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Orginal Article

EFFECTIVENESS OF VARIOUS DISINFECTANTS ON ALGINATE IMPRESSION MATERIAL: IN-VITRO EXPERIMENTAL STUDY

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ABSTRACT

Objectives: *The objective of the study was to highlight a disinfectant which dentists can use to decontaminate alginate impressions in hospitals and clinics before sending it to laboratory.*

Materials and Methods: *This In-vitro experimental study was conducted at the Department of Prosthodontics, Sardar Begum Dental College & Hospital, Peshawar. After Ethical approval Microbiological analysis were carried out at Research Centre Gandhara University, Peshawar. Consent was taken from Male dentate individuals fulfilling the inclusion criteria and maxillary impressions were achieved from them. Palatal portion each impression was divided into four section. Specimens of control group 1 were left unwashed and untreated whereas; Specimens of experimental group 2 were sprayed with simple tap water, specimens of experimental group 3 were washed with 0.5% hypochlorite solution and specimens of experimental group 4 were treated with 1% chlorohexidine solution. These specimen were then arranged on blood agar petri plates stitched with S.mutans and results were collected. For dimensional stability specimen were made from alginate impression materials and were tested according to ADA specification no 19.*

Results: *The control group (untreated) exhibited the highest mean microbial colony count (63.6 ± 15.1 CFU), while disinfection with 1% chlorhexidine resulted in the lowest (1.27 ± 1.53 CFU), reflecting a 98% reduction in microbial load ($p < 0.001$). Treatment with 0.5% sodium hypochlorite also showed a significant reduction (2.2 ± 1.90 CFU; $p < 0.001$, 96.5% reduction), whereas rinsing with tap water (19.87 ± 6.78 CFU) achieved only a 68.8% reduction ($p < 0.001$). In terms of dimensional stability, untreated impressions showed a 10% reduction in dimensions after 1 hour (from 20 mm to 18 mm). In contrast, the 1% chlorhexidine group maintained dimensional stability with only a 2.5% change (20 mm to 19.5 mm), followed by 0.5% hypochlorite (4.5%) and tap water (4%). All changes were statistically significant ($p < 0.05$), with 1% chlorhexidine showing the most favorable balance of antimicrobial efficacy and minimal dimensional distortion.*

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INTRODUCTION

Dental impressions play an important role in prosthetic dentistry as they provide base for customized dental prosthesis and restorations. Dental impressions can become contaminated by various microorganisms including opportunistic oral

Conclusion: *Chlorohexidine 1% is proved to be a potent disinfectant as compared to tap water and 0.5% sodium hypochlorite for irreversible hydrocolloid alginate impression material.*

Key words: *Alginate, Dental Impression Materials, Disinfection, Chlorhexidine, Sodium Hypochlorite, Dimensional Measurement Accuracy*

pathogens, posing a potential threat to both dental professionals and patients. Opportunistic microflora is a diverse group of microorganisms harmlessly residing on mucosal surfaces and skin. They remain inactive and need favorable conditions to grow which potentially leads to an infection. These opportunistic microbes can contaminate the impression either during the intraoral procedure or during its handling in dental clinics and transportation to laboratory. Human micro-oral flora consists of more than six hundred opportunistic communicable species, which is responsible for contamination of dental impressions either through blood or saliva[1]. These microorganisms are transferred from oral cavity mainly to the dentists, supporting staff and dental technician[2].

Alginate impression material, an irreversible hydrocolloid, is commonly used because it is easy to manipulate, cost-effective, and accurate when handled properly[2]. However, it is more susceptible to infection compared to other impression material due to its texture, composition and its ability to imbibe saliva. In fact, microorganisms tend to adhere to alginate three to four folds more than they do adhere to silicone impression material reason being higher porosity and its ability to absorb water and saliva which allows greater microbial penetration[3]. According to Masoumeh et al, 67% of impressions sent to dental laboratories are contaminated with various microorganisms[6].

Al-Jabrah et al. concluded that dental professionals and their supporting staff are more susceptible to getting infections from dental impressions due to cross-contamination. This study suggested that all dental impressions should be disinfected before sending to laboratory for further processing. Rinsing impressions for disinfection with tap water was recommended practices until 1990s[4]. Nowadays, various chemical and compositions are used to disinfect Dental impressions in clinical practice against number of microorganisms[5]. Ideally disinfectant should effectively remove bacteria without altering dimensions & surface texture of the impression and be capable of immediately disinfecting the impressions before pouring it with gypsum[9]. According to International Dental Federation (IDF) all the dental impressions sent to laboratory should be disinfected[10]. In India 75.9% of impressions are washed only and 24% are disinfected properly[11]. Disinfection in some countries is optimum while it is not practiced in several others[11].

