Research Article

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Analysis of Effectiveness of Disinfectants on Contaminated Dental Casts: An In vitro Study

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ABSTRACT

Aim: The aim of this study is to determine, whether saliva has a role in the contribution of bacterial growth on the surface of dental casts over a period of time and whether disinfection can minimize the bacterial growth of contaminated casts with saliva.

Materials and Methods: 60 standardized dental stone cylinders were fabricated with master model dies of 24mm in length and 6mm in diameter. The specimens were divided into 5 groups of 12 each. The first group (control) of 12 specimens was neither contaminated with saliva nor treated with disinfectant. The second group of 12 specimens was contaminated with 25 ml of saliva for 5 minutes and was not treated with any of the disinfectants. The third group of 12 specimens was contaminated with 25 ml of saliva for 5 minutes and was rinsed with running tap water for 20 seconds. The fourth group of specimens was contaminated with 25 ml of saliva for 5 minutes and soaked in 5% sodium hypochlorite for 20 seconds. The fifth group of 12 specimens was contaminated with 25 ml of saliva for 5 minutes and soaked in 2% glutaraldehyde for 20 seconds. The treated dental stone cylinders were placed in individual test tubes containing 2.5 ml of sterile phosphate buffer solution and a final dilution of 10^{-4} was achieved. These were transferred on the sheep blood agar plates which were inoculated and incubated at 37^0C for 24 hours.

Results: Results demonstrated that all specimens contaminated with saliva and not disinfected had a large number of colony forming units (CFU) covering the blood agar plate. The specimens that were contaminated with saliva and rinsed only with water showed a diminished number of colony forming units. The specimens that were contaminated with saliva and soaked in 5% sodium hypochlorite had significantly less number of colony forming units when compared to that with only with tap water. All dental stone cylinders that were soaked for 20 seconds in 2% glutaraldehyde yielded no growth. The controls that were not contaminated with saliva had minimal growth on only few agar plates. The results of the ANOVA demonstrated that there was a significant difference between saliva-contaminated dental stone cylinders of group II and group V as compared to the other groups, indicating that soaking the dental stone cylinders in 5% sodium hypochlorite or soaking in 2% glutaraldehyde for 20 seconds had less chance of growth with micro-organisms.

Conclusion: Thus bacterial growth occurs on saliva contaminated dental casts, hence requires an effective method of disinfection.

Keywords: Dental stone, Disinfection, Sterilization, Colony forming units (CFUs).

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INTRODUCTION

Sterilization is the destruction of all microbial forms, including viruses and bacterial spores. Disinfection refers to the destruction of pathogenic microorganisms alone. Sterilization is best done with heat or chemicals. In the order of preference and reliability, the sterilization or disinfection methods are: steam under pressure, prolonged dry heat, ethylene oxide gas, boiling water, and chemical agents. Chemical disinfectant products must be effective in killing vegetative forms of pathogenic organisms, including influenza and enteroviruses and the tuberculin bacillus within 30 min. Disinfectants that meet these criteria may produce both high and intermediate levels of biocidal activity, depending on the length of exposure.[1]

Impression disinfection has become an important point in day to day clinical practice. Various ADA councils suggested that the materials, impressions, and intra oral prostheses need mandatory disinfection procedure before being sent to a laboratory.[2] Lynn Powell has reported that organisms are transmitted from impressions to casts and from dentures to pumice, where they continue to live. However, identification of the organisms that are actually transferred from the dental office to the laboratories or the frequency of the presence of organisms has not been reported.[3]

The standard procedure of rinsing impressions under running tap water immediately after removal from the mouth eliminates gross contamination along with most saliva and blood. Surface disinfection to inactivate infectious agents would reduce the potential for disease transmission to dental personnel from contaminated impressions.[4] To address these cross contamination concerns, the American Dental Association issued guidelines for disinfecting impressions in 1988, 1991 and 1996. These guidelines recommend using an ADA accepted spray or immersion disinfectant, depending on material, for the duration suggested by the manufacturer. Other disinfection protocols also have been developed to prevent diseases such as hepatitis B, tuberculosis, herpes and AIDS.[5]

Methods for chemical disinfection and ultrasonic cleaning of some impressions will probably sacrifice the dimensional accuracy of the impression due to heat and or time lapse. Untreated impressions are usually poured with some form of gypsum material for processing of dental restorations by laboratory personnel.[6] Topical methods of disinfecting casts are difficult to control, while immersion methods are potentially destructive. Thus an additional method to control cross contamination between patients and laboratory personnel is needed.[7] Hence potential contaminated dental casts need to be routinely disinfected by an easy to use inexpensive and non-damaging method. The two fold purpose of the present study is to determine whether saliva has a role in the contribution of bacterial growth on the surface of dental casts over a period of time and whether disinfection can minimize the bacterial growth on contaminated casts with saliva.

