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Disinfection of Bacterially Contaminated Hydrophilic PVS Impression Materials

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Abstract

Purpose: This study evaluated disinfection of bacterially contaminated hydrophilic polyvinylsiloxane (PVS) and polyether impressions.
Materials and Methods: Four light-bodied PVS (Examix, Genie, Take 1, Aquasil) and one polyether (Impregum) impression materials were evaluated using three disinfectants (EcoTru [EnviroSystems], ProSpray [Certol], and bleach [diluted 1:9]) as spray and immersion disinfections for 10-minute exposures. *Pseudomonas aeruginosa* ATCC 15442, *Salmonella choleraesius* ATCC 10708, and *Staphylococcus aureus* ATCC 6538 was the microbial challenge. Test specimens were prepared using aluminum molds with ten tapered cones. Mucin covered each cone, followed by 0.01 mL of each bacterium. Impressions were made using low viscosity impression material that was injected over the cones and filled custom trays. One-half of the impressions were spray disinfected, while the others underwent immersion disinfection. Trays that were contaminated but not disinfected served as positive controls, while those not bacterially contaminated or disinfected served as negative controls. The impressions were poured with Silky Rock Die Stone, and after setting, two cones were placed within a sterile capsule and triturated into powder. Four milliliters of TRIS buffer (0.05 M, pH 7.0) containing sodium thiosulfate (0.0055% w/v) were poured in each tube. After mixing, the solution was serially diluted and spread-plated onto selective agars. After incubation, colony counting occurred.
Results: No viable bacteria transferred to casts from either spray- or immersion-disinfected impressions. Negative controls produced no microbial colonies. Positive controls produced on average 3.35×10^5 bacterial cells.
Conclusion: Results suggest the methods used could disinfect contaminated impression materials. Microbial transfer from nondisinfected impressions to cones approached 33.5%.

The Centers for Disease Control and Prevention (CDC), in its infection control guidelines, indicated that dental impressions are potential sources of cross-contamination and should be handled in a manner that prevents exposure of practitioners, patients, and the environment.[1] This requires coordination of offices and dental laboratories concerning proper disinfection.

Impressions can be contaminated with bacteria, viruses, and fungi. After removal from the mouth, they require thorough cleaning and disinfection with a hospital-level disinfectant with a tuberculocidal claim followed by an adequate rinse.[1-3] The best time to clean and disinfect impressions is as soon as possible after finishing the impression procedures.[3,4] Impression

manufacturers often make general recommendations for disinfection of their products.

In its guidelines, the CDC identified areas for additional research, which included identifying the most effective methods to disinfect dental impression materials. The CDC also requested investigations concerning the survivability of pathogenic microorganisms on a variety of dental materials, including impressions and the resulting casts.[1]

Many reports describe the effect disinfection has on the reproducibility of different types of impression materials.[5-11] Reversible and nonreversible hydrocolloids, polyethers, and some addition silicone materials are more hydrophilic than other types of impressions and are more suspect to dimensional changes when exposed to solutions.[12-15] With limited exposure time, elastomeric impression materials are dimensionally stable and considered more resistant to changes caused by disinfectants.[16,17]

Fewer studies have investigated the antimicrobial outcomes of disinfecting impression materials or the resulting stone casts.[18-21] Rates of microbial kill vary by the type of disinfectant, disinfection method, exposure time, and impression material used.[19-25]

Immersion is preferred to spraying due to the better coverage of impressions with disinfectant.[3,4] Recommended solutions include hypochlorite, iodophors, glutaraldehydes, and phenols. Limiting the exposure time to 10 to 15 minutes was also recommended; however, dental offices do not often closely monitor disinfection times.[3-6,25-29] In addition, offices consider spraying to be a simpler, more easily accomplished procedure.[3,4]

The purpose of this study was to determine the efficacy of three disinfecting solutions and two disinfecting methods, spray or immersion, on hydrophilic polyvinylsiloxane (PVS) impression materials and one polyether impression material via estimating the number of microorganisms transferred from contaminated and disinfected impressions to stone casts.

