

Efficacy of Various Disinfecting Agents on the Reduction of Bacteria from the Surface of Silicone and Polyether Impression Materials

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Abstract -The aim of the present study was to evaluate the effectiveness of different disinfectants on the reduction of two resistant bacteria from the surface of impression materials. Impressions were made of a sterile metal model of the edentulous maxillary arch which had been contaminated with *Staphylococcus aureus* and *Enterococcus faecalis*. The impressions were cultured before and after disinfection with 0.525 % sodium hypochlorite, Gludex and Mikrozid spray disinfectant. For each of the three impression materials and the two microorganisms, spray disinfectant was found to be less effective than either sodium hypochlorite or Gludex.

KEY WORDS: Impression material; microbiology; disinfection; disinfectants

INTRODUCTION

The risk of infections transmitted by saliva and blood is considered a potential occupational hazard in dentistry¹ and cross-infection is a concern for the profession². The practice of prosthodontics presents major obstacles to the prevention of cross contamination³ as any instrument or piece of equipment used in the oral cavity or on orally soiled prosthetic device or impression is a potential source of cross-infection⁴. Impression materials in contact with the oral tissues, saliva and possibly blood may act as a media for the potential transfer of organisms from patients to dental personnel⁵. Impressions may put laboratory technicians at risk and could result in cross contamination between dental prostheses and patients⁶. To adequately protect all dental personnel and patients, a suitable barrier technique should be used to disinfect all prostheses, devices, impressions and other associated materials used in the mouth that enter or leave the dental laboratory⁷.

The American Dental Association (ADA) have made a series of recommendations regarding infection control procedures for impressions entering the dental laboratory^{2,8}. These guidelines recommended spray or immersion disinfection with a high level disinfectant or sterilant. In 1991 and 1996 The ADA guidelines were modified and recommended spray or immersion disinfection with an ADA accepted disinfectant for the manufacturer's recommended contact time^{9,10,11}. Current ADA recommendations for disinfection of impressions indicate that they should be rinsed to remove blood, saliva and debris and disinfected before being poured in stone or sent to the dental laboratory². Immersion and spray disinfection as well as various disinfection solutions have been tested and shown to be effective for this purpose^{2,10,12}.

Reversible and irreversible hydrocolloids, polyethers and some newer addition silicone materials are hydrophilic¹³. The disinfection process should be adequate but should not adversely affect the dimensional accuracy or surface detail of the impression. Several variables can affect impression materials, including the composition and concentration of the disinfectant, the exposure time and the compatibility of various disinfectants with specific impression materials⁹. Lepe and Johnson¹³ found that overnight immersion of polyether or addition silicone impressions in 2 % glutaraldehyde significantly affected their occlusogingival dimensions, as well as the mesio-distal dimensions of the addition silicone. It is well known that polyethers and newer hydrophylic materials tend to absorb water and some authors have noted distorted characteristics after these materials are disinfected for prolonged periods^{14,15}.

Several previous studies have found that immersion disinfection with 2 % glutaraldehyde is efficient and does not have a pronounced adverse side effect on the wettability and mass change of polyether and addition silicone materials^{16,17,18}. Sodium hypochlorite has been at least as effective as other disinfectants for impressions^{5,6,19}. It has been shown to be effective against both bacteria²⁰ and viruses, including HIV²¹ and Hepatitis B²². It was reported to cause little or no distortion or damage to impressions if diluted 1:10 (0.525 %) with a contact time of 10 minutes or less^{23,24}. When a short immersion technique is used, there is a concurrent loss of antimicrobial activity. There is limited information available concerning the microbiological effects of short term disinfection techniques to reduce the negative side effects on the polyether and silicone impression materials. The purpose of this study is to evaluate the effectiveness of various disinfecting agents on the reduction of bacteria from the surface of polyether and silicone impression materials.

