Original Article

Efficacy and Effects of two Application Methods of Glutaraldehyde Disinfectant Solution on Alginate Impression

Haque SM[1], Nipa KN[2], Kamruzzaman[3], Islam P[4], Jahan L[5], Basak DK[6], Sultana N[7]

Abstract

Background: *Prevention of cross-infection between dental surgery and the laboratory is of paramount importance to protect patients and staff. Dental impressions are considered potentially infectious items as they are contaminated with the patient's saliva and blood. If present in high enough numbers, pathogens can survive several days on impressions and then be transferred onto set gypsum material. Appropriate disinfection of the impression tray must inhibit cross-contamination between sick people, oral surgeons, and laboratory technicians caused by the impression material. Even though impression materials cannot withstand heat disinfection, people should be molecularly decontaminated, which may cause the dimensional accuracy of a dental impression to be degraded or lost. Because washing the impression with water sometimes doesn't wipe away pollutants, disinfecting the impression and further rinsing the disinfectant off is required. In addition to chlorine combinations, phenol and iodide combinations have also been used as antiseptics; glutaraldehyde is only one of the few that has been used as a cleaning agent recently. Objective: This study was conducted to determine the efficacy and effects of two different glutaraldehyde disinfectant solution application methods on alginate impression. Method and Materials: A total of 167 maxillary alginate impressions were disinfected using the spray technique in group A, whereas a total of 167 maxillary alginate impressions were disinfected using the soaking approach in group B. Surface changes were determined by measuring the distance between two defined locations on the assumption exterior with slide callipers in millimetres (mm) with both the aid of a dental compound microscope loop and recording the results in millimetres (mm). Results: After culturing the sample pre-disinfected and rinsed with distilled water, 94 (56.29 per cent) samples were analyzed to have no growth in group A and 102 (61.08 per cent) samples were found to have no development in group B. The remainder of the samples contained microflora, Acinetobacter, and Pseudomonas SPP, and the p-value was 0.73, indicating that they were not statistically important findings. 100% of the samples from both groups were completely independent of the microorganisms after already being disinfected with 2% Glutaraldehyde for 15 minutes. There was no statistically significant difference in mean surface change between alginate samples disinfected using the spray approach and those sanitized using the immersive experience method (p 0.05).Conclusion: As a result, although the two purification strategies (spray and immersion) used to remove microorganisms from the alginate impression surface (two per cent glutaraldehyde solution) were effective, the immersion method caused a further acceleration in the rate than the spray technique.*

Key wards: *Alginate Impression, Cross-infection, Glutaraldehyde Solution, Micro-organisms, Surface dimensions.*

Introduction

In prosthodontics, taking impressions of the oral cavity is standard practice. It is nearly hard to match bad teeth before taking an impression of the mouth. As a result, the importance of the impression cannot be overstated. The oral cavity serves as a point of admittance for microbial cells into the body. Enteral organisms can always be found in large numbers in saliva. At the time of taking the impression, fluids contain pathogens that contaminate both the tray and the impression material used in the procedure. Such pathogens are capable of causing cross-infection among dentists, dental consumables, and laboratory technicians. Cross-infection and surface modification of the impressions can be prevented by using the proper method of disinfection of the impression material, which is essential.[1]

The prevention of cross-infection between dental surgery and the laboratory is critical for the protection of patient populations and laboratory personnel.[2] Infection control experts believe tooth impressions to be exposed to infectious components because they are contaminated with the participant's saliva and blood. Depending on their population density, microbes could even sustain appearances over many occasions before being converted to set gypsum material.[3] Because impression materials are unable to withstand thermal disinfection, individuals should be pharmacologically sanitized before use. While all pathogens have been killed, purification has been accomplished, resulting in the most thorough bacterial removal of pollutants that would be currently possible. While cleansing has become less deadly than pasteurization, it is intended to kill disease-causing microscopic organisms while leaving intact bacterial endotoxins in the treated area.[4] A disinfectant's effectiveness against vegetative bacteria, tubercle bacilli, fungal spores, lipid- and non-lipid-containing infectious agents, and microorganisms' pathogens is determined by its level of efficacy against each of these pathogens.[5]

Immersion disinfection of dental impressions is the benchmark, even though spray sanitizing methods exist as an alternative.[6] The antimicrobial effects of spraying and immersing methods were nearly equal, whereas simple water flushing had no discernible disinfectant influence on the bacteria.[7]

A range of different substances is effective for assumption sterilization, provided that each type is applied to the

1. Dr. Shehab Monirul Haque, BDS, MS (Prosthodontics), Assistant Professor, Prosthodontic department, Udayan dental college, Rajshahi, Bangladesh.

