Original Article

Study on antimicrobial effect of disinfecting solutions on alginate impression materials

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ABSTRACT:

Oral cavity contains 600 species of microbes named oral flora.Dental impressions get contaminated with micro-organisms from patient's blood or saliva. So, impressions are recommended to disinfect before their further working steps. A study was carried out to find out a more effective disinfectant solution between 1% sodium hypochlorite and 2% glutaraldehyde solution on irreversible hydrocolloid impression and thereby identify their efficacy against microbial transmission to the resultant dental cast. In this study, hydrcolloid impression material was selected as the experimental elements because these are the materials which are mostly used in our country. These are disinfecting solution which are worldwide used. The findings suggested that the transmission rate from impression to the casts was 7% in control group ($P<0.001$). It was reduced to (0.6%) when disinfected by 1% sodium hypochlorite solution whereas transmission rate was minimum (0.08%) when disinfected by 2% glutaraldehde solution. The most important things is that it will be very easy for the clinician to produce this solution and disinfect the impression by these solutions and there will have no effect on the dimension of the impression or the cast

Introduction:

The risk of transmission of infectious agent via saliva and blood of the patient is a well established occupational hazard in daily dental practice. Dental impressions can act as a transmitting media for infectious agents to dental personnel or to the dental casts prepared from them. A significantly high level of microbial adherence is obtained from patient's mouth by Irreversible hydrocolloid impressions (Moura et a[2].). As it is a widely used and popular impression material in Bangladesh, effective infection control procedures is necessary to prevent the transmission of infection and to reduce impression derived cross-contamination.

A study concluded that, all members of the dental profession are at a risk at least three times greater than the general population of contacting infection and developing the carrier state, which clearly indicates the urgency of disinfection of all dental impressions prior to delivery to the dental laboratories (Al-Jabrah et al[3]).

The impressions taken by the dentist are frequently sent to distant dental laboratories to be molded into various types
of dental stone or plaster. In most of the cases, the impressions are commonly not disinfected by the dentist, just rinsed with running water expecting that the impressions will be disinfected by the dental technician when received.

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Impressions, casts, impression trays, record bases, occlusal rims, articulators and dental prostheses all can transmit pathogenic microorganisms from the dental office to the dental laboratory. It has been reported by many authors that organisms are transmitted from Infection control is important in the practice of dentistry because dental healthcare workers and patients are exposed to a wide variety of microorganisms via blood and oral/respiratory secretions. These microorganisms may include hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), *Mycobacterium tuberculosis*, S*taphylococci, Streptococci*, and other viruses and bacteria. In Egypt about 3 million people are treated yearly in Ministry of Health dental clinics. Several studies suggested that exposure to dental procedures is a risk factor for HCV in Egypt. (OSAP, Infection control in dentistry guidelines).

Hypochlorite (NaOCl) has efficacy to destroy a wide range of micro-organisms and is effective against the Hepatitis B and HIV viruses. Their activity is reduced in the presence of organic matter, and they are corrosive at concentrations necessary for environmental disinfection (NZDA Code of Practice: Control of Cross Infection in Dental Practice, April 2002.)

From the findings of the renowned scientists, it is clear that, impression and the model may become the source of cross infection. SO, it is very important at the present situation that the impression should be disinfected for the safety of the lab. personnel as well, as the as practicing doctors. Alginate, one of irreversible impression material is randomly used in our country for some of its advantageous points like hydrophilic in nature, cheap, record of fine details etc. However, very few reports have been published on the disinfecting process of the impression and its casts. According to the reports in some local medical journal in our country that cross infection by B- virus is increasing in an alarming rate. It is reported that the models made of from these impressions may become the source of such cross infection. Thus the objective of this stud was to:

To evaluate the antimicrobial effect of disinfecting solutions like 1% Sodium hypochlorite solution and 2% Gluteraldehyde solution on the irreversible hydrocolloid (alginate) impression and the transmission of the microorganisms on the cast made from these impressions after they are treated for two minutes with these disinfecting solutions.