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MATERIALS AND METHODS

Three different elastomeric impression materials were tested [Oranwash L (Zhermack, Italy), Impregum Penta Soft (3M Espe, Germany), P2 (Kulzer Germany)]. Facultative microorganisms such as *S. aureus* and *E. faecalis* are considered by many to be the most resistant species in the oral cavity^{25,26}. The two resistant microorganisms, *S. aureus* and *E. faecalis* were selected for this study to evaluate the effectiveness of disinfectants.

A metal model of an edentulous maxillary arch was sterilized in an autoclave. The model was then contaminated by immersion in 0.9 % saline containing approximately 10^7 bacteria/ml of either *Staphylococcus aureus* (ATCC 25923) and *Enterococcus faecalis* (ATCC 29212) for 10 minutes. Silicone impression material was hand-mixed for 30 seconds following the manufacturer's instructions, while the polyether impression materials were mixed in an automatic mixing machine (3M Espe Pentamix). An impression was made of the contaminated model and was allowed to set at room temperature for 3 minutes, using a sterile metal rim lock tray. The impression was then removed and rinsed slowly for 15 seconds with 250 cc of sterile water to simulate the rinse performed by the dentist after removing an impression from a patient's mouth. After gently shaking off excess water, viable bacterial transfer was verified by sampling the impression sites of the right and left maxillary first molars with a sterile cotton swab soaked in D/E Neutralizing Broth (Becton Dickinson, Cockeysville, MD). Each swab was placed in D/E Neutralizing Broth and swirled vigorously to dislodge bacteria.

After initial sampling, impressions were treated with three different test agents [0.525 % sodium hypochlorite (prepared at Gazi University, Department of Pharmacology), Gludex (2 % glutaraldehyde, Cimedix, Turkey), Mikrozyd spray (25g ethanol 94 %, 35g 1-propanol, Schülke and Mayr, Germany)]. Impressions were immersed for 10 minutes both in a solution of freshly prepared 0.525 % sodium hypochlorite or 2 % glutaraldehyde disinfectant solution. Alternatively impressions were sprayed with a sprayed disinfecting agent to fully cover all surfaces. The sprayed impressions were then sealed in a zip lock plastic bag and stored at room temperature for 10 minutes.

After the disinfection process, the impressions were again rinsed with 250 cc. of sterile water for 15 seconds and then were gently shaken to remove excess disinfectant and water. The sites of right and left maxillary second molars in the impression were then cultured as before. Five similar metal models of edentulous maxillary arch were used in the study and the models were re-inoculated with bacteria after each impression. Five impressions were made for each microorganism and for each disinfectant and two samples were taken from each impression before and after the disinfection procedures (n=10). Five impressions per organism went through the disinfection procedure but using with sterile water as a negative control. The pre-impression and post-impression cultures were plated on trypticase soy agar (BBL, Becton Dickinson, Cockeysville, MD) and all plates were incubated at 37°C for 24 to 48 hours, depending on the rate of growth. After incubation, all plates were examined for growth and standard microbiologic methods were used to identify and enumerate recovered bacteria.

In the present study, for the determination of the effects of the disinfectants the success proportions in mortality was used. These success proportions or the mortality rates are between 0 and 1. The scores were not normally distributed therefore, for all statistical analysis nonparametric techniques were used. The effects of disinfectants were compared for each bacteria and material combination. The effect of disinfectants and the required pair-wise comparisons were analysed by using nonparametric k-independent sample test (k :The number of disinfectants =4) with Kruskal-Wallis statistics. Differences were considered significant at $p < 0.001$.

RESULTS

The results of the present study and the comparisons are clearly shown in Table 1. The effects of disinfecting agents for each bacteria and material combination, the mean ranks of disinfectants, Chi-square statistics value and the significant level were presented in Table 1. The distribution of success proportions of disinfectants for each bacteria and material combination are shown in Figure 1 and 2. All the disinfecting agents used in this study have high reduction rates compared with the control which generally showed low reduction rates for each bacteria and material combination.

