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Original Article

Comparative Evaluation of the Efficacy of Preprocedural Mouthrinse and Spray Disinfectant in Reducing Oral Microflora on Corrective Complete Denture Impression: A Crossover Clinical Study

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Abstract

Background: The study was taken up with the aim to identify the micro-organisms transferred on the surface of corrective complete denture impression and to compare the effectiveness of preprocedural mouthrinse in reducing oral microflora in corrective complete denture impression over spray disinfectant.

Materials and Methods: The study was conducted on maxillary complete denture impressions made on 30 completely edentulous subjects. A total of 90 impressions were made, and 90 custom trays were fabricated for 30 subjects. These custom trays were divided into three groups: 30 corrective impressions were secured without using mouthrinse (control group), 30 corrective impressions obtained were disinfected with 2% glutaraldehyde (Cidex) for 20 min, and 30 corrective impressions were made after making the subject rinse with hydrogen peroxide mouthwash (hydroxyl) with specified dilution (1:4) for 30 s. The identification of micro-organisms was done using catalase test, oxidase test, and Gram-staining. Student's *t*-test was used for statistical analysis of the study.

Results: For the control group, 8 patients showed growth of coagulase −ve *Staphylococcus* and *Streptococcus* viridans; 8 patients showed only *Streptococcus* viridans; in 14 patients only coagulase −ve *Staphylococcus* was found. The data obtained revealed that 18 impressions out of 30 were rendered fully sterile by spray disinfection while the remaining 12 impressions showed a decrease in the colony count of coagulase −ve *Staphylococcus* and *Streptococcus* viridans. Preprocedural mouthrinsing resulted in total elimination of coagulase −ve *Staphylococcus* and *Streptococcus* viridans in 28 impressions while the remaining impressions showed a definite reduction in colony count of these two micro-organisms. 93.33% of the maxillary corrective complete denture impressions secured after preprocedural mouthrinse with hydrogen peroxide showed total elimination of coagulase −ve *Staphylococcus* and *Streptococcus* viridans. Only 60% showed total elimination of micro-organisms for impressions secured after spray disinfection.

Conclusion: The study showed that preprocedural mouthrinsing resulted in significant reduction in viable micro-organisms on the surface of the impression.

Key words: Maxillary impression, Preprocedural mouthrinsing, Spray disinfectant

INTRODUCTION

Dentistry is predominantly a field of surgery involving exposure to blood, saliva, and other potentially infectious materials, and therefore, requires a high standard of infection control and safety.[1]

Analysis of prosthodontic setups shows that many of the instruments and support equipment carry the potential to transmit disease but is not amenable to adequate sterilization or disinfection. Dental practitioners, auxiliaries, and laboratory personnel are subject to significant risk with respect to infectious disease, which can be spread by saliva or blood as droplets and aerosols, or by direct contact.[2]

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Impressions are laden with micro-organisms after removal from the oral cavity, and some of these organisms have the potential for disease transmission.[3,4] Potential pathogens have been isolated from the impressions and organisms have been shown to survive up to 5 h on an impression.[5] The prevalence of these diseases and their potentially harmful effects mandate adherence to infection control procedures in the dental office and laboratory. Dental office personnel may not follow the recommended protocols for disinfecting impressions and other items that come in contact with a patient.[6] Therefore, prosthodontists and the associated personnel are at an added risk of transmission of the infection spreading through contaminated impressions and the casts thus obtained.[7]

Since sterilization of impressions is expensive, time consuming, and potentially damaging to the material, spray disinfection with various chemicals has become a practical alternative.[8] Various studies have focused the intention toward the destruction of micro-organisms with various disinfectants as regard their duration without causing dimensional changes.[1,4,9]

The use of mouthrinse is an effective and feasible way to reduce viable bacteria in the oral cavity.[10] Preprocedural mouth rinsing seems to be one of the most effective methods of controlling the spread of bacteria in the dental office.