Dimensional stability of impression materials are very important as these impressions are used to make casts, on which prosthesis are made. These prosthesis needs to be sugly fitting to the prepared tooth and to the gingival ridge of edentulous patient.

Various studies have explored the impact of different disinfectants on the dimensional stability of alginate impression materials. Ralph (1990) found that hypochlorite and glutaraldehyde solutions, commonly used as disinfectants, can cause dimensional changes in alginate impressions. Zahid (2017) further investigated this, concluding that Chlorox® (sodium hypochlorite) is the most stable disinfectant for newer alginates, with Cavex CA 37® showing the least change in weight. Al-Nema (2018) also found that sodium hypochlorite can lead to significant dimensional changes, while chlorhexidine and iodine solutions can accelerate the setting time of the material. Lastly, Al-Omari (1998) reported that chlorhexidine, paraldehyde, and phenol derivatives can all cause dimensional changes in alginate impressions, with the latter two also affecting the surface quality of the resultant casts.

In our country so far there is no data available as far as dental impression disinfection is concerned. This study was conducted to highlight a disinfectant which dentists can use to decontaminate alginate impressions in hospitals and clinics before sending it to laboratory.

MATERIALS AND METHODS

This In-vitro experimental study was conducted at the Department of Prosthodontics, Sardar Begum Dental College & Hospital, Peshawar, from June 2021 to December 2021. Ethical approval for the study was obtained from the board of advanced study and research as well as the ethical commit-

tee of Gandhara University in Peshawar, Khyber Pakhtunkhwa, Pakistan. Male dentate individuals having, 20-50 years of age, with no visible carious lesions were included in this study while Individuals with incompetent lips, salivary gland pathologies, systemic disorders and smokers and alcoholics were excluded from this study. Informed consent was obtained from each patient fulfilling the inclusion criteria. Sample size was 100 which was done using convenient sampling technique. Details of the groups and their treatment is shown in table 1.

Preparation of petri dishes from media

Blood agar base I powder of 12 grams was mixed in 300 millilitres of distilled water and stirred until mixed completely. The mix was then sterilized at 121°C under 1.5 bar pressure in an autoclave (MAC-230 100V -1KW, SANYO Elclave). The sterilized culture media was cooled to 45^{0}C under laminar flow and mixed with 10ml of warm defibrinated sheep blood which was kindly provided by microbiology lab Khyber teaching hospital, Peshawar, Khyber Pakhtunkhwa. Sterile petri dishes were arranged in laminar flow cabinet (streamline EN 1822.1). 10-15ml blood agar media was dispensed to each petri dish with of prepared while it is still warm, bubbles if any were removed with loop and left till cool. The petri dishes after cooling were covered and stored at 0°C in refrigerator (MIDAS international LB-LWB1.5-5X33R) until use[52].

Stainless steel Mold

A stainless-steel die was fabricated according to ADA specification no 19 which consisted of three parts; a block carrying the mould and a ring used to confine the mixed materials to the mould and a die used to place the materials in place as shown in Figure 1. Mould had three horizontal laser engravings lines of 30mm with an interline space of 3mm and two horizontal laser engravings of 6mm as shown in figure 2.

Mixing of Alginate impression materials

Hygedent Normal Set was used in our study. Impression materials were mixed manually according to manufacturer's recommendations using elastic bowl and metallic spatula with figure of eight strokes carefully to achieve a creamy voids free mix for recording good details.

Impression

Tray selection was done for each participant. Selected tray was then loaded with mixed alginate impression material using metallic flat spatula from posterior towards anterior. Loaded tray was then inserted into participant's mouth and first seated posteriorly then anteriorly, so that material should not flow towards soft palate. Tray was held in position until material gets fully set. Once set peripheral seal was broken first with the help of finger prior removing the impression tray and tray will then be removed with a rapid firm snap[5,15].

Disinfection Efficacy Assay

Mid palatal region of every impression was divided into 4 equal sections with the help of precise cutter. These sections were assigned to each of the four groups as explained in table 1. Specimens of control group 1 were untreated and unwashed, specimen of experimental group 2 were with tap water whereas, experimental group 3 and experimental group 4 were spraying with their respective disinfectant solution. Specimen of all groups were placed in plastic bag for ten (10) minutes and labelled to avoid possibility of evaporation of disinfection solution.