Of the disinfecting agents used, Gludex was shown to be the most effective solution in the elimination of *S. aureus* and *E. faecalis* from the surface of Oranwash L, followed by sodium hypochloride, Mikrozyd spray disinfectant and the control. The difference between each disinfecting agents and the control group were found to be statistically significant ($p < 0.001$). For the Penta Soft material Gludex was found the most effective disinfectant in reducing the amount of *S. aureus* followed by sodium hypochloride, Mikrozyd spray disinfectant and the control. The differences between the three disinfecting agents were not found statistically significant ($p > 0.001$), however statistically significant difference was found between the disinfectants and the control ($p < 0.001$). For the Penta Soft material, all disinfecting agents had a similar effect against *E. faecalis*. Although the disinfection values of the control group were less than those of other disinfecting agents, no statistically significant difference was observed between the groups ($p > 0.001$). For the P2 impression material, the most effective disinfecting agents were Gludex and sodium hypochloride. Spray disinfectant was less effective than Gludex and sodium hypochloride and the control group was the least effective of all.

DISCUSSION

A number of bacteria, fungi, and viruses present in the dental environment have been linked to debilitating and life-threatening diseases²⁷. Microbial contamination of dental materials and prostheses has been documented by a number of workers^{28,29}. Impressions are laden with microorganisms after removal from the oral cavity and some of these organisms have the potential for disease transmission. Potential pathogens have been isolated from impressions³⁰ and organisms have been shown to survive up to 5 hours on an impression¹.

Table 1. The mean ranks, Chi-square statistics value and the significant level of disinfectants on impression materials for each microorganisms.

	ORANWASH L				PENTA SOFT				P2			
	GLUDEX	SOD,HP	SPRAY	CONTROL	GLUDEX	SOD,HP	SPRAY	CONTROL	GLUDEX	SOD,HP	SPRAY	CONTROL
<i>S.AUREUS</i>	32.50	26.80	17.10	5.60	27.20	25.10	24.20	5.50	28.50	28.50	19.50	5.50
	Chi-square=32.618, p<0.001				Chi-square=27.639, p<0.001				Chi-square=33.033, p<0.001			
<i>E. FAECALIS</i>												
	Chi-square=31.404, p<0.001				Chi-square=6.154, p=0.104				Chi-square=32.681, p<0.001			

* Continuous horizontal lines between two or three disinfectants indicate that there was no significant difference (p>0.001)
Discrete horizontal lines indicate that there was significant difference between the disinfectants (p<0.001)

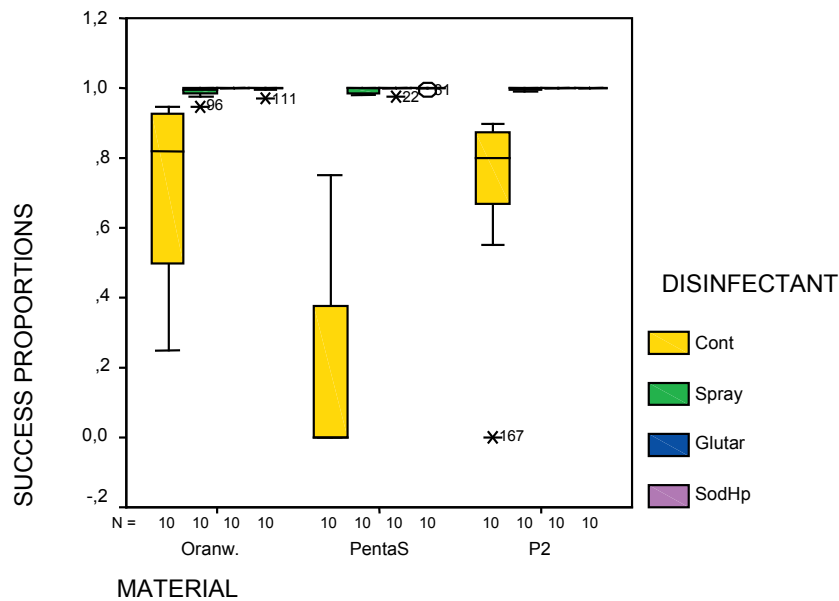


Figure 1. The effect of disinfecting agents on the reduction of *S. aureus* counts after disinfection.