According to the Center for Disease Control, "blood and saliva should be thoroughly and carefully cleaned from impression material that has been used in the mouth. Contaminated materials, impression, and intra-oral devices should also be cleaned and disinfected before being handled in the dental laboratory and before they are placed in a patient's mouth."[11] It is imperative that the recommendations for disinfecting dental impressions, presented by the Center for Disease Control be followed.[12] Therefore, the study was taken up with the aim to identify the micro-organisms transferred on the surface of corrective complete denture impression and to compare the effectiveness of preprocedural mouthrinse in reducing oral microflora in corrective complete denture impression over spray disinfectant.

MATERIALS AND METHODS

The study was conducted in the Department of Prosthodontics, Bhojia Dental College and Hospital, Baddi, Himachal Pradesh, on maxillary complete denture impressions made on 30 suitable completely edentulous subjects of either sex without any recent history of common cold, sore throat, and antibiotic medication.

The study participants were given clear explanation about the objective of the study. Ethical clearance was obtained from the concerned authorities of the institution. Voluntary informed consent was obtained from all the subjects.

Initial impression was made with impression compound. For each subject, three custom trays were fabricated with autopolymerizing acrylic resin on the cast obtained from the impression for each subject. Thus, a total of 90 custom trays were fabricated for 30 subjects. These custom trays were divided into three groups, and corrective impression was secured with zinc-oxide eugenol impression paste.

- Group I (control group): 30 corrective impressions were secured without using mouthrinse
- Group II: 30 corrective impressions obtained were disinfected with 2% glutaraldehyde, (Cidex) for 20 min
- Group III: 30 corrective impressions were made after making the subject rinse with hydrogen peroxide mouthwash (hydroxyl) with specified dilution (1:4) for 30 s.

Saliva sample was collected with sterile swab from each of the zinc-oxide impressions. It was plated on 5% sheep blood agar and then on MacConkey agar. Thereafter with the help of Nichrome loop sterilized on the flame, streaking of both the plates was done. Following this, the plates were kept in candle jar (5% carbon dioxide) and immediately transported to the laboratory.

The candle jar was kept in incubator at 37°C for 48 h. After 48 h, the plates were reviewed for colonies.

Catalase test, oxidase test and Gram-staining were done for all plates.

Catalase test is primarily used to differentiate between genera *Staphylococcus* from *Streptococcus*. Certain bacteria have enzyme catalase which acts on hydrogen peroxide to release nascent oxygen. In catalase test, first of all, a drop of 3% hydrogen peroxide is put on a slide. Then with the help of cover slip, the colonies were taken and touched them with 3% H_2O_2. There was bubble formation due to the release of nascent oxygen. *Staphylococcus* is catalase positive and *Streptococcus* is catalase negative.

The principle of oxidase test is to determine the presence of an enzyme cytochrome oxidase which catalyses the oxidation of reduced cytochrome by molecular oxygen. In oxidase test, a slide containing oxidase disc of Hi-Media was taken. Then, the colony was taken from the MacConkey agar plate with a cover slip and touched them with oxidase disc.

Gram staining (or Gram's method) is a method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative). It is based on the chemical and physical properties of their cell walls. Primarily, it detects peptidoglycan, which is present in a thick layer in Gram-positive bacteria.

Staining Mechanism

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell envelope), which are stained purple by crystal violet (CV), whereas Gram-negative bacteria have a thinner layer (10% of cell envelope), which are stained pink by the counter-stain. There are four basic steps of the Gram stain:

- Applying a primary stain (CV) to a heat-fixed smear of a bacterial culture. Heat fixing kills some bacteria but is mostly used to affix the bacteria to the slide so that they do not rinse out during the staining procedure
- The addition of a mordant, which binds to CV and traps it in the cell (Gram's iodine)
- Rapid decolorization with alcohol or acetone, and
- Counterstaining with safranin. Carbol fuchsin is sometimes substituted for safranin since it will more intensely stain anaerobic bacteria but it is much less commonly employed as a counterstain. CV dissociates in aqueous solutions into CV^+ and chloride (Cl^-) ions. These ions penetrate through the cell wall and cell membrane of both Gram-positive and Gram-negative cells. The CV^+ ion interacts with negatively charged components of bacterial cells and stains the cells purple.

