Clinical Evaluation of the Efficacy of Removing Microorganisms to Disinfect Patient-Derived Dental Impressions

Hiroshi Egusa, DDS, PhD[a]/Takao Watamoto, DDS, PhD[b]/Takuya Matsumoto, DDS, PhD[c]/Keiko Abe, DDS[d]/Munemasa Kobayashi, DDS[e]/Yoshihiro Akashi, DDS[e]/Hirofumi Yatani, DDS, PhD[f]

***Purpose*:** Disinfection of dental impressions is an indispensable procedure for the control of cross-contamination; however, there is limited information on the efficacy of disinfection under clinical conditions. The objective of this study was to clinically evaluate the disinfection efficacy of commercially available agents in removing oral pathogens from patient-derived impressions. ***Materials and Methods*:** Impressions from 54 patients were divided into groups and either left undisinfected or underwent 1 of 5 disinfection treatments: (*1*) 2% glutaraldehyde (GA), (*2*) 1% sodium hypochlorite (SH), (*3*) 0.25% benzalkonium chloride (BC), (*4*) 1 ppm ozonated water (OW), or (*5*) the Hygojet/MD520 system (HJ). An impression culture technique using a brain heart infusion agar medium was used to visualize the microbial contamination on the surface of the impression cultures. The persistent presence of oral pathogens on the impression cultures was examined using selective isolation agar plates. ***Results*:** The isolation frequencies of streptococci, staphylococci, *Candida*, methicillin-resistant *Staphylococcus aureus*, and *Pseudomonas aeruginosa* species from undisinfected impressions were 100%, 55.6%, 25.9%, 25.9% and 5.6%, respectively. Disinfection with HJ and BC removed the microorganisms with the greatest efficacy, followed by GA, SH, and OW. Potential bacterial contamination could be detected even after disinfection had been performed. Combined use of BC plus GA or SH removed oral pathogens almost completely from dental impressions. ***Conclusions*:** This investigation showed that potential contaminants are still present, even after general disinfection procedures. Therefore, either HJ or the combined use of BC with GA or SH is recommended for clinical and laboratory use. *Int J Prosthodont 2008;21:531–538.*

Impression materials that come into contact with oral tissues, saliva, and possibly blood can act as fomite media for the potential transfer of organisms from patients to dental personnel.[1–3] To avoid the contamination of dental office staff and dental technicians, it is recommended that impressions be disinfected immediately after their removal from the mouth.[4–6] Various disinfectants are commercially available,[7,8] and specific recommendations concerning their use are based primarily on the verification by in vitro studies of the microbiologic effectiveness of disinfection procedures.[9–14] It should be noted that the effects of disinfectants on artificially contaminated impression materials (in vitro) may differ from those on patient-derived impressions (in vivo) because of the presence of salivary and serum proteins on the impression surface or individual differences in oral flora composition. Although a number of organizations, such as the American Dental Association,[5,6] the British Dental Association,[4] and the Japan Prosthodontic Society (http://www.hotetsu.com), have issued recommendations for the prevention of cross-infection, clinical studies of the carriage of oral microorganisms on the impression surface and the efficacy of disinfectants in removing them from patient-

[a]*Assistant Professor, Department of Fixed Prosthodontics, Osaka University Graduate School of Dentistry, Osaka, Japan.*
[b]*Research Associate, Department of Fixed Prosthodontics, Osaka University Graduate School of Dentistry, Osaka, Japan.*
[c]*Associate Professor, Department of Oro-Maxillofacial Regeneration, Osaka University Graduate School of Dentistry, Osaka, Japan.*
[d]*Clinical Instructor, Department of Fixed Prosthodontics, Osaka University Graduate School of Dentistry, Osaka, Japan.*
[e]*PhD Candidate, Department of Fixed Prosthodontics, Osaka University Graduate School of Dentistry, Osaka, Japan.*
[f]*Professor and Chair, Department of Fixed Prosthodontics, Osaka University Graduate School of Dentistry, Osaka, Japan.*

***Correspondence to:** Dr Hiroshi Egusa, Department of Fixed Prosthodontics, Osaka University Graduate School of Dentistry, 1-8 Yamadaoka, Suita-city, Osaka 565-0871, Japan. Fax: +81-6-6879-2947. E-mail: egu@dent.osaka-u.ac.jp*