MATERIALS AND METHOD

Sample size:

One hundred and twenty impressions were collected, six from each patient.

GROUPING OF SAMPLE:

1. Group-A: Impressions immersed in distilled water-20 impressions .
2. Group B: Impressions immersed in 1% sodium hypochlorite solution-20 impressions.
3. Group C: Impressions immersed in 2% gluteraldehyde solution-20 impressions.
4. Group A1: Gypsum cast prepared from another 20 group-A impressions.
5. Group B1: Gypsum cast prepared from another 20 group-B impressions.
6. Group C1: Gypsum cast prepared from another 20 group-C impressions.

In this study, 20 patientsof both sexes having similar oral hygiene (oral hygiene index-3), similar gingival index (gingival index-1), similar periodontal index (periodontal index-1) were included. All the instruments were sterile.Defective impressions and Defective casts were excluded.

In this study, antimicrobial efficacy was measured according to Bustos et al.[7], Atabek et al.[8]

The reduction rate of colony forming units (CFU)/ml was compared with the control group.

Transmission rate was measured according to Junevicius, Pavilonius and Surna[1], Sofou et al[4].

Bacterial transmission rate (colony forming units (CFU)/ml) was compared to the gypsum cast with the control group.

STUDY PROCEDURE

Each of the patient was evaluated by a thorough medical and dental history as well as clinical examination according to the history sheet. Treatment plan and study procedure were explained to the patient. After confirmation of their full cooperation, 20 subjects of them were finally selected. Study procedure consisted of Prosthodontic laboratory procedure and Microbiological laboratory procedure.

Prosthodontic laboratory procedure:

Total 120 irreversible hydrocolloid (alginate) impressions were taken from 20 patients by Lygine TM (Lot -95453 and 97260) Dentamerica, USA. Six maxillary arch impressions were taken from each patient with a 48 hours interval. The intervals were given to reorganize the oral floras because their count might be reduced after taking each impression. Impression material was proportioned, mixed with distilled water and manipulated according to manufacturer's recommendations. After setting, the impressions were taking out from the patient's mouth and randomly arranged in groups-A, B, C, A_1, B_1, and C_1. All groups consisted of 20 impressions. Group A impressions were rinsed with 250 ml distilled water for 10 minutes then $2cm^2$ area from anterior segment including teeth and palate were removed aseptically with a sterile blade from the impressions. Collected samples were transferred to sterile conical flasks containing 20 ml sterile normal saline and vibrated with vibrator for 2 minutes to separate the microorganisms from impression surface. 2ml of saline suspension from each conical flask were collected in a sterile test tube and covered with sterile cotton plugs. Group B impressions were rinsed with distilled water to remove food debris and saliva then immersed in 1% sodium hypochlorite solution in sterile beakers for 2 minutes. After disinfection procedure impressions were again immersed in 0.5% sodium thiosulphate solution for 15 seconds to neutralize chlorine molecule then $2cm^2$ area from each impressions were separated and sample were collected by following same procedure of group A. Group C impressions were rinsed with distilled water for 10 seconds then immersed in 2% glutaraldehyde solution for 2 minutes As the solution is available at 2.54% concentration, it was diluted to achieve 2% concentration. After disinfection, impressions were rinsed with distilled water to remove glutaraldehyde from the impression surface, $2cm^2$ areas were separated and 2ml samples from each impression was prepared. For groups A_1, B_1, and C_1, laboratory procedure were similar to groups A, B, and C up to water rinsing or disinfection procedure then gypsum product was poured into the impressions and allowed to set for 1 hour. After removing the casts from the impressions $2cm^2$ areas were snapped off from the cast with an aseptic way by a sterile hacksaw blade. Snapped off areas would be as similar as the separated areas of groups A, B, and C. Samples of group A_1, B_1, C_1 were immersed in 20 ml of sterile normal saline for 30 minutes then vibrated for 2 minutes to separate the microorganisms from the casts. 2ml of the samples were collected in sterile test tubes and covered with sterile cotton plugs. Group A samples were served as control group and all samples should be leveled. Collected samples were transferred to Microbiology laboratory within one hour (Bustos et al[7]., Atabek et al[8], Junevicius, Pavilonius and Surna[1], Sofou et al[4].).