Iodine (I^-) interacts with CV^+ and forms large complexes of CV and iodine (CV-I) within the inner and outer layers of the cell. Iodine is often referred to as a mordant but is a trapping agent that prevents the removal of the CV-I complex, and therefore, colors the cell.

When a decolorizer such as alcohol or acetone is added, it interacts with the lipids of the cell membrane. A Gram-negative cell will lose its outer lipopolysaccharide membrane, and the inner peptidoglycan layer is left exposed. The CV-I complexes are washed from the Gram-negative cell along with the outer membrane. In contrast, a Gram-positive cell becomes dehydrated from an ethanol treatment. The large CV-I complexes become trapped within the Gram-positive cell due to the multilayered nature of its peptidoglycan. The decolorization step is critical and must be timed correctly; the CV stain gets removed from both Gram-positive and negative cells if the decolorizing agent is left on too long (a matter of seconds).

After decolorization, the Gram-positive cell remains purple and the Gram-negative cell loses its purple color. Counterstain, which is usually positively charged safranin or basic fuchsin, is applied last to give decolorized Gram-negative bacteria a pink or red color.

Identification of Micro-organisms

Catalase positive *Staphylococcus* is identified by slide coagulase test. In slide coagulase test, a drop of normal saline 0.85% was put. Then with a nichrome loop, a colony from the McConkey/blood agar was taken and mixed with normal saline. After it, a drop of plasma was added and looked for agglutination. If agglutination was present, it indicated coagulase positive *Staphylococcus aureus* and in the absence of agglutination, the micro-organism identified was coagulase-negative *Staphylococcus* which is a normal microflora in humans.

Catalase negative *Streptococcus* viridans (normal microflora) are identified by its partial discoloration on blood agar and greenish tinge around colonies. If there is complete hemolysis of catalase negative *Streptococcus*, it indicates *Streptococcus pyogenes* which is a pathogen. If no hemolysis occurs, it indicates Group D *Streptococcus*.

In oxidase test, change to violet/purple color indicated oxidase positive micro-organisms.

Gram-positive bacteria are arranged in chains, few in packs and few in bunches.

Statistical Analysis

Student's *t*-test was used for statistical analysis of the study. The $P < 0.05$ was accepted as indicating statistical significance and $P \leq 0.001$ was noted as highly significant. Student's *t*-test was used to find a significant difference between two means. The results were averaged (mean ± standard deviation) for each parameter.

RESULTS

Subject categorization was done (Table 1) and a total of 90 impressions were made for 30 patients.

The micro-organisms identified in 30 patients are presented in Figures 1 and 2, Table 2. For the control group, 8 patients showed growth of coagulase −ve *Staphylococcus* and *Streptococcus* viridans; in 8 patients only *Streptococcus* viridans was found. In 14 patients, only coagulase −ve *Staphylococcus* was found. The data obtained revealed that 18 impressions out of 30 were rendered fully sterile by spray disinfection while the remaining 12 impressions showed a decrease in the colony count of coagulase −ve *Staphylococcus* and *Streptococcus* viridans. The most striking feature was the total elimination of coagulase –ve *Staphylococcus* and *Streptococcus* viridans in 28 impressions after preprocedural mouthrinsing while the

Table 1: Distribution of groups

Groups	Procedure	Total
I	No preprocedural mouthrinsing or spray disinfection of corrective impression	30
II	Spray disinfection of corrective impression	30
III	Corrective impression after preprocedural mouthrinse	30