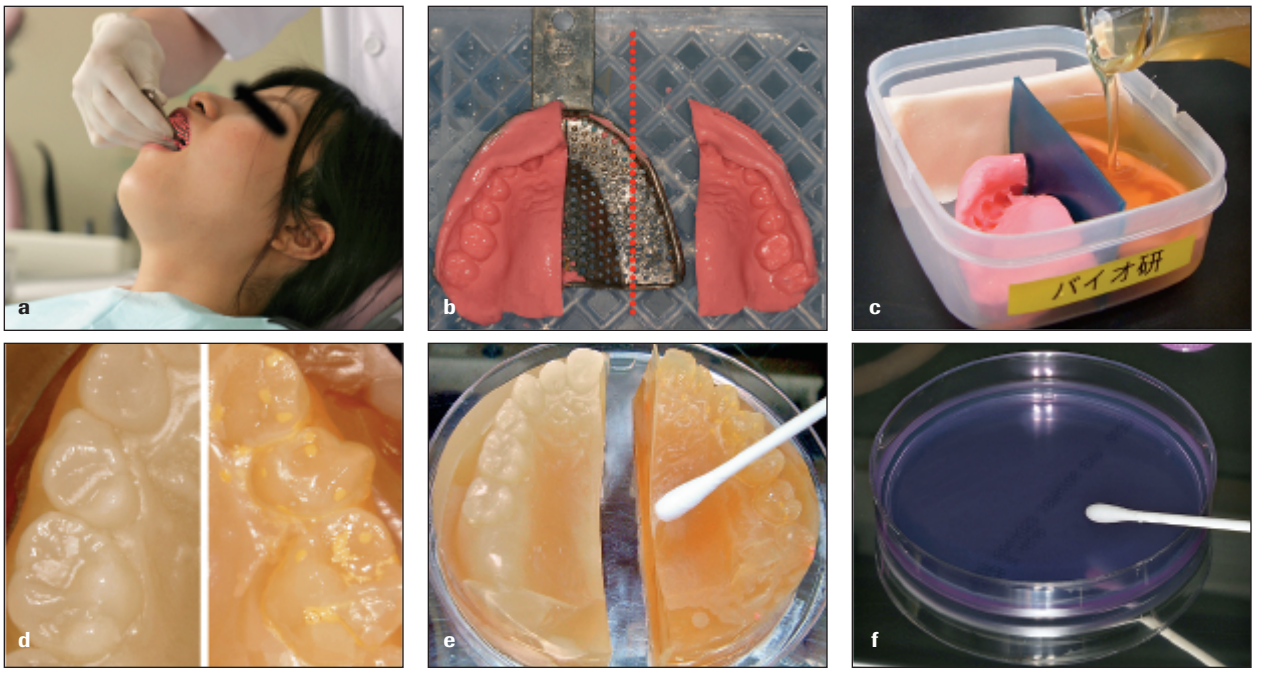

Fig 1 Experimental procedure involving detection of oral microorganisms on dental impressions and evaluation of disinfection treatments in removing the microorganisms (see Materials and Methods for details).

derived impressions are lacking. Therefore, there are presently no conclusive recommended disinfection procedures for dental impressions.

The goals of this study were: (*1*) to clinically examine the carriage of oral pathogens on the impression surface, highlighting important human pathogens such as *Candida*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Pseudomonas aeruginosa*; and (*2*) to validate the efficacy of several commercially available disinfectants—2% glutaraldehyde, 1% sodium hypochlorite, 0.25% benzalkonium chloride, 1 ppm ozonated water, and the Hygojet/MD520 system—in removing microorganisms from patient-derived alginate impressions.

Materials and Methods

Subjects and Materials

The present study was conducted in accordance with a protocol approved by the Ethical Committee of the Osaka University Graduate School of Dentistry, and informed consent was obtained from all subjects. The subjects included 54 adults (37 women and 17 men; mean age, 53.6 years; range, 24 to 83 years), randomly selected from the patients of the Department of Fixed Prosthodontics at Osaka University Dental Hospital, with the following inclusion criteria: (*1*) no complete denture on either jaw; (*2*) more than 10 teeth present in the maxilla; (*3*) age over 20 years; and (*4*) had not received oral hygiene/toothbrushing instructions. Seven of the subjects wore removable partial dentures.