Materials and methods

The experimental procedures used generally followed those of Huizing et al[19] and Flanagan et al.[20]

Impression materials and disinfectants

Five impression materials were evaluated; four were PVS materials—Examix (GC America, Alsip, IL, Lot #0402051), Genie (Sultan Healthcare, Englewood, NJ, Lot #24513), Take 1 (Kerr, Bioggio, Switzerland, Lot #4—1027), and Aquasil (Dentsply, York, PA, Lot #030225). The fifth impression material was Impregum Penta Soft (3M ESPE, St. Paul, MN, Lot #B179450). For all impression materials, type III light-bodied viscosities were used. All materials came from single lots and were prepared according to manufacturer recommendations.

Three disinfectants were used: EcoTru (0.20% parachlorometaxylene, EnviroSystems, Mooresville, NC), ProSpray (water-based dual phenolic, 0.28% o-phenylphenol and 0.03% o-benzyl-p-chlorophenol, Certol, Commerce City, CO), and diluted regular bleach (5.52% sodium hypochlorite, one part bleach to nine parts sterile distilled water, Clorox, Oakland, CA). All products came from single lots and were freshly prepared, stored, and handled according to their manufacturers' recommendations. Testing included both spraying (total of 0.6 mL/specimen) and immersion. Exposure in all cases was for 10 minutes. Immersion disinfectants were single-use solutions.

Test bacteria

Test bacteria included *Pseudomonas aeruginosa* ATCC 15442, *Salmonella choleraesius* ATCC 10708, and *Staphylococcus aureus* ATCC 6538. All came from the American Type Culture Collection (Manassas, VA). This collection is commonly used to establish a hospital-level disinfectant as defined and required by the US Environmental Protection Agency (EPA).[2]

Inoculum preparation

After removal from frozen stocks, each bacterium was aerobically incubated in trypticase soy broth supplemented with 0.25% w/v glucose (TSB-G) at 37°C for 24 hours. Two additional daily transfers followed. Finally, 1.0 mL inoculums were immersed in 250 mL of TSB-G and incubated as described. After 24 hours, solutions were centrifuged (10,000 rpm, 20 minutes at 4°C). Two rinses were made with physiological buffered saline (PBS, pH 7.2) followed by centrifugation and finally a suspension in 5.0 mL of PBS. Using previously established standard viable cell count curves as references, additional PBS was added to the solution to adjust the final OD^{375} values to those that corresponded to 1.0×10^8 bacteria per mL for each bacteria. Using the prepared inoculums, 2.0 mL of each bacterial type were combined into a single sterile tube and vortexed for 15 seconds. Using 0.01 mL of this combination will result in final concentrations of approximately 1.0×10^6 for each bacterium. Most disinfection testing uses an initial bacterial concentration (challenge) of 1,000,000 cells.[19,20] Disinfection is considered to occur when there is a three-log reduction (or 99.9%) or around 1000 viable cells remaining.[2]

Specimen molds and contamination

An aluminum mold was used to prepare the specimens. The mold has ten cones, each being 4 mm in diameter at the base, 2 mm in diameter at their tops, and 10 mm in height (Figs 1 and 2). The aluminum molds were sterilized by standard steam autoclaving between uses.

First, 0.01 mL of mucin 0.5% w/v (Fisher Scientific, Pittsburgh, PA) was added to each metal cone. Ten minutes later, 0.01 mL of the bacterial mixture, which contained an estimated 1.0×10^6 of each of the three bacterial types, was added as evenly as possible over each cone. The cones then dried for 10 minutes to be ready for the impression procedures.

All impression mixing was according to manufacturer recommendations. Light-body viscosity impression material was first injected on each contaminated cone and then into a custom tray with 3 mm of relief (Triad True Tray, light cured, Dentsply, St. Charles, MO). Custom trays underwent steam sterilization before use, and impression setting time was for twice the recommended period to ensure complete setting.

Impression disinfection

Six impressions were made using each type of impression material for a total of 30 impressions of the mold for the disinfection groups. The 30 impressions were divided into two groups:

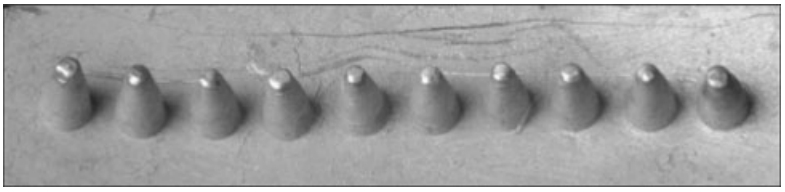

Figure 1 Aluminum mold used to contaminate impression materials.