AIMS AND OBJECTIVES

1) To compare the effectiveness of different disinfectants on dental casts.

2) To analyze the most effective disinfectant for reducing number of colony forming units on dental casts.

MATERIALS AND METHODOLOGY

This in-vitro study was carried out in the Department of Prosthodontics, in association with the Vista Microbiological Laboratories. A healthy dentulous patient's saliva was obtained, commercially available sheep blood agar and phosphate buffered saline were used, to determine whether bacterial growth on the dental casts can occur and to evaluate the effectiveness of disinfectants on bacterial contaminated casts.

ARMAMENTARIUM

a) INSTRUMENTS USED

1. High Cobalt-Chromium Metal dies – 24 mm length and 6 mm diameter (Dentalloy, Chennai, India)
2. Rubber Bowl and Straight spatula (Classic dental products, Shahdara, New Delhi, India)
3. Incubator (Metzer opto, Mumbai, India)
4. 25 microliter pipet (Orbit autopipet-Ahmedabad, India)
5. Digital colony counter (EI Products, Panchkula, India)

6. Tweezer (Vaishnavi surgicals, Jalandhar, India)
7. Mechanical Vibrator (Confident, Chennai, India)

b) BIOLOGICAL MATERIALS USED

1) Type III Dental stone (Kalabhai, Vikhroli west, Mumbai, India)
2) Distilled water (Avi Scientific, Mumbai, India)
3) Addition silicone material (Aquasil. Dentsply, Surrey, UK)
4) Saliva (Undiluted) – From patient
5) Sterile test tube (Borosil, Nungambakkam, Chennai, India)
6) Phosphate buffered saline solution (Sigma-Aldrich, Missouri, USA)
7) Blood agar plates (Himedia, Tarnaka, Secunderabad, India)
8) Tap water
9) Glutaraldehyde 2% (SD fine Chem Ltd, Mumbai, India)
10) 5% sodium hypochlorite (Asian acrylates, Mumbai, India)

METHODOLOGY

Fabrication of master model

High Cobalt-Chromium Metal master dies (Fig.1) of 24 mm length 'and 6 mm in diameter were fabricated.

Preparation of dental stone cylinders

Impressions of master model dies were made in addition silicone elastomeric impression material. The dies were removed from the addition silicone impression material. They were poured with type III dental stone. The dental stone was allowed to set for one hour and the stone cylinders were removed. Out of which only 60 standard gypsum specimens was picked for the study. These dental stone cylinders (Fig.2) were preserved at room temperature and humidity for 24 hours and then divided into five groups consisting of 12 each.

Group I - 12 specimens were designated as controls without salivary contamination and without disinfectant.
Group II - 12 specimens were dipped in saliva for 5 minutes without any treatment.
Group III - 12 specimens were immersed in saliva for 5 minutes and rinsed in tap water for 20 sec.
Group IV – 12 specimens were immersed in saliva for 5 minutes and soaked in 5% sodium hypochlorite for 20 seconds.
Group V - 12 specimens were immersed in saliva for 5 minutes and soaked in 2% Glutaraldehyde for 20 seconds.

After this each of the cylinders was placed in sterile test tubes for 20-seconds containing 2.5 m1 of sterile phosphate buffered saline (Fig.3). The suspension was then agitated. To facilitate colony forming units, final volume of 250-microliteres with 10^{-4} dilution was obtained. To determine the number of bacteria in 10^{-4} dilution, 100- microliter from the total solution of 250-microlitere was transferred to a Sheep blood agar plates to obtain a lawn of growth on the surface of the plate. The total number of colony forming units (CFU) per plate would be equal to the CFU x2.5×10^{-4}. The blood agar plates were incubated at 37 °C for 24 hours followed by counting of colony forming units with digital colony counter (Fig.4) The data was analyzed using statistical software with ANOVA and DUNCANS non-parametric MULTIPLE RANGE TEST for specific comparison. .