Table 2: Colony count in each subject

Colony count for control group	Colony count after disinfection	Colony count after mouthwash
Streptococcus viridans>10^5 mg/ml	3.7×10^4 organisms/ml	Sterile
Coagulase−ve *Staphylococcus*>10^5 mg/ml	Sterile	Sterile
Coagulase−ve *Staphylococcus*>10^5 mg/ml	Sterile	Sterile
Coagulase−ve *Staphylococcus*>10^5 mg/ml	Sterile	Sterile
Coagulase−ve *Staphylococcus*>10^5 mg/ml	Sterile	Sterile
Streptococcus viridans>10^5 mg/ml and coagulase−ve *Staphylococcus*>10^5 mg/ml	4.8×10^4 org/ml	Sterile
Streptococcus viridans>10^5 mg/ml	Sterile	Sterile
Coagulase−ve *Staphylococcus*>10^5 mg/ml	Sterile	Sterile
Streptococcus viridans>10^5 mg/ml and coagulase−ve *Staphylococcus*>10^5 mg/ml	7.2×10^4 org/ml	Sterile
Streptococcus viridans>10^5 mg/ml	5.5×10^4 org/ml	Sterile
Streptococcus viridans>10^5 mg/ml	8.8×10^4 org/ml	Sterile
Streptococcus viridans>10^5 mg/ml and coagulase−ve *Staphylococcus*>10^5 mg/ml	5.4×10^4 org/ml	Sterile
Coagulase−ve *Staphylococcus*>10^5 mg/ml	2.4×10^4 org/ml	Sterile
Streptococcus viridans>10^5 mg/ml and coagulase−ve *Staphylococcus*>10^5 mg/ml	6.4×10^4 org/ml	Sterile
Streptococcus viridans>10^5 mg/ml and coagulase−ve *Staphylococcus*>10^5 mg/ml	4.5×10^4 org/ml	Sterile
Coagulase−ve *Staphylococcus*>10^5 mg/ml	7.8×10^4 org/ml	Sterile
Coagulase−ve *Staphylococcus*>10^5 mg/ml	Sterile	Sterile
Coagulase−ve *Staphylococcus*>10^5 mg/ml	Sterile	Sterile
Coagulase−ve *Staphylococcus*>10^5 mg/ml	Sterile	Sterile
Coagulase−ve *Staphylococcus*>10^5 mg/ml	Sterile	Sterile
Streptococcus viridans>10^5 mg/ml and coagulase−ve *Staphylococcus*>10^5 mg/ml	Sterile	Sterile
Coagulase−ve *Staphylococcus*>10^5 mg/ml	4×10^4 org/ml	3.2×10^4 org/ml
Streptococcus viridans>10^5 mg/ml	Sterile	Sterile
Streptococcus viridans>10^5 mg/ml	Sterile	Sterile
Streptococcus viridans>10^5 mg/ml and coagulase−ve *Staphylococcus*>10^5 mg/ml	Sterile	Sterile
Streptococcus viridans>10^5 mg/ml and coagulase−ve *Staphylococcus*>10^5 mg/ml	7×10^4 org/ml	5×10^4 org/ml
Streptococcus viridans>10^5 mg/ml	Sterile	Sterile
Coagulase−ve *Staphylococcus*>10^5 mg/ml	Sterile	Sterile
Coagulase−ve *Staphylococcus*>10^5 mg/ml	Sterile	Sterile
Streptococcus viridans>10^5 mg/ml	Sterile	Sterile

remaining 2 impressions showed a definite reduction in colony count of these two micro-organisms.

About 93.33% of the maxillary corrective complete denture impressions secured after preprocedural mouthrinse with hydrogen peroxide showed total elimination of coagulase −ve *Staphylococcus* and *Streptococcus* viridans. For impressions secured after spray disinfection, 60% showed total elimination of micro-organisms.