15 impressions were spray disinfected (an average of 0.6 mL of disinfectant), while disinfection of the other 15 was through immersion in 75 mL of each disinfectant. Spray and immersion disinfection involved only fresh solutions for 10 minutes. The process involved all three types of disinfectants. There were ten impressions in each of the control groups—a positive control (bacterial contamination, but no disinfection) and a negative control (no contamination or disinfection).

Specimen preparation

Silky-Rock Die Stone (Type IV, Whip Mix Corporation, Louisville, KY) was mixed according to the manufacturer's instructions using sterile distilled water and sterile mixing bowls and spatulas. Stone was vibrated into the impressions and allowed to set for 45 minutes.

After separation, the stone cones were broken from the cast using a sterile hemostat (Fig 3) resulting in a total number of 400 stone cones. Pairs of cones were placed in ethylene oxide sterilized capsules with pestles (Henry Schein, Port Washington, NY) and processed in an amalgamator for 5 seconds, resulting in powdered cone material. Then 4.0 mL of TRIS buffer (0.05M, pH 7.0) containing sodium thiosulfate (0.0055% w/v, Matheson Coleman, Norwood, OH) was added to each capsule. The capsules were then processed for 5 seconds, and the resulting slurry was placed into a sterile test tube.

The sodium thiosulfate served as a neutralizer for residual disinfectant. The neutralization test employed in this study followed methods suggested by Russell[30] and Sutton et al.[31]

Determination of neutralizer (disinfectant) efficacy (NE) was accomplished by comparing the recovery of identical inocula from the neutralizing solution in the presence or absence of a 1:10 dilution of the biocide (EcoTru Professional, Prospray, or diluted bleach, 1:10). The tubes were left at room temperature for 15 minutes. One of the test microorganisms prepared in the same manner as for impression contamination was added to the tubes and mixed. The amount of bacterial suspension added produced a final concentration in each tube of 5.0×10^3 bacteria. The tubes again sat at room temperature for 15 minutes. Then a spiral platter applied 0.05 mL from each tube onto duplicate plates of selective media (Bacto Pseudomonas F agar for the *P. aeruginosa*, MacConkey agar for the *S. cholerasius,* and Mannitol salt agar for the *S. aureus*). Aerobic incubation at 37°C for 48 hours followed. Colony counting then occurred. Neutralizing toxicity (NT) was evaluated by comparing the recovery of the challenge organisms in the neutralizer-exposed population and the neutralizer with biocide population.

NE and NT ratios came from the geometric mean of the recovery in the different populations. The minimal acceptable NE and NT ratios were 0.75. In the experiment performed, NE and NT ratios for the three test bacteria ranged from 0.85 to 0.89.

Plating and analyses

The slurries were serially diluted (to 10^{-4}) with TRIS buffer, followed by spread plating of 0.05 mL aliquots onto selective agar media for each dilution. Included were Bacto Pseudomonas F (*P. aeruginosa*), MacConkey (*S. choleraesius*), and Mannitol salt agar (*S. aureus*), resulting in a total of 3000 agar plates. All media came from Fisher Scientific (Fisher Scientific, Waltham, MA). Aerobic incubation was at 37°C for 48 hours.

After incubation, colony counting was done, and the total number of each bacteria present was determined. The values for each cone pair were converted to CFU per mL, and the five pairs averaged.

Results

No bacterial growth occurred from any stone cones coming from contaminated and disinfected impression materials. Both disinfecting methods, spraying and immersion, and all three disinfectant solutions successfully killed all challenge bacteria on each of the five types of impression materials. Sampling

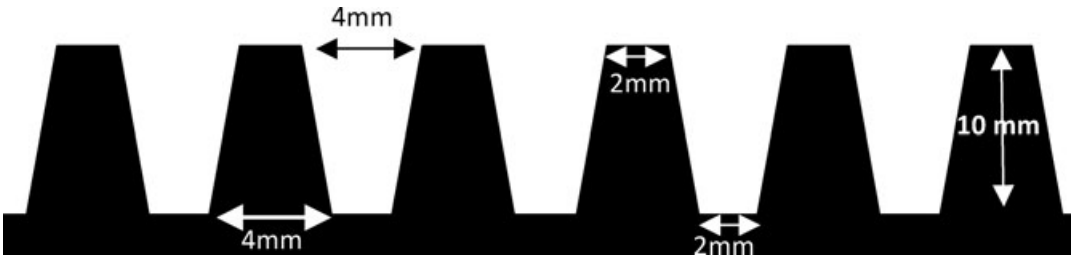

Figure 2 Diagrammatic illustration showing a cut section view of the master mold used for the bacterial viability test.