RESULTS

For samples from all five groups, a dilution of 10^{-4} was made and one swab was taken for the culture. The results and their statistical values are shown in the following tables.

All specimens contaminated and not treated had a large number of CFUs covering the blood agar plate. Each blood agar plate was checked. The specimens that were contaminated and rinsed only with water showed a range of 50 - 200 colonies at 10^{-4} dilution. Half of the blood agar plates that were cultured from the dental stone cylinders and soaked in 5% sodium hypochlorite showed no colonies and the remaining had only a few (1 to 12) CFUs at 10^{-4}. The controls that were not contaminated with saliva and disinfectant had minimal growth on only two agar plates and no growth on remaining 10 agar plates. All the dental stone cylinders that were soaked for 20 seconds in 2% glutaraldehyde yield no growth.

Table - I: shows comparison of effectiveness of each disinfectant on number of colony forming units.

	Control group	Saliva contamination & without any treatment (Group-I)	Rinsing with Tap water Group-III)	Soaking in 5% sodium hypochlorite (Group-IV)	Soaking in 2% glutaraldehyde (Group-V)
1.	0	450	198	0	0
2.	0	354	170	8	0
3.	0	368	133	12	0
4.	0	450	150	4	0
5.	0	450	136	10	0
6.	2	600	59	0	0
7.	0	400	112	0	0
8.	0	450	124	4	0
9.	4	450	192	4	0
10.	0	396	152	0	0
11.	0	450	144	2	0
12.	0	450	84	0	0

Table II: Summary statistics of number of microbial colonies seen at 10^{-4} dilution in five groups.

Groups	N	Mean	Std. Dev.	SE	Median
Control group	12	0.50	1.24	0.36	0.00
Saliva contamination group	12	439.00	62.09	17.92	450.00
Tap water group	12	137.83	40.42	11.67	147.00
5% sodium hypochlorite group	12	3.67	4.25	1.23	4.00
2% glutaraldehyde group	12	0.00	0.00	0.00	0.00

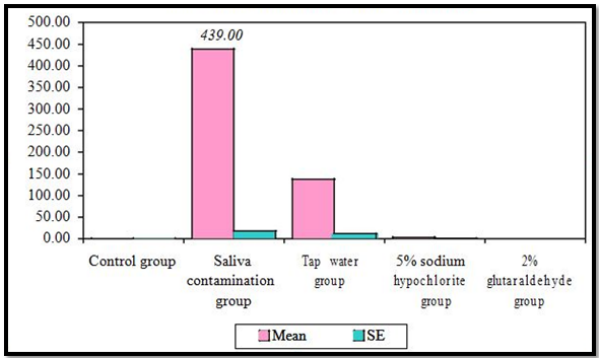
Table III: Comparison of five groups with respect to number of microbial colonies seen at 10^{-4} dilution by Kruskal Wallis ANOVA

Groups	Mean	Std.Dev.	SE	Median	Sum of ranks
Control group	0.50	1.24	0.36	0.00	200.00
Saliva contamination group	439.00	62.09	17.92	450.00	654.00
Tap water group	137.83	40.42	11.67	147.00	510.00
5% sodium hypochlorite group	3.67	4.25	1.23	4.00	298.00
2% glutaraldehyde group	0.00	0.00	0.00	0.00	168.00
H-value	52.7295				
p-value	0.00001*				

*p<0.05

The p-VALUE(<0.0001) indicates non homogeneity of means . Obviously glutaraldehyde 2% is the most efficient disinfectant with least number of CFU. Of the two disinfectants solutions evaluated, only 2% glutaraldehyde was able to be effective with notable reduction of colony forming units in all trials when compared to soaking in 5% sodium hypochlorite solution and tap water. The last two solutions followed closely in effectiveness.

Graph I: (One Dimensional) : Comparison of five groups with respect to number of microbial colonies seen at 10^4 dilution



Graph - II: (Two Dimensional) : Comparison of five groups with respect to number of microbial colonies seen at 10^{-4} dilution

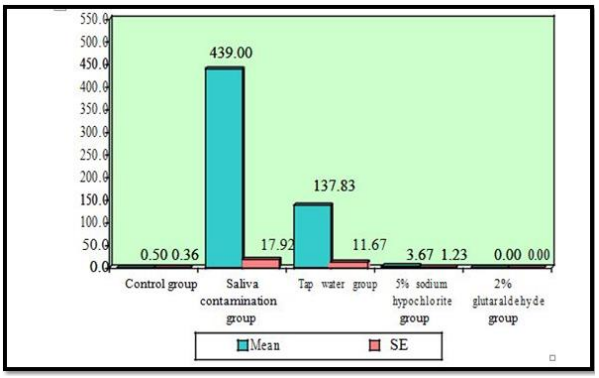


Fig 1: Metal master dies.