Pairing of samples was done (Tables 3 and 4). According to statistical analysis a highly significant ($P < 0.001$) reduction in colony count of bacteria was observed in pair 3, a significant reduction in pair 2 and a reduction in pair 1. Thus, greatest reduction in the colony count was found in the pair comparing preprocedural mouthwash and disinfection.

DISCUSSION

The aim of the study was to assess the efficiency of a 30-s preprocedural mouthrinse with hydrogen peroxide mouthwash (1:4) over 2% glutaraldehyde spray disinfection for 20 min in reducing viable coagulase −ve *Staphylococcus* and *Streptococcus* viridans in maxillary corrective complete denture impression secured with zinc-oxide eugenol impression paste.

The disinfection of impressions is a standard recommendation for infection control procedures in prosthodontics. Concerns have been expressed about the effects of disinfection on the impression materials. Research has shown that disinfection process may cause degradation or distortion of the impressions. Nevertheless, the American Dental Association (ADA)

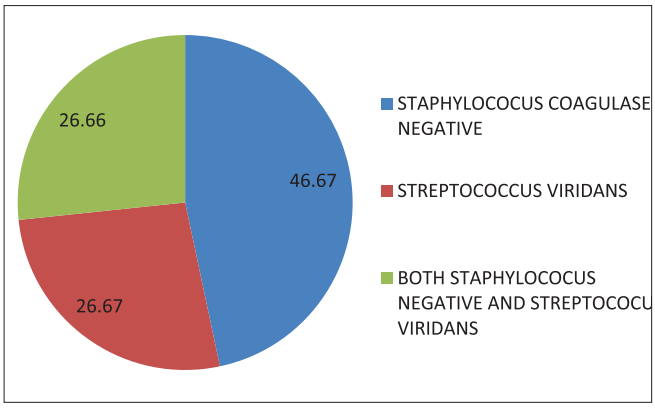

Figure 1: Micro-organisms identified

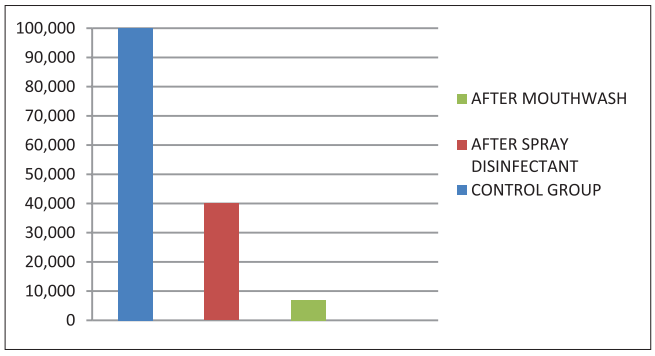

Figure 2: Colony forming units

currently recommends immersion or spray disinfection with an ADA-accepted disinfectant for the manufacturer's recommended contact time.[13] Kern *et al.* demonstrated the effectiveness of glutaraldehyde in reducing micro-organisms on impression materials.[14]

Look *et al.* reported that although 2% glutaraldehyde achieved total viral inactivation in <1 min, short disinfectant sprays, in general, are not an appropriate disinfection method.[2]

de Albuquerque *et al.* in their study found that single preprocedural chlorhexidine mouthrinse is effective in reducing salivary micro-organisms.

The authors demonstrated that low-concentration, 30-s chlorhexidine mouthrinses could be an easy and inexpensive method to help reduce postoperative infections by lowering oral counts of *S. aureus* and mutans group streptococci.[15]

Wennström and Lindhe J studied the effect of hydrogen peroxide release during mouth rinsings on the composition of the microbiota of developing plaque in humans and the amount and pathogenicity of the plaque formed. The authors suggested that hydrogen peroxide released by mouthwashes during rinsing may prevent or

Table 3: Pairing of samples (*t*-test)