Microbiological laboratory procedure:

In microbiology laboratory (Department of Microbiology, BSMMU, Dhaka) 20 micro-liter of all samples were transferred aseptically in sheep blood agar plate (locally made by BSMMU, Department of microbiology) by wire loop. Agar plates were labeled and incubated at 37^0C for 24 hours in aerobic condition(5% CO_2) in Memmect incubator (West Germany). After 48 hours, microbial colonies was calculated with the help of a magnifying glass and multiplied to express them in Colony Forming Units(CFU)/ml. (Bustos et al. [7], Atabek et al.[8], Sofou et al.[4]).

Data collection:

All the collected data were transferred to microbiological laboratory work data collection sheets on the basis of grouping and specific parameters like antimicrobial efficacy and transmission of microorganisms to dental cast of irreversible hydrocolloid impression materials.

RESULTS

The in vivo study was intended to evaluate the antimicrobial efficacy of 1% Sodium hypochlorite and 2% Glutaraldehyde disinfectant solutions on Irreversible hydrocolloid (Alginate) impression materials. Parameters of the study were antimicrobial efficacy and microbial transmission. The findings of study obtained by analysis are presented bellow:

Table I: Descriptive statistics of the comparison of bacterial count (CFU/ml) among the groups on alginate impression before and after disinfection procedure.

Groups(n=20)	(MeanïSD)	Min	Max	P Value
Group-A	8743±3294	3750	15000	
Group-B	490 ±372.85	0	1250	0.0001
Group-C	47.5±71.6	0	250	

Statistical analysis was done by ANOVA

Group A- Impressions rinsed with distilled water(control group)
Group B- Impressions disinfected by 1% sodium hypochlorite solution.
Group C- Impressions disinfected by 2% glutaraldehyde solution.

**= P value <0.0001 considered as highly significant
n = number of subjects.

The mean bacterial count was 8743±3294 cfu/ml with ranged from 3750 to 15000 cfu/ml in group A. The mean Bacterial count was 490±372.85 cfu/ml with ranged from 0 to 1250 cfu/ml in group B. In group C, the mean bacterial count was 47.5±71.6 cfu/ml with ranged from 0 to 250 cfu/ml. The mean difference of bacterial count was statistically significant (p<0.0001) among three groups.

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Table II: Descriptive statistics of the comparison of bacterial count (CFU/ml) transmission from alginate impression to gypsum cast before and after disinfection procedure.

Groups(n=20)	MeanïSD	Min	max	P Value
Group-A_1	615±300	200	1250	
Group-B_1	52.5±83.47	0	250	**0.0001****
Group-C_1	7.5±18.3	0	50	

Statistical analysis was done by ANOVA

Group A_1- Gypsum casts prepared from group A impressions.
Group B_1- Gypsum casts prepared from group B impressions.
Group C_1- Gypsum casts prepared from group C impressions.
**= P value <0.0001 considered as highly significant
n = number of subjects.

The mean Bacterial transmission was 615±300 cfu/ml with ranged from 200 to 1250 cfu/ml in group A_1. The mean bacterial transmission was 52.5±83.47 cfu/ml with ranged from 0 to 250 cfu/ml in group B_1. In group C_1, the mean Bacterial transmission was 7.5±18.3 cfu/ml with ranged from 0 to 50 cfu/ml. The mean difference of bacterial transmission was statistically significant (p<0.0001) among three groups.