The alginate impression material (Aroma Fine DFII, GC Corporation), rubber bowl, spatulas, polyethylene containers (Tupperware), and boxing wax were sterilized with ethylene oxide.[15] Other instruments and materials (impression trays, water, etc) were sterilized by autoclaving.

Impressions and Disinfection

An alginate impression was made of each subject's maxillary arch. As a negative control, an alginate impression was also made of a maxillary arch of a standard typodont with rubber-simulated soft tissue sterilized by ethylene oxide. After setting for 2 minutes in the subject's mouth (Fig 1a), the impression was removed and split sagittally down the middle (Fig 1b). The separated impressions were either left undisinfected (controls) or underwent 1 of the disinfection treatments shown in Table 1. The combined use of 0.25% benzalkonium chloride and either 2% glutaraldehyde or 1% sodium hypochlorite (40 times dilution of 10% benzalkonium chloride by either 2% glutaraldehyde or 1% sodium hypochlorite solution) was also tested.

The carriage of oral flora on the impressions and the efficacy of the disinfection treatment in removing the microorganisms were evaluated using a modified impression culture technique.[16,17] The 2 pieces of the

Table 1 Disinfection Treatments Used in the Present Study

Treatment	Manufacturer	Procedure
Running tap water	–	Rinsing under running water for 30 seconds
2% glutaraldehyde (STERIHYDE L)	Maruishi Pharmaceutical	Immersion for 10 minutes
1% sodium hypochlorite	Yoshida Pharmaceutical	Immersion for 10 minutes
0.25% benzalkonium chloride	Nihon Pharmaceutical	Immersion for 10 minutes
1 ppm ozonated water (L CLEAN TT-15MDS)	Tamura TECO	Immersion and flowing solution for 10 minutes
Hygojet/MD520 system	Dürr Dental	In accordance with instructions

impression ("control" and "treated" sample) were then placed in a sterile polyethylene container with 2 compartments separated by boxing wax (Fig 1c). Next, brain heart infusion (BHI) agar medium (5.2% BHI and 3.7% Bacto-Agar, Difco Laboratory) prepared at 50°C was poured onto the surface side of the impression (Fig 1c). After cooling for 1 hour at 4°C, the hardened BHI agar was aseptically separated from the impression and incubated at 37°C aerobically for 48 hours. Photographs of the impression culture surface were taken and the existence of colonies was determined by visual observation (Fig 1d). The colony area on the impression culture surface was analyzed by PopImaging software version 3.61 (Digital Being Kids). The percentage reduction of the colony area following disinfection treatment was calculated with the following formula:

$$\% \text{ reduction area of colony} = [1 - \{(\% \text{ of colony area on disinfection treated sample})/(\% \text{ of colony area on untreated sample})\}] \times 100$$

The Steel-Dwass multiple comparison test using the SAS system was used to assess differences in the % reduction of colony area among the groups of disinfection treatments. *P* values of less than .05 were considered to be significant.

Selective Isolation of Oral Microorganisms

The colonies on the surface of the BHI impression culture for control or treated samples were collected by swabbing with a sterile cotton swab (Fig 1e) and were then suspended in 1 mL of sterile phosphate-buffered saline. The colony suspension was plated on 5 selective agar medium plates (Fig 1f)—mitis-salivarius agar (Becton Dickinson), *Candida* GE agar (Nissui), mannitol salt agar (Becton Dickinson), OPAII *Staphylococcus* agar (Becton Dickinson), and *P aeruginosa*–selective agar medium (Becton Dickinson)—to detect the presence of *Streptococcus mutans* and other streptococci, *Candida*, staphylococci, MRSA, and *P aeruginosa*, respectively. After 48 hours of incubation under aerobic conditions at 37°C, the existence of positive colonies for each selective medium was determined visually according to the manufacturer's instructions.