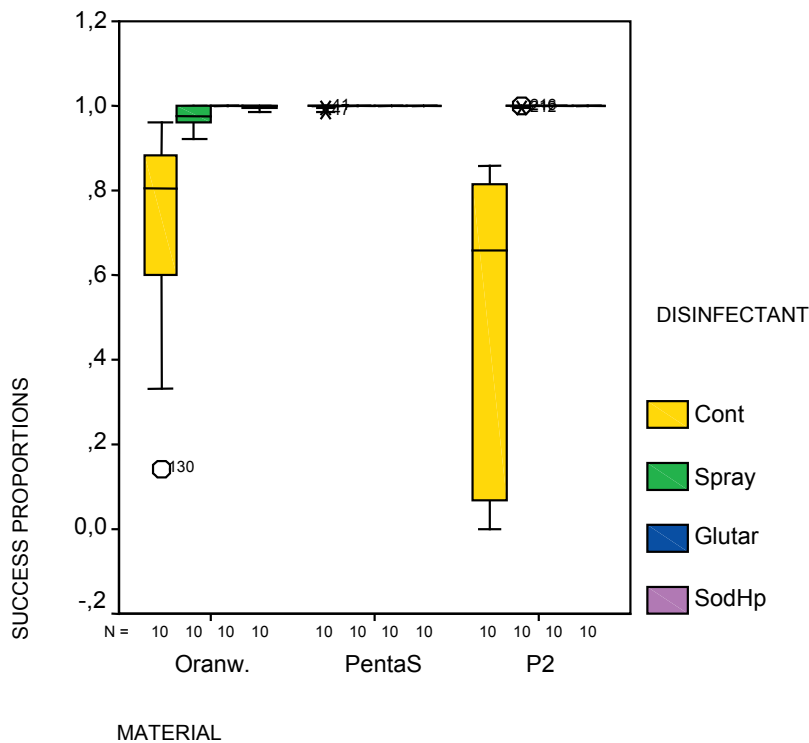


Figure 2. The effect of disinfecting agents on the reduction of *E. faecalis* counts after disinfection.

Some pathogenic contaminants include bacteria such as *E. coli*, *S. aureus*, *E. faecalis*, *P. aeruginosa*, *S. mutans* and the yeast *C. albicans*. The microorganisms (*S. aureus*, *E. faecalis*) chosen for this study are widely used to evaluate the effectiveness of disinfectants. *S. aureus*, a pathogen causing respiratory infections, has often been isolated from dentures and the oral cavity^{26,31,32}. *S. aureus*, especially methicillin-resistant *S. aureus*, is resistant to antibiotics so that the nosocomial infection can become a serious problem in clinical management³³. *E. faecalis*, a facultatively anaerobic gram-positive coccus, is a normal commensal adapted to the ecologically complex environment of the oral cavity and gastrointestinal tract³⁴. *E. faecalis* is known

to be a resistant species. It can withstand certain chemical agents, including some antibiotics, which would be highly toxic to other organisms³⁵.

In 1991 ADA Council on dental materials, Instruments and Equipment recommended the immersion disinfection of polyether materials in either hypochloride, iodophor or glutaraldehyde¹⁰. A 10-minute, 1:10 dilution of 5.25 % sodium hypochloride was listed as a recommended surface disinfectant². To prevent the possible distortion of the silicone and polyether impression materials, a disinfection time of 10 minutes was used in the present study.