2. Dr. Kamrun Nahar Nipa, BDS, MS (Prosthodontics), BCS (Health), Lecturer, Department of Prosthodontics, Shaheed Suhrawardy Medical College and Hospital, Dhaka, Bangladesh.

3. Dr. Kamruzzaman, BDS, MS (Prosthodontics), Lecturer, Department of Oral and Maxillofacial Surgery, Sir Salimullah Medical College and Mitford Hospital, Dhaka, Bangladesh.

4. Dr. Piarul Islam, BDS, BCS (Health), MS (Prosthodontics). Assistant Professor, Dhaka Dental College, Dhaka.

5. Dr. Laila Jahan, BDS, MS (Prosthodontic), Assistant professor, Prosthodontics department, Dhaka dental college, Dhaka, Bangladesh.

6. Dr. Dilip Kumar Basak, BDS, MPH (Reproductive child health), Clinical Research Coordinator, Bio Dev Services Private Limited, Dhaka, Bangladesh.

7. Dr. Nahid sultana, BDS, MSc (Public Health), Graduate Public Health Student, Department of Community Medicine, International Medical University, Kuala Lumpur, Malaysia.

impression by the manufacturer's recommendations. Though it's been noted that simply washing the impression with liquid somehow doesn't disable pathogens, it has also been confirmed that deodorizing the impression and then further rinsing the disinfectant off is required to ensure the complete removal of pollutants. Because chemical disinfection is a surface phenomenon, the ground of the impression must be cleaned to consider removing any visible particles before getting immersed in the solvent mixture to ensure the maximum type of exposure with the cleaning solution occurs.

Perception sensations must be thoroughly cleaned before being sent to the laboratory by current infectious disease specifications. To prevent cross-infection from patient to dentist as well as exposure of laboratory personnel, disinfection is a critical step in the process. If the procedure is followed to the letter, disinfection has no effect on the accuracy or surface details reproduction from the impression if it is done correctly. Some chemicals, such as alcohols, chlorine combinations, phenol, and iodide combinations, have been used as disinfectants in previous studies, but glutaraldehyde has only been used in a few of them. As a result, this study was carried out to determine the efficacy and effects of two different application methods of glutaraldehyde disinfectant solution on alginate impression.

The hypotheses were whether immersion of alginate impression into Glutaraldehyde solution would eliminate that many microscopic organisms as an exploration of alginate impression into Glutaraldehyde spray would and that involvement of alginate impression into Glutaraldehyde solvent would cause a greater transformation in specular reflection than an exploration of alginate perception into Glutaraldehyde spray would. The results showed that both hypotheses were correct.