Results

Oral Microorganisms on Dental Impressions

The use of the BHI impression culture detection method produced a large number of obvious colonies on the samples of the alginate impressions. These colonies varied in color, size, and form, indicating different types of microorganisms were present on the surface. They were distributed predominantly over the areas of the palate and the dental arch (Figs 1d, right, and 2). In contrast, no live colonies were observed on the BHI impression cultures from the negative controls from sterilized typodonts, thus indicating the adequacy of the sterilization procedures.

Selective agar culture demonstrated that streptococci and staphylococci colonies were detected on the nondisinfected impressions from all subjects and 55.6% of subjects, respectively (Fig 3). In addition, of the 54 nondisinfected impression samples investigated, the detection of opportunistic pathogens of MRSA, *Candida*, and *P aeruginosa* was confirmed in 14 (25.9%), 14 (25.9%), and 3 (5.6%) samples, respectively (Fig 3).

Effects of Disinfection on Removal of Microorganisms from Impression Surface

The split-impression culture method demonstrated that all disinfection procedures investigated reduced the colony growth area versus the nondisinfected control samples (Fig 2, Table 2). The percent reduction of colony area was the greatest following disinfection with the Hygojet/MD520 system and 0.25% benzalkonium chloride, followed by 2% glutaraldehyde, 1% sodium hypochlorite, and 1 ppm ozonated water. Disinfection using the Hygojet/MD520 system and 0.25% benzalkonium chloride resulted in the complete removal (100% reduction) of the visible colony area for all samples investigated (Table 2, Figs 2e and 2f). In contrast, treatment with rinsing under running water did not effectively reduce the colony growth area (Table 2, Fig 2a). In 3 of 8 subjects, the percentage colony growth area with this treatment was larger than that without treatment (control).