After 10 minutes, microbial swabs were taken from specimens of each group using a sterile cotton swab and were stitched to already prepared & stocked blood agar plates. These petri dishes were then kept in incubator at 37ºC±1for 24 hours. Microbial colonies were counted with a Pen Marker Method after 24 hours and data were recorded in already prepared data collection charts. These microbiological procedures were carried out under aseptic conditions in laminar flow cabinet to prevent contaminations in petri dishes and to avoid bias in our results.

Dimensional stability:

Mixed Alginate impression material was packed in the specially designed mould (Figure 1) and the die was used to press the mixed materials into tray. The whole assembly was kept in water at 37°C during the setting time, to simulate the humid oral environment. Prepared specimen without any distortions were divided later into four groups using lottery method. Set alginate specimen were treated with the respective solutions and packed in zip lock bags. Dimensions were measured initial after setting

at 0 minutes, 15 minutes, 30 minutes, and 1-hour using digital Vernier-Callipers.

The data were compiled as well as analysed in SPSS version 20. Descriptive statistics were used to describe, organize and summarize data. All results were presented in the form of tables and graphs. One-way-ANOVA-and-post-hoc-Tukey test were applied for checking significance amongst control and experimental groups with significance level kept at < 0.05.

RESULT

In this study the control group (unwashed and untreated) showed elevated microbial growth amongst all groups, whereas experimental groups showed decontamination values. Whereas, experimental group 4 showed least microbial growth amongst control and all experimental groups. (Table 2)

Figure 3 is the Box and whiskers plot showing mean and standard deviations of collected data and whiskers plots signify their standard deviations. As evident from the plot, control group 1 specimen has higher colonies which were decreased in experimental groups.

In paired statistics, every group was compared with control group which was unwashed and untreated. Mean results of each experimental group in paired analysis were all significant with control group. (Table 3)

Dimensional stability values of control and various experimental groups are given in table 4 and figure 4. As compared to the baseline readings control group 1 showed significant loss of dimensional stability, however experimental groups showed a lesser amount of loss in dimensional stability. Amongst the experimental groups, experimental Group 4 (1% chlorohexidine) showed minimum changes in dimensional stability.

Figure 5 displays the values of dimensional stability of control and various experimental groups of different samples at 0 minutes, 15 minutes, 30 minutes and 1 hour.

DISCUSSION

Dental care providers meet potentially harmful microbes during their routine dental practice. Patients visiting dental care facilities for their treatment are imperative source of microorganisms for the dental

Table 1: Showing distribution of samples for various test amongst different groups.

Groups	Disinfection	Dimensional change	Total
Control Group 1 (Un-washed & Un-treated)	15	10	25
Experimental Group 2 (Tap water)	15	10	25
Experimental Group 3 (0.5% hypochlorite solution)	15	10	25
Experimental Group 4 (1% Chlorohexidine solution)	15	10	25
Total:	60	40	100

Table 2: Showing means, standard deviations and significance in control and experimental groups.

Groups	N	Mean	St. Deviation	St. Error Mean	Significance
Control group 1 (untreated/unwashed)	15	63.6000	15.10345	3.89969	0.001
Experimental group 2(tap water)	15	19.8667	6.78093	1.75083	
Experimental group 3 (0.5% hypo chloride)	15	2.2000	1.89737	.48990	
Experimental group 4 (1% chlorohexidine)	15	1.2667	1.53375	.39601	

Table 3: Showing paired statistics (Post Hoc Tukey) amongst control and various experimental groups

		Mean	Std. Deviation	Std. Error Mean	Sig.
Pair 1	Control group 1 (untreated/unwashed) - Exp group 2(tap water)	43.73333	15.32256	3.95627	.000
Pair 2	Control group 1 (untreated/unwashed) - Exp group 3 (0.5% hypo chloride)	61.40000	14.63264	3.77813	
Pair 3	Control group 1 (untreated/unwashed) - Exp group 4 (1% chlorohexidine)	62.33333	14.65638	3.78426	

care providers and hence for the rest of community[12]. Studies done previously indicated that surfaces of a dental impression taken out of the mouth of a patient is contaminated with bacteria, fungi and other microbes. Different impression materials have different level of contaminations[12]. Impressions taken from patient's oral cavity with alginate contain potential hazardous microorganisms[1]. These microorganisms are adaptable, opportunistic and their main source of spread is oral cavity. The current study evaluated whether tap water, 0.5% hypo chloride or 1% chlorohexidine proficiently exterminate all types of microorganisms from alginate impression taken for the study.1% chlorohexidine showed promising results compared to 0.5% hypo chloride and tap water. 1% chlorohexidine showed significant results comparatively in10 minutes, eradicating maximum

Fig 1: Different parts of Mould for Dimensional stability.