DISCUSSION

This was a prospective comparative study, designed to evaluate the antimicrobial efficacy of 1% sodium hypochlorite and 2% gluteraldehyde disinfecting solutions in removing microorganisms from the surface of irreversible hydrocolloid impression material. Immersion disinfection of impressions were performed with a 2 minutes application time and chance of microbial transmission to dental cast was also evaluated. 20 subjects were selected from the patients and the stuffs of the prosthodontics department. Total 120 impressions were collected (06 from each subject). Impressions were divided into six groups, each consists of 20 impressions, which were treated in different disinfection resume. After microbiological incubation for group A, mean bacterial count was 8743+ 3294 colony-forming unit (CFU)/ml, with the range from 3750 to 15000 CFU/ml. For group B, mean bacterial count was 490+372.85 CFU/ml with the range from 0 to 1250 CFU/ml. For group C, mean bacterial count was 47.5+71.6 with the range from 0 to 250 CFU/ml) The mean bacterial count difference between the groups was statistically highly significant ($p<0.0001$). Table: 1 shows the results and the comparison of group A, B & C.

Bustos et al[7]. (2010) during their study (vivo) found $0.7x10^3 \pm 1.05x10^3$cfu microbial colony count on the alginate impression surface for the control group. After a 5 minutes disinfection treatment with 0.5% sodium hypochlorite solution the colony count was detected $0.017x10^3 \pm 0.22x10^3$cfu.A Atabek et al[8]. noticed 100% reduction of microorganisms from the alginate impression surface after disinfected them by 1% sodium hypochlorite solution for 3 minutes where as the mean colony count was $300x10^3$cfu/ml for the untreated control groups. In A study conducted by Beyerle et al[9]. (1994) to evaluate the efficacy of different concentration of sodium hypochlorite disinfection solutions on alginate impression ,detected 5.7x105 cfus of S.aureas before disinfection which reduced to 6.4x100cfus after getting a 1 minute disinfection treatment with 0.5% NaOcl. Moura et al[2]. (2010)in their study found mean colony count 45.35±6.83cfu for untreated control group.When they treated alginate impressions with2.5% NaOcl solution in humidified box for 10 minutes colony count reduced to 11.45±12.49 cfu. Egusa et al[5]. (2008) mentioned only 15.8% microbial reduction on median surface of alginate impression when washed with running tap where as 30.15% reduction was noticed when treated with 1% NaOCL solution for 10 minutes.

All of the above studies show the similar result regarding to current study because all control groups impressions show higher microbial count than the study groups disinfected with different concentration(0.5%-2.5%) of Naocl solution.

Bustos et al[7]. (2010) used 2% glutaraldehyde solution with 5 minutes application time to disinfect alginate impression ,which reduced microbial count to mean cfu=$0.020x10^3 \pm 0.04x10^3$ from the untreated control group's count mean cfu=$0.71x10^3 \pm 1.05x10^3$.Egusa et al[5]. (2008) described a 42.8% reduction of microbial colony count on alginate impression surface when they were treated with 2% gluteraldehyde solution for 10 minutes where as only 15.8% colony count reduction achieved when rinsed with tap water. The antimicrobial efficiency of 2% glutaraldehyde solution is also supported by this present study.

Both of the studies conducted by Egusa et al[5]. supported the antimicrobial efficiency of 2%gluteraldehyde solution over/than/on 1% NaOcl solution as like as present study but Bustos et al (2010) reported the similar efficacy of both disinfectant on alginate and silicon impression.

Table: 2 shows the comparison of bacterial count transmitted from alginate impressions (group A, B & C) to Gypsum casts (group A_1, B_1& C_1). Mean bacterial count for group A_1 was 615±300 CFU/ml within the range from 200 to 1250 CFU/ml. the mean bacterial count 52.5±83.47 CFU/ml within the range of 0 to 250 CFU/ml for group $B_1$and the mean bacterial count 7.5±18.3 CFU/ml within the range of 0 to 50 CFU/ml for group C_1 This comparison showed statistical significance ($p<0.0001$).When transmission of bacterial count compare with group A, group A1 shows 7% transmission whereas after disinfection procedure group B1 shows 0.6% and group C1 shows 0.08% microbial transmission.

impression to dental cast in present study is supported by other previous studies.