Materials and Methods

From January 2019 to December 2019, a prospective comparative experimental investigation has been carried out at the Department of Prosthodontics, Faculty of Dentistry, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh, in collaboration with the Bangladesh Dental Association. Using the Hygiene TM (Lot -95453 and 97260) from Dentamerica, USA, a total of 334 alginate impressions of the maxillary arch were taken from 334 patients with the maxillary entablature. Maxillary impressions were taken from patient populations who have been undergoing treatment in the prosthodontic department at the time of the procedure. The impression material was mixed with sterile water to prevent contamination from mixing water, and it was modified by the product's company standards. The impressions were chosen to take out of the patient's mouth after they had been allowed to set completely for several hours. All impressions were rinsed with distilled water to remove any food particles or dust particles that may have been left behind. Group-A is comprised of the impressions that have always been rinsed for 2 minutes with 250 mL of distilled water after which they had been dried. Approximately 20 mL of sterile normal saline was applied to the impression surface and then wobbled for 2 minutes to detach any microscopic organisms from the ground of the impression. In the following step, 2 ml of saline suspension from each impression was collected using an auto pipettor (Top Pette pipettes by Dragon Lab in Beijing) into an autoclavable test tube and covered with an autoclavable cover as a pre-disinfection sample. In the following 15 seconds, the impressions have always been sprayed with a 2 percent glutaraldehyde degreasing solution 10 times in a 15-second period. To remove the disinfectant solution from the alginate surface, impressions were washed thoroughly for 2 minutes with 250 mL of distilled water. A second time, 20 mL of sterile normal saline was immersed onto the impression surface, which was then vibrated for 2 minutes to detach the microorganisms from the impression surface and rinsed away. The auto pipettor was used to collect 2 ml of saline suspension from each impression for use as a post-decontamination specimen, which would then be coated with either a sterile lid to prevent contamination. During the second minute of the procedure, the impressions from Group B were rinsed with 250 mL of distilled water. A 20 mL solution of sterile normal saline might have been added to the impression load-carrying-carrying fluid, which was then shaken vigorously with a vibrator for 2 minutes to detach any microorganisms from the impression surface. Once each impression had been collected as a pre-disinfection sample, 2 mL of saline suspension from each impression was placed in a sterile test tube and covered with a sterile cover. Impressions were immersed in a 2 percent glutaraldehyde disinfectant solution for 2 minutes, after which they were rinsed with 250 mL of distilled water for another 2 minutes to remove any remaining cleaning solution. Vibrating the impression surface for 2 minutes with a turntable detaching the microorganisms from the impression surface after adding residue left fluid to 20 mL sterile normal saline helped to disconnect individuals. Post-disinfection samples were obtained from each impression as well as placed in a sterile test tube with an aseptic lid. Cudex (India), Jonson (India), and Jonson and Jonson (India). It was then diluted with distilled water to accomplish a 2 per cent density since it was only accessible at a 2.45 per cent concentration when it was purchased. Upon collection, secretions have been transported to the laboratory where they have been subjected to additional microbial contamination clinical laboratories.

Surface details determination procedure

Following the decontamination procedure, each sample was examined under a magnification loop to ensure was indeed free of contaminants. The interpoint measurement was performed by the slide caliper in millimeters (mm) again to detect any variability and detailed information on the tissue surface of the alginate impression. When using the dental magnifying loop (420, 3.5x zoom), the description was evaluated carefully with

the great assistance of the loop's headlight but instead recorded as post-disinfection calibrated information. The interpoint intervals among both 2 categories have always been assessed before and after sterilization, and the results have been compared. As previously stated, group A contains alginate samples sprayed with a 2 per cent glutaraldehyde decontamination remedy, while group B contains alginate samples that have been submerged in a 2 percent glutaraldehyde decontamination solution.

Microbiological laboratory procedure

A total of 2 microliters of pre- and post-disinfected fluids were transferred aseptically in sheep blood and MacConkey agar plates using the inoculation loop technique in the department of microbiology. Agar plates were labeled and incubated at 37oC for 24 hours in an aerobic environment (in the concentration of 5 percent CO2) in a Memmect incubator under aerobic conditions (in the presence of 5 percent CO2) (West Germany). Microscopically, microbial colonies were observed after 48 hours of cultivation. McConkey agar media were colored greenish by Pseudomonas SPP, while Acinetobacter (diplococci) were colored pinkish with a white body. The bacteria of mucus, such as cocci, were developed in culture media and produced a grape-like infrequent cluster when increased on agar media. The absence of production findings indicates that microorganisms didn't progress in culture media. In the case of constant variables, the unpaired t-test was used, while the Chi-square test was used in the case of categorical variables. Statistics were considered statistically significant when P values were less than 0.05.