***A; Block carrying the mould, *B: Ring, to confine mixed materials to mould, *C: Die used to press materials in mould**

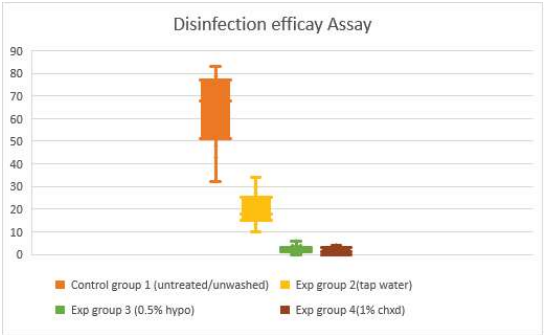

Fig 3: Showing no mean and standard deviation in control and experimental groups.

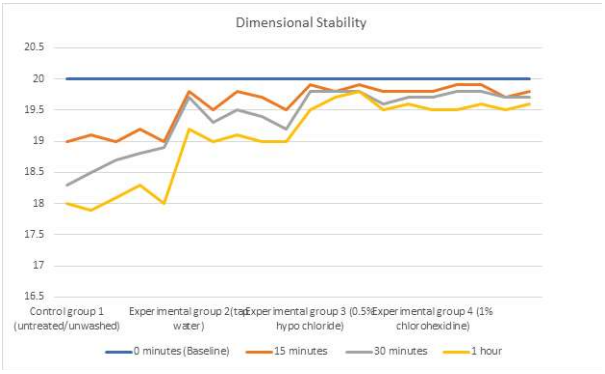

Fig 5: Showing baseline and readings of various samples at different intervals of control and experimental groups.

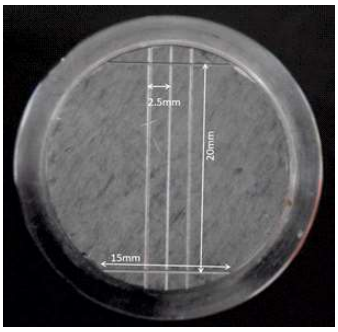

Fig 2: Mold for dimensional stability of alginate

***showing Three vertical engravings and two horizontal lines**

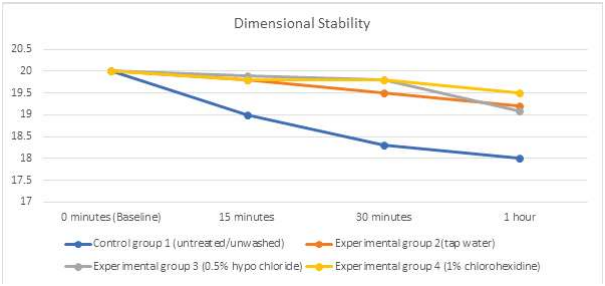

Fig 4: Showing means of each group at different time intervals.

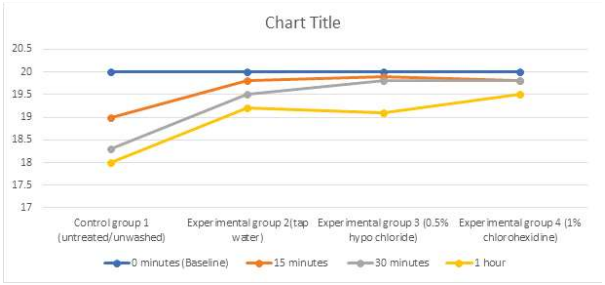

Fig 6: ?????????????????.

Table 4: Showing mean Dimensional stability values of control and experimental groups.

Group	0 Minutes (Baseline)	15 Minutes	30 Minutes	1 Hour
Control Group 1 (Untreated/Unwashed)	20	19	18.3	18
Experimental Group 2(Tap Water)	20	19.8	19.5	19.2
Experimental Group 3 (0.5% Hypo Chloride)	20	19.9	19.8	19.1
Experimental Group 4 (1% Chlorohexidine)	20	19.8	19.8	19.5

microbes from alginate impression samples.