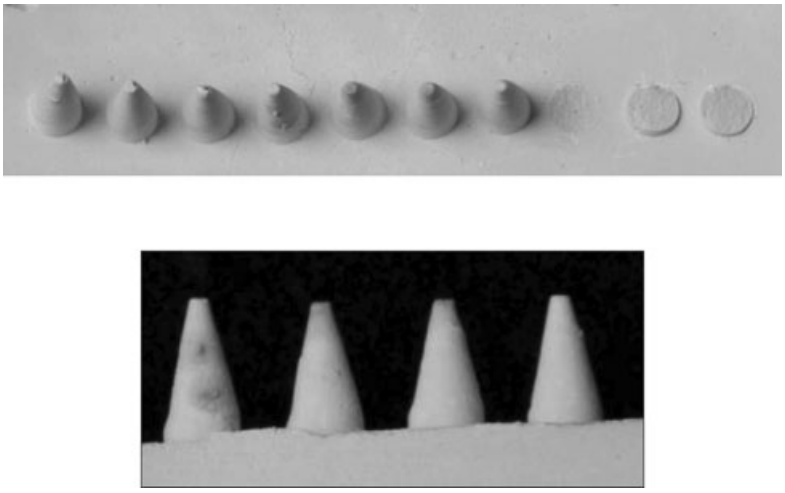

Figure 3 Examples of casts used to measure effects of disinfection top and side views; shown are the residual bases of several removed cones (top).

of inoculums indicated that 95.67% of the calculated number of each of three bacteria was present at the time of use. No viable bacteria of any type were present in any of the stone casts coming from negative control impression materials. Bacteria were measured using three types of selective agar media.

In the positive control group, an average of 3.35×10^5 bacterial cells were produced, with an overall recovery average of 33.5% occurring among the various stone casts. Specific recoveries were 35.6% (*P. aeruginosa*), 32.9% (*S. choleraesius*), and 32.0% (*S. aureus*). The numbers of recovered bacteria (colony counts) were compared to the numbers of bacteria thought to be present in the inoculums. Table 1 reports on the transfer and survival of bacteria from contaminated impression to stone casts (positive controls).

Discussion

It is difficult to compare the results of this study to previously published efforts. Many studies have evaluated the response of aqueous impression materials like alginate to disinfection. In one study, alginate impressions containing disinfectants like chlorhexidine and quaternary ammonium compounds proved to be highly effective in completely preventing the transfer of microorganisms to the test tubes, while unsupplemented alginates had no antimicrobial effect.[20] Few studies involved comparisons of PVS impression materials to polyether impression material concerning responses to disinfection.[24,32]

A very limited number of studies estimated the number of microorganisms transferred from contaminated and then disinfected impressions to resultant casts. One study reported *Streptococcus faecalis* transfer from contaminated PVS impressions to two types of Type IV gypsum die stones; the bacteria remained viable for periods up to 7 days. Incorporation of a chlorine disinfectant in the stone reduced the numbers of viable cells present in the gypsum specimens essentially to zero.[19]

Another study concluded that recovery of microorganisms from the stone cast shows that dental casts can be a medium of cross contamination between patients and dental personnel.[29] Comparing the results of this study to the two previous studies does not raise concerns about the complete microbial kill noted. Other studies report less success in microbial kill;[16,21,24]

Table 1 Percentage of viable bacteria in stone cones as compared to the number calculated to be present in inocula-positive controls*