Result

Table I: Presence of microbes after culturing of the samples pre-disinfected washed with dist

Micro-organism	Pre-disinfection		Total	p-value
	Group A (n=167) N (%)	Group -B (n=167) N (%)		
No growth	94(56.29)	102(61.08)	196	0.374^{ns}
Acinetobacter (Scanty Growth)	4(2.40)	8(4.79)	12	0.240^{ns}
Normal Flora	57(34.13)	49(29.34)	106	0.347^{ns}
Pseudomonas SPP (Scanty Growth)	12(7.19)	8(4.79)	20	0.357^{ns}
Total	167(100)	167(100)	334	

- Group A= Maxillary alginate impression sprayed with 2% glutaraldehyde disinfection solution
- Group-B= Maxillary alginate impression immersed in 2% glutaraldehyde disinfection solution.
- X2 test was done for significance.

Data from samples that had previously been infected but had been cleaned with distilled water are shown in **Table 1**. No progress was identified in 94 of the 102 samples in group A, and in 102 of the samples in group B. Normal flora, Acinatobector (Scanty Growth), and Pseudomonas SPP (Scanty Growth) p-value was 0.05, which was not statistically significant and non-pathogenic in the rest of the samples.

Table II: Findings of the Microbial count after post-disinfecting 2% Glutaraldehyde between two groups.

After 2% Glutaraldehyde	Study group		Total	p-value
	Group A (n=167) n (%)	Group-B (n=167) n (%)		
No growth	167(100)	167(100)	334	
Acinetobacter (Scanty Growth	00	00	00	
Normal Flora	00	00	00	
Pseudomonas SPP (Scanty Growth)	00	00	00	
Total	167(100)	167(100)	334	

- Group A= Maxillary alginate impression sprayed with 2% glutaraldehyde disinfection solution
- Group-B= Maxillary alginate impression immersed in 2% glutaraldehyde disinfection solution.

Table III: Mean surface change of alginate impression in spray method (n=167).

	Group=A		p-value
	After Distilled Water Wash (n=167)	After Spraying 2% Glutaraldehyde (n=167)	
	Mean(±SD)	Mean(±SD)	
Surface change (in mm)	3.38(±0.81)	3.59(±0.80)	0.01

- Group A= Maxillary alginate impression sprayed with 2% glutaraldehyde disinfection solution.
- p-value reached from paired sample t-test.

Samples post-infection with 2 percent Glutaraldehyde were inoculating, and in **Table II**, 167(100%) samples were observed to have no growth in group A and 167(100%) in group B. There was no plausible pathogen between the two groups of incubators after 48 hours. For the Spray Process, the mean surface change of alginate impression, 3.38 mm after distilled water was washed and 3.59 (0.80) mm after spraying 2 percent glutaraldehyde (p 0.05) was statistically significant **(Table III)**.

Table IV: Mean surface change of alginate impression in immersion method (n=167).

	Group-B		p-value
	After Distilled Water Wash (n=167)	After immersing in 2% Glutaraldehyde (n=167)	
	Mean(±SD)	Mean(±SD)	
Surface change (in mm)	3.43(±0.80)	3.73(±0.80)	<0.001

- Group-B= Maxillary alginate impression immersed in 2% glutaraldehyde disinfection solution.
- p-value reached from paired sample t-test.

Table IV shows the average surface change of alginate impressions in the dispersion method, 3.43(0.80) mm after a distilled thoroughly rinse and 3.73(0.80) mm after a glutaraldehyde soaking (p 0.05).

Table V: Mean surface change in alginate impression between two methods (n=334).

	Study samples		p-value
	Group A (n=167)	Group-B (n=167)	
	Mean(±SD)	Mean(±SD)	
After Distilled Water Wash	3.38(±0.81)	3.43(±0.80)	0.96^{ns}
After 2% Glutaraldehyde disinfection	3.58(±0.81)	3.73(±0.80)	0.50^{ns}

- Group A= Maxillary alginate impression sprayed with 2% glutaraldehyde disinfection solution
- Group-B= Maxillary alginate impression immersed in 2% glutaraldehyde disinfection solution.
- Surface change (in mm).
- p-value reached from unpaired sample t-test.

Table V shows that the mean surface change of alginate samples disinfected by spray and immersion was not significantly different (p 0.05).