There have been many previous studies concerning the disinfection of impressions^{12,36,37}, yet nearly all of these studies have looked at the effect of disinfection process has on the impression integrity or on the physical characteristics of the casts produced^{16,23}. However, few studies have investigated the efficacy of the disinfection process and the disinfectants used^{6,12,18,38}. The aim of this study was to determine effectiveness of three disinfecting agents in the elimination of two bacteria on silicone and polyether impression materials. A control group of samples treated with sterile water for 10 min. served as a negative control.

All the disinfecting agents used in the current study had more success with high reduction rates than the control although significant differences were also found between the disinfecting agents for each bacteris and material combination. It was observed that each disinfectant displayed significantly different effect on Oranwash L, however Gludex and 0.525 % sodium hypochlorite had a similar effect on P2 polyether impression material. Furthermore it was found that there was no significant difference between the effects of all the disinfectants in the reduction of *S. aureus* and *E. faecalis* on Penta Soft impression material. Organisms may reside in surface porosities, which may provide a protective microenviroment. The surface chemistry of some impression materials may inhibit certain disinfectants, and some disinfectants may be better absorbed into the impression material. This may be the reason why the effects of the disinfectants were different on silicone and polyether impression materials. Schwartz *et al*³⁹ also indicated the importance of the surface chemistry of the impression materials on the effectiveness of the disinfecting agents.

Sodium hypochlorite is one of the world's oldest and most widely used disinfectant⁴⁰. Rueggeberg *et al*.³⁸ reported that impressions contaminated with *S. sobrinus* showed no growth of bacteria after a 10-minute immersion in 0.5 % sodium hypochlorite solution. Westerholm *et al*.⁴¹ reported that 0.525 % sodium hypochlorite was only effective against *S. aureus* and added that 5.25 % sodium hypochlorite was the most effective disinfectant, however Schwartz *et al*⁹ found that 0.525 % sodium hypochlorite was effective against *S. aureus*, *S. choleraesuis*, *P. aeruginosa* and mixed oral flora. Jennings and Samaranayake⁵ compared the efficacy of three commercially available disinfectants - chlorhexidine gluconate, glutaraldehyde, and sodium hypochlorite - in removing microorganisms from irreversible hydrocolloid impression material. They found that, disinfection of irreversible hydrocolloid for 30 minutes with 2 % glutaraldehyde or 0.0125 % sodium hypochlorite resulted in virtual elimination of the organisms tested. These results are based on different microorganisms, different impression materials, methodologies and exposure times than used in this study and are difficult to compare. However in the present study 0.525 % sodium hypochlorite and 2 % glutaraldehyde (Gludex) solution were applied only for 10 minutes and under the conditions of this study sodium hypochlorite and glutaraldehyde were found to be the effective disinfectants in the elimination of *S. aureus* and *E. faecalis* from the surface of silicone and polyether impression materials.

To be effective, a disinfectant must produce consistently high kill levels on every impressiom materials³⁹. In the current study it was found that rinsing and a 10 min. immer-

sion in a sterile water control was ineffective disinfection for all tested bacteria on polyether and silicone impression materials. Beyerle *et al*⁶ reported the similar results for the irreversible hydrocolloid impressions.

CONCLUSION

Three disinfectants and a control (sterile water for 10 min.) were tested for the efficacy in bacterial counts on a silicone and two polyether impression materials contaminated with *S. aureus* and *E. faecalis*. Within the limitations of this study, it could be concluded:

1. 10 minutes of immersion in 2 % glutaraldehyde (Gludex) and 0.525 % sodium hypochlorite was effective for disinfection and great reduction was achieved against both microorganism.
2. For each of the three impression material and two microorganism, Mikrozyd spray disinfectant was found to be less effective than both Gludex and 0.525 % sodium hypochlorite.
3. The number of microorganisms decreased in control group among each material group, but was not enough for effective disinfection.

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