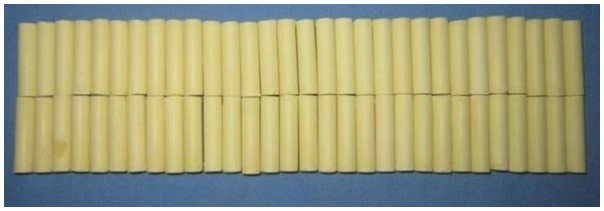


Fig 2: Dental stone cylinders.

Fig 3: Dental stone cylinders dipped in sterile phosphate buffered saline.

Fig 4: Digital colony counter.

DISCUSSION

Prosthodontics is one field of dentistry where prevention of cross contamination seems to be an insurmountable problem as majority of materials in dental laboratories are contaminated with bacteria transmitted by blood and saliva. Prevention of cross contamination should be prime consideration. The potential hazards of dentists acquiring or transmitting infectious diseases during delivery of dental care have been identified in recent dental literature.[8, 9] An indirect source of disease transmission is contaminated impressions.[10] The models made of dental stone

poured against contaminated impressions may be a medium for cross contamination between patients and dental personnel.[11]

In this study dental cast is used because it is transferred numerous times between the dental laboratory and the dental office. It has been shown that gypsum casts made from contaminated impressions can be medium for cross contamination between patients and dental personnel. The procedure of disinfecting the contaminated dental prosthesis before entering the laboratory is known as the "Barrier System", Barrier protection is a key part of any infection control and Occupational Safety and Health Administration (OSHA) exposure control program.[12]

Studies have shown that topical methods of disinfecting casts are difficult to control, while immersion methods are potentially destructive.[13] In this study, the disinfectants used were tap water, 5% sodium hypochlorite and 2% glutaraldehyde solution. The exposure time was chosen to be short i,e 20 sec for water , 20 sec for 5% Sodium hypochlorite and 20 sec for glutaraldehyde. The efficacy of disinfectant depends on length of treatment time and concentration. Undiluted saliva was used in this study because it is what the acrylic resin bases and dental stone cast would be contaminated with clinically.

Saliva was contaminated with specimens for 5 minutes in 2.5 ml solution of phosphate buffered saline to facilitate counting of colony forming units, and then dilutions were accomplished to reduce the relative numbers to manageable quantities. To determine the number of bacteria from the total solution was transferred to a sheep blood agar plate. The blood agar plates were incubated at 37^0C for 24 hours in the incubator.

All specimens contaminated with saliva and not treated, had a large number of colony forming units covering the blood agar plate. The observed pattern of disinfection on specimens that were contaminated and rinsed only with tap water showed a range of colonies. The specimens that were contaminated with saliva and soaked in 5% sodium hypochlorite had significantly less number of colony forming units when compared to that with only water. The specimens treated with 2% glutaraldehyde had no growth.

The use of water as a disinfectant in this study was justified by the fact that water was readily available at Chairside and was easily accessible to all practitioners. But use of tap water alone was not very effective in reducing the colony forming units. So the additional step was instituted, i.e soaking in 5% sodium hypochlorite significantly reduced number of colony forming units.

The concept of cold sterilization has modernized disinfection.[14] All three bodies advise soaking in 2% Glutaraldehyde as most effective disinfectant. The BDA recommends soaking for 3 hours, the FDI and ADA for 10 hours.[15] It has several advantages over other disinfectants,

i. Mild odor
ii. Readily available through many retail sources,
iii. The solution remains fully active for two weeks after activation
iv. Microbiologic testing indicates that the 2% solution of activated glutaraldehyde is effective in the destruction of fungi, viruses, and bacteria including mycobacterium tuberculosis when specimen immersed for 10 minutes.