Discussion

The results of this study demonstrated that after culturing the sample disinfected with 2 percent Glutaraldehyde, microorganisms in groups A and B showed no growth. Between the two groups, no pathogens were discovered to exist. Al Shikh and Milosevic (2020).[6] found that aldehyde-based spray and immersion disinfection procedures are the most effective and gold standard.6 When immersed for 10 minutes in 2 percent glutaraldehyde, these germs were nearly eliminated, according to Egusa et al. (2008).[8] Glutaraldehyde is a fixative reagent used to fix proteins in samples. Because the surface of the proteins preserved on impressions is fixed by glutaraldehydehyde, it may have an antibacterial impact on the oral flora residing in the depths of these proteins.[9] Glutaraldehyde at 2 percent after a 10-minute exposure duration completely eliminated microorganisms in Bustos et al. (2010) research.[10] According to impression material and disinfectant solution, Al Shikh, and Milosevic (2020) also found post-disinfection bacterial growth.[6] After glutaraldehyde spray disinfection, neither PVS nor polyether impression showed any development. Commercially available dental impression materials, according to Ulgey et al. (2020), cannot be disinfected. Dentures might be harmed by cleaning methods. When it comes to disinfecting impression materialsfor Pseudomonas, Ulgey et al., (2020) found that spray was 100% efficient and the most dependable technique of disinfection of impression materials of all examined methods.[11]

The mean surface change of alginate imprint in Spray Technique 3.38 (0.81) mm after a distilled water wash and 3.59(0.80) mm after spraying 2 percent glutaraldehyde (p 0.05) was statistically meaningful in this investigation. When cleaning impressions under running tap water, germs can be removed, but they would also disseminate throughout the impression surface of the material, and disinfectant is consequently necessary (Egusa et al. 2008).[8] A new generation of spray disinfectants has already been developed that can give excellent disinfection while avoiding the drawbacks of soaking treatments, like the risk of damaging dimensional stability (Doddamani et al. 2011).[12]

There was statistical significance in surface changes of the alginate imprint in the soaking procedure between distilled water and 2 percent glutaraldehyde (p -0.05). Both immersion exposure times were found to contain 2 percent glutaraldehyde, according to Bustos et al. (2010). The surface morphology of the disinfection samples did not differ from that of the non-infected (control group) samples.

The average surface alteration of alginate samples disinfected by spray and immersion was found to be indistinguishable (p 0.05) in this investigation. Disinfecting the hydrocolloid impressions with 2% glutaraldehyde for 5 minutes worked well for Bustos et al. (2010).[10] Physical qualities like as dimensional stability and surface integrity can indeed be minimized by decreasing immersion duration. If certain findings can be extended to the disinfection of full-mouth impressions, it will be necessary to undertake a clinical investigation. Muzaffar D (2015) found that if the disinfecting procedure requires the impression to be immersed in a disinfectant solution for much more than an hour, alginates are ineffective.[13] According to Ulgey et al. (2020), the usage of disinfecting treatments for 15 minutes can be accepted as suitable due to the cheaper damage throughout all frequencies of impression.[11] Disinfecting and trying to pour instantly after disinfection improves the structural accuracy of conventional irreversible hydrocolloid impressions, but this wasn't the situation with prolonged material properties, according to a study (Nassar et al. 2011) on the dimensional stability of irreversible hydrocolloid impression materials.[14] They concluded that the stability of irreversible hydrocolloids may depend on the choice of disinfectant and disinfection procedure. The surfaces of alginate imprints do have a high level of microbial adhesion (Correia-Sousa J. et al., 2013).[15]

Conclusion

All such germs contained in the alginate impression are

completely eradicated using the spray and immersion methods of disinfection solution containing 2 percent glutaraldehyde (GDA). In contrast to the spray approach, the immersion procedure produces a greater alteration in the surface of the water. As a result, it is preferable to sterilize the alginate impression by spraying it with glutaraldehyde at a concentration of 2 per cent.

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Conflicts of interest
The authors declare that they have no conflict of interest.

Ethical Approval
Ethical clearance for the study was taken from the institutional review board before the commencement of the study.

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Correspondence

Dr. Shehab Monirul Haque, BDS, MS
Assistant Professor,
Prosthodontic Department
Udayan Dental College
Rajshahi, Bangladesh.