	Bacteria								
	Pseudomonas aeruginosa			*Salmonella choleraesius*			*Staphylococcus aureus*		
Impression type	Incoulum	Recovered	Percent recovered	Incoulum	Recovered	Percent recovered	Incoulum	Recovered	Percent recovered
Examix	1.0×10^6	3.49×10^5	34.9	1.0×10^6	3.31×10^5	33.1	1.0×10^6	3.16×10^5	31.6
Genie	1.0×10^6	3.38×10^5	33.8	1.0×10^6	3.34×10^5	33.4	1.0×10^6	3.26×10^5	32.6
Take 1	1.0×10^6	3.67×10^5	36.7	1.0×10^6	3.08×10^5	30.8	1.0×10^6	3.12×10^5	31.2
Aquasil	1.0×10^6	3.59×10^5	35.9	1.0×10^6	3.22×10^5	32.2	1.0×10^6	3.10×10^5	31.0
Impregum	1.0×10^6	3.67×10^5	36.7	1.0×10^6	3.50×10^5	35.0	1.0×10^6	3.36×10^5	33.6
Average	1.0×10^6	3.56×10^5	35.6	1.0×10^6	3.29×10^5	32.9	1.0×10^6	3.20×10^5	32.0

*Recovered values were the average number of bacteria from each of five pairs of stone cones.

however, most involved alginate impression materials. Alginate is categorized as an irreversible hydrocolloid impression material. The set alginate material is porous and exhibits properties of synergies and imbibition. Elastomeric impression materials do not exhibit these properties.[32,33]

In our study, the fact that all materials were elastomeric impression materials can explain why they all gave the same results. Test parameters used in this study are not directly comparable to any previously published work, but closely related to the work done by some studies to determine the possibility of transmitting microorganisms to stone models via elastomeric impression materials. Sofou et al[32] had closely similar results to our study. The difficulty in comparing this research work to others is understandable, considering most studies of impression disinfection involve alginate materials and generally monitor physical characteristics of treated materials and not microbial kill and/or transfer to casts. Like almost all other research efforts, this study had limitations. Evaluation involved only three types of vegetative bacteria. The combination of the three, however, does serve as part of the official EPA microbial challenge to evaluate disinfectants used on hard surfaces. Not tested were bacterial endospores, viruses, and yeasts. The bacteria cultured from the casts were compared (colony formation, colony type/size/color, and gram-staining results) to the inoculating bacteria to ensure that the bacteria did not have any changes during their migration/movement from the impressions to the casts.

Evaluation included four types of PVS impression materials and one polyether impression material. Other types of impression materials may be available; however, material costs were limiting factors, so this collection of five impression materials served as a representative grouping. Dental practitioners commonly use more than 20 disinfectant solutions. Material costs and time constraints limited the disinfectant list; however, testing did include three major chemical groupings of disinfectants recommended in the literature.[3-6,25-29] Testing did not include a negative control "disinfectant" such as sterile tap water.

Evaluation of disinfection of impression materials, the effects on material integrity, and efficacy of microbial kill levels remain active areas of research. The CDC, in its infection control guidelines, indicated the need for research studies to identify effective disinfection methods that are relatively benign to impression reproducibility. The CDC was also interested in measuring the survival of potentially pathogenic microorganisms on a variety of contaminated dental materials. In this study, handling of impressions attempted to duplicate actual clinical procedures including timing of disinfection—would delaying disinfection cause a decrease in the numbers of viable cells remaining? This could be a possibility if the contaminating bacteria remained on the surfaces of the impression and did not penetrate inward due to the hydrophilic properties of these materials or inside the stone casts. Additional studies will be needed to investigate the effect of delayed disinfection on the microbial transfer to the resulting definitive casts and the effect of spray versus immersion disinfection on the physical properties and dimensional accuracy of hydrophilic PVS/polyether impression materials and the resulting definitive casts.

Conclusions

In accordance with the limitations of this study, the following conclusions can be made:

1) No bacterial growth was noted from any stone cones resulting from disinfected impressions materials;
2) No bacterial growth on the selective media used was noted from any stone cones resulting from impression materials not contaminated or disinfected;
3) On an average, 33.5% of inoculated bacteria became incorporated and remained inside positive control stone dies;
4) Ten minutes of either spray or immersion disinfection is an effective time to disinfect the investigated impression materials;
5) Disinfectants investigated in this study will effectively disinfect PVS and polyether elastomeric impression materials.

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