This study utilizes diluted solutions and the most effective disinfectant is 2% Glutaraldehyde followed by Sodium hypochlorite. Stern M.A et al in 1981 evaluated dental stones after repeated exposure to various spray disinfectants. Mainly they concentrated on abrasion and compressive strength of stone.[16] Results showed 2% Glutaraldehyde spray decreased the compressive strength of type-III stone by 26%, phenols increased compressive strength of Type-IV stone by 18% and iodophore had no effect. This study supports spray disinfection. Various minimum acceptable CFUs reduction have been suggested. Donald L Mitchell criteria used in this study to determine that a solution was effective for use as a disinfectant for dental casts is that when it reduced CFU counts. The solution meeting this criterion is shown in (Table - I).

So overall in this study, 2% glutaraldehyde was much effective disinfectant for shorter duration exposure of 20 sec, when compared to rinsing in tap water and soaking in 5% sodium hypochlorite. Further research needs to be done with regard to surface damage from rinsing in tap water, scrubbing

with soap and water, or soaking in 2% glutaraldehyde for 20sec.

CONCLUSION

Within the limitation of this study, the following conclusions were derived.

1) Bacterial growth on dental stone cast is seen when the cast is contaminated with saliva.
2) Rinsing the specimens in tap water for 20 seconds diminishes the bacterial growth.
3) Soaking the dental stone cylinders in 5% sodium hypochlorite or soaking in 2% glutaraldehyde for 20 seconds reduces the bacterial growth of saliva contaminated dental stone significantly than rinsing only with running tap water.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

REFERENCES

1) Council on dental therapeutics: "Guidelines for infection control in the dental office and the commercial dental laboratory". J Am Dent Assoc.1985;110(6):969-72.

2) Mitchell DL, Hariri NM, Duncanson MG Jr, Jacobsen NL, McCullum RE. "Quantitative study of bacterial colonization on the dental casts". J Prosthet Dent. 1997;78(5):518-21.

3) Powell GL, Runnells RD, Saxon BA, Whisenant BK. "The presence and identification of organisms transmitted to dental laboratories". J Prosthet Dent. 1990;64(2):235-7.

4) Merchant VA, McNeight MK, Ciborowski CJ, Molinari JA. "Priliminary investigation of a method for disinfection of dental impressions". J Prosthet Dent. 1984;52(6):877-9.

5) Kugel G, Perry RD, Ferrari M, Lalicata P. "Disinfection and communication practices; A survey of U.S. Dental Laboratory". J Am Dent Assoc. 2000; 131(6):786-92.

6) Leung RL, Schonfeld SE."Gypsum casts as a potential source of microbial cross-contamination". J Prosthet Dent. 1983;49(2):210-1.

7) Twomey JO, Abdelaziz KM, Combe EC, Anderson OL. "Calcium hypochlorite as a disinfecting additive for dental stone". J Prosthet Dent. 2003;90(3):282-8.

8) Stern MA, Johnson GH, Toolson LB. "An evaluation of dental stones after repeated exposure to spray disinfectants". Part I: Abrasion and compressive strength. J Prosthet Dent. 1991;65(5):713-8.

9) Samaranayake LP, Hunjan M, Jennings KJ. "Carriage of oral flora on irreversible hydrocolloid and elastomeric impression materials". J Prosthet Dent. 1991;65(2):244-9.

10) Vandewalle KS, Charlton DG, Schwartz RS, Reagan SE, Koeppen RG. "Immersion disinfection of irreversible hydrocolloid impression with sodium hypochlorite; Part II – effect on gypsum". Int J Prosthodont. 1994;7(4):315-22.

11) Westerholm HS 2nd, Bradley DV Jr, Schwartz RS."Efficacy of various spray disinfectants on irreversible hydrocolloid impressions". Int J Prosthodont. 1992;5(1):47-54.

12) Brace ML, Plummer KD."Practical denture disinfection". J Prosthet Dent. 1993;70(6):538–40.

13) Abdelaziz KM, Combe EC, Hodges JS. "The effect of disinfectants on the properties of dental gypsum: 1. Mechanical properties". J Prosthodont. 2002;11(3):161-7.

14) Sarma AC, Neiman R. 'A study on the effect of disinfectant chemicals on physical properties of die stone". Quintessence Int. 1990;21(1):53-9.

15) Johansen RE, Stackhouse JA Jr."Dimensional changes of elastomers during cold sterilization". J Prosthet Dent. 1987;57(2):233-6.

16) Stern MA, Whitacre RJ. "Avoiding cross – contamination in Prosthodontics". J Prosthet Dent. 1981;46(2):120-2.