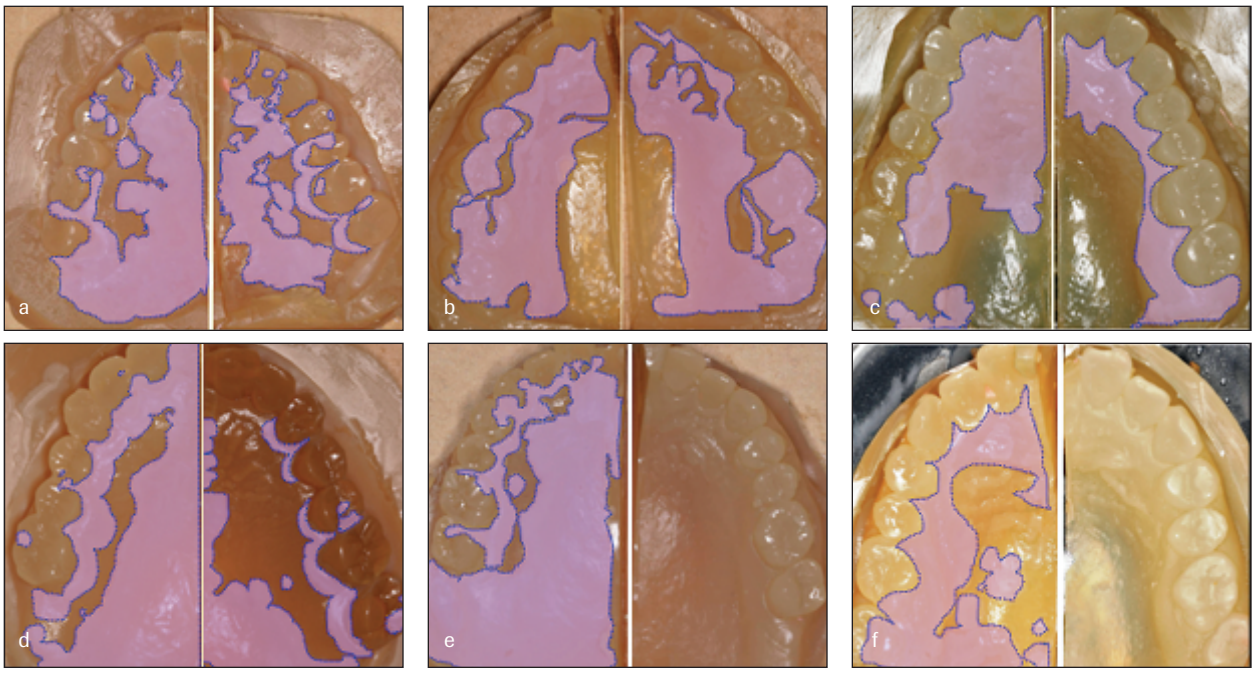

Fig 2 Colonies of oral flora grown on the BHI impression culture surface with or without disinfection of patient-derived impressions. Visible colony growth areas are highlighted in light purple. Left panels (control) show impression cultures without disinfection treatment. Right panels show impression cultures following treatment with (a) running water, (b) 1 ppm ozonated water, (c) 1% sodium hypochlorite, (d) 2% glutaraldehyde, (e) Hygojet/MD520 system, or (f) 0.25% benzalkonium chloride.

Table 2 Percent Reduction in Colony Growth Area on Surfaces of Patient-Derived Impressions Following Disinfection

Treatment	No. of examined subjects	% reduction of colony area		
		Median	Interquartile	*P**
Running tap water	8	15.8	−62.4 to 45.4	
1 ppm ozonated water	6	22.1	9.2 to 28.3	
1% sodium hypochlorite	12	30.1	17.0 to 44.6	
2% glutaraldehyde	10	42.8	11.7 to 70.5	
0.25% benzalkonium chloride	8	100	100 to 100	
Hygojet/MD520 System	10	100	100 to 100	

*No significant differences were observed among samples connected by bars ($P < .05$).

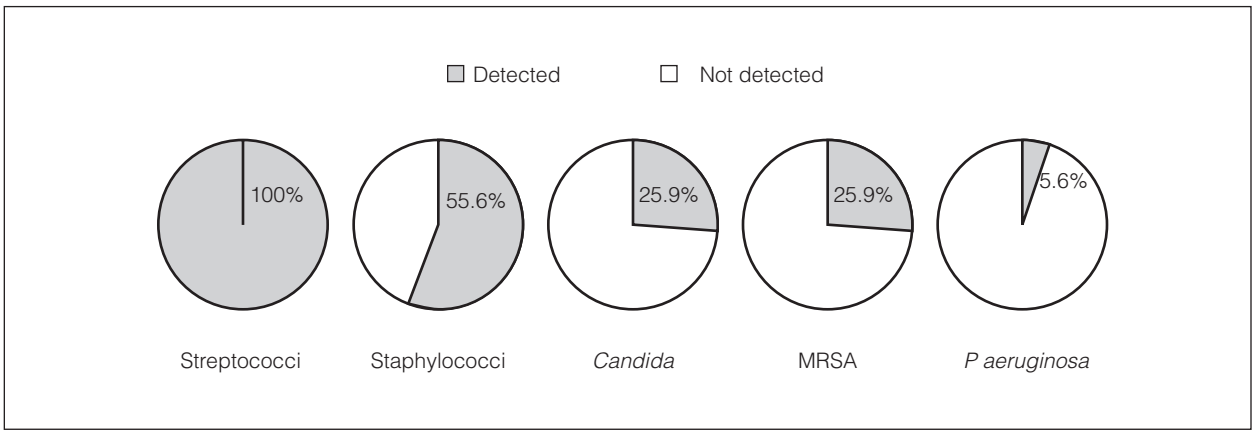

Fig 3 Detection of streptococci, staphylococci, *Candida*, MRSA, and *P aeruginosa* on 54 patient-derived impression samples without disinfection treatment.

Table 3 Effects of Disinfection Treatments on Oral Pathogens on Dental Impressions*

	Streptococci		Staphylococci		*Candida*		MRSA		*P aeruginosa*	
Treatment	U	T	U	T	U	T	U	T	U	T
Running tap water (n = 7)	7	7	5	5	2	1	—	—	—	—
2% Glutaraldehyde (n = 11)	11	9	4	2	3	—	5	2	1	—
1% Sodium hypochlorite (n = 12)	12	9	6	5	1	—	2	—	—	—
0.25% Benzalkonium chloride (n = 8)	8	1	5	—	2	—	3	—	—	—
1 ppm ozonated water (n = 5)	5	5	2	—	2	1	1	—	1	1
Hygojet/MD520 system (n = 11)	11	3	10	—	4	—	3	—	1	—

U = untreated; T = treated; — = not detected.
*Total no. of subjects in whom streptococci, staphylococci, *Candida*, MRSA, or *P aeruginosa* were detected from their impressions with or without disinfection treatment.