Related study about bacterial transmission to dental cast from patient derived (short time disinfected) casts (group A_1, B_1& C_1) before & after disinfection procedure was showed statistical significance ($p<0.0001$).

CONCLUSIONS:

After completion of study is concluded that both disinfectant solutions (1% sodium hypochlorite and 2% glutaraldehyde) significantly reduced microbial count from alginate impression surface. Among them 2% glutaraldehyde showed more antimicrobial effect than 1% sodium hypochlorite. It is also concluded rate of bacterial transmission from alginate impression to cast was significantly reduced incase of 1% sodium hypochlorite solution than 2% glutaraldehyde solution.

References

1.Junevicius. J, Pavilonis .A, Surna .A. Transmission of Microorganisms from Dentists to Dental Laboratory Technicians through Contaminated Dental Impressions. Stomatologija, Baltic Dental and Maxillofacial Journal, 2004, 6:20-23.

2.Moura D. C.V. S, Moura. W.L , França .F.M.G, Martins .G.A.S, Feltrim .P.P, Zanetti .R.V .Disinfection of irreversible hydrocolloid impressions with sodium hypochlorite steam: Assessment of antimicrobial efficacy. Rev. odonto ciênc. 2010;25(2):182-187.

3. Al-Jabrah O, Al-Shumailan Y, Al-Rashdan M. Antimicrobial effect of 4 disinfectants on alginate, polyether, and polyvinyl siloxane impression materials.Int J Prosthodont. 2007 May-Jun;20(3): 299-307.

vitro study of transmission of bacteria from contaminated metal models to stone models via impressions.Clin Oral Investig. 2002 Sep;6(3):166-70.

5. Egusa H, Watamoto T, Matsumoto T, Abe K, Kobayashi M, Akashi Y, Yatani H.: Clinical evaluation of the efficacy of removing microorganisms to disinfect patient-derived dental impressions. Int J Prosthodont. 2008 Nov-Dec;21(6):531-8.

6. Anil .S BDS, MDS, Manju Gilbert .M BDS, Philip .T BDS, Haridas .A BDS , Beena .V.T BDS. HIV; AIDS and Dentistry - Control of Cross Infection K. D. J. Vol. 16 No. 2 Page 609.

7. Bustos .J; Herrera .R; González .U; Martínez .A &. Catalán .A. Effect of Inmersion Desinfection with 0.5% Sodium Hypochlorite and 2% Glutaraldehyde on Alginate and Silicone: Microbiology and SEM Study. Int. J. Odontostomat., 4(2):169-177, 2010.

8. Atabek .D DDS, PhD; Alacam.a DDS, PhD ; Tuzuner .e . DDS, PhD ;Sibel Polat. S DDS, PhD, Sipahi .a.b PhD. In-Vivo Evaluation of Impression Material Disinfection with Different Disinfectant Agents. Hacettepe Diş Hekimliği Fakültesi Dergisi Cilt: 33, Sayı: 2, Sayfa: 52-59, 2009.

9. Beyerle .M.P, Hensley .D.M, Bradley .D.V, Schwartz .R.S, Hilton .T.J. Immersion disinfection of irreversible hydrocol-loid impressions with sodium hypochlorite. Part I: microbi-ology. Int J Prosthodont 1994; 7:234-8.

Bhat .V S, Shetty .M S, Kamalakanth .S.K Infection control in the prosthodontic laboratory Year : 2007 | Volume : 7 | Issue : 2 | Page : 62--65.

10. Leung .R.L, Schonfeld .S.E. Gypsum casts as a potential source of microbial cross-contamination. J Prosthet Dent 1983; 49: 210-211.