hemmati MD et al. Antimicrobial Effects of SeptiTurbo, Deconex (Solarsept) and 0.525% Sodium Hypochlorite Spray on Alginate Impression Materials. Journal of Dental School 2017; 35(3): 163-169.
15. Toyuz L Z G, Rosen M. Disinfection of alginate impression material using disinfectants as mixing and soak solutions. J. Dent 199 1.
16. Choudhary G. K. et al. Disinfectant Efficacy of 0.525% Sodium Hypochlorite andEpimax on Alginate Impression Material. The Journal of Contemporary Dental Practice, January 2018;19(1):113-11.