Disinfection of Oral Pathogens on Dental Impressions

Disinfection with 2% glutaraldehyde or 1% sodium hypochlorite was only partially successful against streptococci and staphylococci (Table 3). This was more effective using the Hygojet/MD520 system or 0.25% benzalkonium chloride and less effective with 1 ppm ozonated water (Table 3). Disinfection using the Hygojet/MD520 system and 0.25% benzalkonium chloride was completely effective against staphylococci, *Candida*, and MRSA. Treatment with rinsing under running water only minimally altered the detection of streptococci, staphylococci, and *Candida*.

Disinfection Efficacy of Combinations of 0.25% Benzalkonium Chloride

A selective agar culture detected both streptococci and staphylococci colonies on more than 80% of the impression samples of the 5 subjects investigated, even after disinfection by immersion for 10 minutes in 2% glutaraldehyde or 1% sodium hypochlorite solution. On the other hand, disinfection with the combined use of 0.25% benzalkonium chloride and either 2% glutaraldehyde or 1% sodium hypochlorite solution resulted in almost complete removal of these microorganisms (Table 4). In addition, there was no visible colony growth on the BHI impression cultures following the combined use of these disinfection procedures for all samples investigated (data not shown).

Table 4 Effects of Disinfection with the Combined Use of 0.25% Benzalkonium Chloride and 2% Glutaraldehyde or 1% Sodium Hypochlorite on Oral Pathogens on Dental Impressions*

	2% glutaraldehyde		1% sodium hypochlorite	
Pathogen	Alone	Comb.	Alone	Comb.
Streptococci (n = 5)	4	—	4	—
Staphylococci (n = 5)	5	1	5	—
Candida (n = 5)	—	—	—	—
MRSA (n = 5)	—	—	—	—
P aeruginosa (n = 5)	—	—	—	—

— = Not detected.
*Total number of subjects in whom oral pathogens indicated in the table were detected on their impressions following disinfection treatment with either 2% glutaraldehyde or 1% sodium hypochlorite alone or in combination (comb.) with 0.25% benzalkonium chloride.

Discussion

The microbial contamination of patient-derived impressions has been documented; however, few studies have characterized the pathogenic microorganisms on the impressions.[18–20] Patient-derived dental impressions and gypsum casts are contaminated with numerous microbes, including *Candida*, MRSA, and *P aeruginosa*, which are known opportunistic pathogens responsible for nosocomial and/or life-threatening infection in immunocompromised hosts.[17] The current study examined a total of 54 individuals for detection of these pathogens on the patient-derived impressions. The isolation frequency of streptococci, staphylococci, *Candida*, MRSA, and *P aeruginosa* species on undisinfected impressions was 100%, 55.6%, 25.9%, 25.9%, and 5.6%, respectively. This result confirmed the ability of patient-derived dental impressions to sustain pathogenic microbial contamination. The detected organisms are basically opportunistic pathogens, which are transiently found in the oral cavity. *Candida* causes a common opportunistic infection known as oral candidosis, which is seen in immunocompromised patients.[21] *P aeruginosa* is a common nosocomial contaminant, and epidemics have been traced to many items in the hospital environment.[22] MRSA is an important nosocomial pathogen that has recently been reported in patients without typical risk factors for nosocomial acquisition (community-associated MRSA).[23] Outbreaks of community-acquired MRSA infection in

healthy children and adults have been described worldwide.[24] The presence of these persisting pathogens on impressions creates the risk of transmission to dental staff and any other contacts. The possibility exists that further colonization may occur and may result in a serious infection. It is therefore important that all impressions be disinfected prior to being transferred to a laboratory.

Blair and Wassell[8] considered a number of techniques for disinfecting impression materials. They highlighted the fact that no universally recognized impression disinfection protocol is yet available and also showed that the use of some type of disinfectant had increased from 1988, at least in dental hospitals.[7] The recommendations of dental advisory bodies have undergone considerable modification. Until 1991, rinsing of impressions under water was the recommended practice.[25] This has been shown to reduce the counts of the bacteria present on an impression surface by approximately 90%.[26] However, the current results showed that this treatment had no effect on the number of positively detected streptococci, staphylococci, and *Candida* species. Moreover, this treatment increased rather than decreased the colony growth area in some cases. Rinsing under water would help to remove a certain amount of saliva, blood, and debris, but the current result suggested that this treatment cannot effectively remove oral pathogens and may spread a significant number of remaining bacteria over the surface of the impression materials. Therefore, it is not adequate just to rinse the impression under running water without the use of a disinfectant.