Selection of ten (10) minutes an efficient time for disinfection and sealing the specimen in a sterile plastic bag was depended on work done according to pilot study done prior to this research project. As the experimental methodology revealed that treatment time of 10 minutes for alginate impression specimen sprayed with disinfected solution resulted in lesser colony count. This disinfection duration was inspired by a study done by Suprono et al. in 2012[13]. Whereas, alginate impression specimens that were untreated and unwashed resulted in increased colonies forming units of oral microorganisms. Sealing specimen in a sterile plastic bag after spraying it with disinfectant solution is to prevent evaporation of the disinfectant solution and to let the surfaces of the specimen decontaminate properly. This step was also inspired by Suprono et al. in 2012 in which they sealed their specimens in a sterile plastic bag to prevent evaporation and to improve disinfection[3].

In this study the experimental group 2 (washed under tap water) resulted in decreased colony forming units. The results of this group was in simulation with studies done in literature, according to the literature reviewed by Michelle Strange in 2022 rinsing a dental impression after removing from the mouth was a standard practice before 1991[3].

Disinfectant agents carrying chlorine happen to be effectual for disinfection against microorganisms, investigations from literature signified that they are idyllic chemical disinfectant agents for irreversible hydrocolloid impressions[14]. In current research project, 0.5% hypochloride solution was used for disinfection. It is a commonly used household bleach which was used to decontaminate irreversible alginate impression samples and proved potent against oral microbes. The results of our study agreed with a study done by Ghahramanloo et al.[1] in 2009, they concluded that 0.565% hypo chloride efficiently prevented microorganisms development and effectively disinfected alginate impressions[1]. Results of our study was also in agreement with a study by Moura et al. in 2010 in their study they also concluded that sodium hypochlorite 2.5 and 5.25%proved a fruitful decontaminant solution for irreversible hydrocolloid impressions[15].

Chlorohexidine is intermediary decontaminant and broad-spectrum antiseptic agent also used as preserver. Chlorohexidine is bactericidal, viricidal as well as mycobacteriostatic. Chlorhexidine 1% has shown cidal activity against “s.aureus,e.coli,b.surbititis”[16] kollu et al., in 2013 used Lower disinfectant solution concentration as much as 1.0 g/Lchlorhexidine efficiently as water substitute in mixing with alginate powder yielding self-disinfecting alginate impression material[17]. Amongst experimental groups chlorohexidine proved to be potent in eliminating more microbes from alginate impression material samples hence proved more efficient that 0.5% sodium hypochlorite solution. Our study's findings are consistent with those of Gounder et al.'s research,in 2016, in which they evaluated various disinfectant agents for their ability to disinfect as well as their impact on the mechanical properties of alginate impression materials[18]. Our findings are also consistent with those of Qamruddin et al.'s study, where the oral microbiome was effectively disinfected with chlorhexidine[19].

In the blood agar media culture, the collected data were identified as CFU. The marker counter method was used to record the data, and the CFU count, a common way to estimate the number of microbial colonies, was used to indicate the counts. The microbiological investigation showed that the number of CFUs that were recorded after disinfection was significantly lower than the number that were recorded for an undisinfected specimen. This makes the disinfection process an important problem to solve after taking an impression in the dental care facility. The current study meets the goals by showing that rinsing an impression with tap water reduces the number of bacteria in the impression, while treating an impression with either 0.5 percent sodium hypochlorite or 1 percent chlorohexidine almost completely eliminates the number of bacteria in the impression.

LIMITATIONS

In the current study it was observed that only two disinfectant solutions were analyzed. Moreover, disinfection was not checked against specific opportunistic microbes. Disinfectants were only checked for microbes that can grow on blood agar.

CONCLUSION

In light of the current study it is concluded that rinsing with tap water only removes the planktonic

microbes and there still is potential for infection. Chlorohexidine 1% is proved to be a potent disinfectant as compared to 0.5% sodium hypochlorite for irreversible hydrocolloid alginate impression material.

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CONFLICT OF INTEREST
Authors declare no conflict of interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
None declared.

AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	FF, FR, TAK, MG, AK, WA
Acquisition, Analysis or Interpretation of Data:	FF, FR, TAK, MG, AK, WA
Manuscript Writing & Approval:	FF, FR, TAK, MG, AK, WA

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.