Paired samples statistics	Mean	*n*	Standard deviation	Standard error mean
Pair 1				
Colony count for control group	100,000.0000	30	0.00000	0.00000
Colony count after disinfection	22,500.0000	30	30,306.19602	5533.12906
Pair 2				
Colony count for control group	100,000.0000	30	0.00000	0.00000
Colony count after mouthwash	2733.3333	30	10,667.16953	1947.54979
Pair 3				
Colony count after disinfection	22,500.0000	30	30,306.19602	5533.12906
Colony count after mouthwash	2733.3333	30	10,667.16953	1947.54979

Table 4: Pairing of samples (*t*-test)

Paired samples test	Paired differences					*t*	df	Significant (2-tailed)
	Mean	Standard deviation	Standard error mean	95% confidence interval of the difference				
				Lower	Upper			
Pair 1								
Colony count for control group-Colony count after disinfection	77500.00000	30306.19602	5533.12906	66183.48042	88816.51958	14.007	29	0.042*
Pair 2								
Colony count for control group-Colony count after mouthwash	97266.66667	10667.16953	1947.54979	93283.48010	101249.85323	49.943	29	<0.006*
Pair 3								
Colony count after disinfection-Colony count after mouthwash	19766.66667	28806.94760	5259.40500	9009.97565	30523.35768	3.758	29	<0.001**

*P<0.05, **P<0.001

retard the colonization and multiplication of anaerobic bacteria.[16]

Therefore, the use of mouthrinses is an effective and feasible way to reduce viable bacteria in the oral cavity. Preprocedural mouth rinsing seems to be one of the most effective methods of controlling the spread of bacteria in the dental office, and some studies have addressed this topic.[10]

Similarly, in prosthodontics mouthrinsing before impression making with a recommended mouthwash may be employed as an effective infection-control procedure. This procedure may put an end to various contradictions regarding the dimensional stability and accuracy of impression material subject to spray or immersion disinfection. It may be potent in preventing cross-contamination, and therefore, reduces the dangers involved in the spread of certain infectious diseases to the prosthodontists, ancillary, and the laboratory personnel.

Chacra *et al*. in their study found that by decreasing colonization of bacteria, hydrogen peroxide promotes local hygiene. Hydrogen peroxide improves coagulation and decreases the incidence of bleeding without side effects.[17,18] Furthermore, hydrogen peroxide mouth rinse contains no alcohol, and thus, does not dry out the oral cavity.

The hydrogen peroxide mouthwash is generally used to fight and prevent various oral harmful bacteria and infections. It is thought to decrease colonization of bacteria and infection, thereby decreasing the severity and duration of pain. It has further shown enhanced wound healing following gingival surgery.[17]

The results of this study proved the impressive efficacy of hydrogen peroxide mouthwash. In 93.33 % of the maxillary corrective complete denture impressions secured after preprocedural mouthrinse with hydrogen peroxide, there was total elimination of coagulase −ve *Staphylococcus* and *Streptococcus* viridans. While for impressions secured after spray disinfection the success rate was 60%. Thus, indicating preprocedural mouthrinsing better than spray disinfection. The factor that needs to be emphasized here is that superior efficacy of preprocedural mouthrinse is free from any objections regarding the integrity of the impressions obtained in this manner. It is important to mention that the preprocedural mouthrinsing infection-control method, i.e., before impression making in completely edentulous mouth has not been addressed often if not ever in the literature available.

- The limitation of the study was that only healthy patients were included in the sample size. Therefore, only the normal oral micro-organisms were evaluated
- Within the limitations of the study, preprocedural mouthrinsing can be used as an aid to prevent cross-contamination and infection-control procedure in prosthodontics. Uncompromising dimensional accuracy and stability of the impressions obtained in this manner is the major benefit of this procedure. In addition, it has a chemotherapeutic role without any known side effect on the oral hygiene of an edentulous mouth and is quite economical.

CONCLUSION

The study showed that preprocedural mouthrinsing was found to an effective measure for lowering the viable micro-organisms on the surface of the impression.

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