In 1993 Owen and Goolam[27] advocated the use of a disinfecting solution of 2% glutaraldehyde (dip in the solution, rinse, dip again, and cover with damp gauze for 10 minutes) or 1% sodium hypochlorite (spray with the solution, rinse, spray again, and stand under damp gauze for 10 minutes) for irreversible hydrocolloid (alginate) impressions based on a systematic review. More recently, a study supported by the British Dental Association recommended that all impressions should undergo, at a minimum, disinfection by immersion in 1% sodium hypochlorite for a minimum of 10 minutes.[8] Whereas most manufacturers can confirm the disinfectant properties of their products, which are usually evaluated on the basis of the reduction of cultivated microorganisms under in vitro conditions, there are few studies on how effective these products are with patient-derived (in vivo) contaminated impression material. Because there are few scientific studies to support certain recommended practices, these recommendations are based instead on strong theoretical rationale, suggestive evidence, the opinions of respected authorities, clinical experience, descriptive studies, or committee reports.[6] Further studies are therefore required to address the efficacy of disinfectant regimes under clinical conditions.

The current study assessed the efficacy of some commercially available disinfectants under clinical conditions using indices of persistent oral pathogens, such as streptococci, staphylococci, *Candida*, MRSA, and *P aeruginosa*. The data showed that disinfection with 2% glutaraldehyde or 1% sodium hypochlorite was only partially successful against streptococci and staphylococci; disinfection was more effective with the Hygojet/MD520 system or 0.25% benzalkonium chloride and less effective with 1 ppm ozonated water (Tables 2 and 3, Fig 2). Glutaraldehyde is classified as a "high-level disinfectant," which is able to inactivate spores and all other microbial forms, including human immunodeficiency virus (HIV) and hepatitis B virus (HBV). Sodium hypochlorite is an "intermediate-level disinfectant," which may not inactivate spores but will destroy other microbes, in particular tubercle bacilli, HIV, and HBV. It is noted that the persistent presence of oral pathogens could be detected in the impression samples disinfected by even 2% glutaraldehyde or 1% sodium hypochlorite after immersion for 10 minutes, which are currently the recommended procedures. It is important here to differentiate between *sterilization* and *disinfection*. *Sterilization* is an absolute term that means the killing or removal of all microorganisms. *Disinfection* implies the destruction of pathogenic organisms and is relative, depending on, among other factors, the duration of exposure to the disinfecting agent.[7] For example, exposure to 2% glutaraldehyde at room temperature will result in disinfection after 10 minutes but will sterilize only after 10 hours.[27] Patient-derived impressions seem to be almost completely disinfected by either 2% glutaraldehyde or 1% sodium hypochlorite; however, the oral pathogens retained on the surface were not completely removed by these procedures, ie, materials were not sterilized. If potentially existing opportunistic pathogens such as MRSA transfer to healthy personnel, they colonize as a part of the normal flora and cause no ill effects, but they may cause ill effects if transferred to other sites (eg, by breaking the skin) or if passed on to a susceptible person. Therefore, it is important to understand the necessity of careful handling of dental impressions as a potentially infectious material, even after generally recommended disinfection procedures are performed.

The Hygojet/MD520 system showed the highest disinfection efficacy among all of the examined procedures (Tables 2 and 3, Fig 2). The Hygojet/MD520 system uses a disinfectant spray procedure in a closed chamber. The MD520 disinfectant solution is based on a combination of aldehydes, quaternary ammonium compounds, special surfactants, complexing agents, and adjuvants in aqueous solution. The active ingredi-

ents are glutaraldehyde (0.5%) and ammonium chloride (0.25%). Each impression was sprayed for 10 seconds with the disinfecting solution, stored for 10 minutes in a closed chamber, and then rinsed for 10 seconds with water in the Hygojet chamber. From the standpoint of impression accuracy, the Hygojet/MD520 system does not significantly influence the quality of the surface and the hardness of the gypsum and therefore can be recommended for clinical and laboratory use.[28]

Benzalkonium chloride (a quaternary ammonium compound) is classified as a "low-level disinfectant," which is unacceptable for the disinfection of contaminated impressions because of its inability to inactivate spores, HIV, and HBV. Unexpectedly, 0.25% benzalkonium chloride was as effective in removing examined oral pathogens as the Hygojet/MD520 system and was more effective than 2% glutaraldehyde or 1% sodium hypochlorite. The Hygojet/MD520 disinfectant contains the identical concentration (0.25%) of benzalkonium chloride (alkyl dimethyl benzyl ammonium chloride) as an active ingredient, which is a commonly used surface active agent. It is possible that the surface active behavior of the benzalkonium chloride may not only affect the microbial surfaces but also help clear proteins, such as saliva, blood, and debris, from the surface of the impression materials that would otherwise help retain the microorganisms on the impression materials. On the other hand, glutaraldehyde functions as a fixative reagent against proteins. The glutaraldehyde might fix the surface portion of the proteins retained on the impressions, thus resulting in a protective effect on the oral flora existing in the depths of the fixed proteins. However, from a biologic point of view, disinfection only with 0.25% benzalkonium chloride, which is a low-level disinfectant, is not adequate for infection control and therefore should not be recommended. To examine the potentially augmented disinfection effects against HIV and HBV, a combined use of 0.25% benzalkonium chloride and 2% glutaraldehyde or 1% sodium hypochlorite was investigated. These combinations successfully removed oral pathogens from dental impressions, thus suggesting an increased disinfection efficacy of 2% glutaraldehyde or 1% sodium hypochlorite by adding 0.25% benzalkonium chloride (Table 4). Therefore, it is recommended that 0.25% benzalkonium chloride be added to general disinfection solutions such as 2% glutaraldehyde and 1% sodium hypochlorite.

Ozonated water is known to act as an antimicrobial agent against bacteria, fungi, and viruses.[29,30] The current data showed insufficient disinfection efficacy of 10 minutes of immersion in 1 ppm ozonated water in removing persistently present oral pathogens from dental impression surfaces. Ozonated water can be used as a soaking or flowing solution for medical and dental instruments, if used properly. The use of ozonated water in a higher concentration than employed in this experiment still remains to be investigated; however, ozonated water can be mutagenic if used for a long period and in high concentrations.[31] Further studies are needed to verify the effective concentrations for disinfection without the hazardous side effects of ozone and to confirm the surface accuracy of dental impressions following this treatment.

This study showed the efficacy of several disinfection procedures under clinical conditions against oral pathogenic bacteria and fungi. These procedures were not evaluated against viruses, however. Many dental personnel pay particular attention to cross-infection with HIV, which causes acquired immunodeficiency syndrome, and HBV, which poses a greater risk to dental personnel.[32] Further clinical study is thus warranted to verify the disinfection efficacy of the recommended procedures against these viruses. Infection control is a dynamic and ever-changing aspect of medical and dental practice. It is crucial that all dental staff members therefore be made aware of the most recent information and that appropriate procedures be in place to prevent the transmission of infection. Regular monitoring and updating of disinfection procedures in the light of new scientific evidence under clinical conditions are necessary.

Conclusions

The present study confirmed the persistent presence of oral pathogens, including *Candida*, methicillin-resistant *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, on patient-derived dental impressions. These potential contaminants were present on impressions even after the performance of general disinfection procedures, such as immersion for 10 minutes in 2% glutaraldehyde or 1% sodium hypochlorite. Therefore, these impression materials must be assumed to have the potential to transmit infectious agents to all dental personnel who routinely handle them and to any other contacts. From the standpoint of microbiologic effectiveness and dimensional accuracy, the Hygojet/MD520 system can be recommended for clinical and laboratory use. Alternatively, the use of surfactants such as 0.25% benzalkonium chloride together with high- or intermediate- level disinfectants, such as 2% glutaraldehyde or 1% sodium hypochlorite, increases efficacy by possibly removing remaining proteins from the impression surfaces.

Acknowledgments

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