High-Level Microwave Disinfection of Dental Gypsum Casts

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Purpose: To test whether microwave oven irradiation can disinfect gypsum casts in compliance with current disinfection requirements, and to determine whether this procedure would be as effective as a validated method of chemical disinfection of impressions. ***Materials and Methods:*** In 2 in vitro experiments, samples of 5 irreversible hydrocolloid impressions of a disinfected acrylic resin model were contaminated with suspensions of recommended test organisms *Staphylococcus aureus* and *Pseudomonas aeruginosa,* respectively. In 1 in vivo experiment, 10 impressions were made of 10 subjects. All impressions were poured and the resulting casts cut in 2 halves. One half of each cast was exposed to 5 minutes of irradiation in a microwave oven at 2,450 MHz and 900 W. The other halves were left untreated as controls. In a second in vivo experiment, 10 impressions were disinfected by immersion in a 0.07% solution of NaOCl at pH of about 10 for 3 minutes, and then poured. All casts were incubated aerobically in Bacto tryptic soy broth at 37°C for 6 hours and assessed for bacterial growth by counting colony-forming units per milliliter (cfu/mL) of the culture. The results were analyzed using the Wilcoxon signed-rank test. ***Results:*** Untreated gypsum casts showed cfu/mL counts with a median log value of 6, while microwave-irradiated ones had median cfu/mL counts of 0. Casts poured from chemically disinfected impressions demonstrated cfu/mL counts with a median log value of 4. ***Conclusion:*** Under the described conditions, microwave-irradiated gypsum casts satisfy current disinfection requirements, but gypsum casts poured from chemically disinfected impressions do not. *Int J Prosthodont 2005;18:520–525.*

In prosthodontics, objects potentially contaminated with pathogenic microorganisms are transported between the dental laboratory and the dental clinic. It has been claimed that to avoid cross-contamination, specific disinfection measures should be followed.[1-5] In the literature, the usual solution to this problem has been to chemically disinfect the impressions,[6] and the efficacy of such disinfectants has been the subject of several studies.[7–10] However, there are a number of problems associated with their use. They take time and expense to perform in a dental practice. Moreover, all chemical disinfectants are potentially harmful to the health of the user and to the environment, and they may have an unpleasant odor. Furthermore, they are not readily compatible with irreversible hydrocolloid,[6,11–13] which is one of the most frequently used impression materials.[14] Consequently, to a large extent, disinfection procedures on impressions are not followed in clinical practice.[15–17] When they are, their clinical efficacy on the microflora appears to be inadequate or questionable.[18]

Even a cast from a properly disinfected impression may subsequently become contaminated by a technician or clinician.[14] Also, the prosthesis will become

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contaminated by the patient after trial and adjustment in the mouth and will recontaminate the cast after repositioning. In practice, contaminated gypsum casts are not possible to disinfect chemically. If elimination of possible cross-contamination is considered a requirement, then disinfection measures should be applied throughout all phases of treatment to both the cast and the prosthesis.

The results of a pilot study have indicated that disinfection of gypsum casts may be accomplished by means of microwave irradiation.[19] Unlike mere disinfection of the impression, this method, if effective and practicable, would eliminate cross-contamination via the cast, because it can be repeated at every stage as required.

The hypotheses tested in this study were:

1. A commercially available household microwave oven, used with a short exposure time, would generate sufficient irradiation on gypsum casts to effect disinfection, as defined by the current infection control guidelines for the dental laboratory[1] and the European Standard EN 1040.[20]
2. This procedure would be as effective as a validated method of chemical disinfection of impressions.

Materials and Methods

The general design of this study is illustrated in Fig 1.

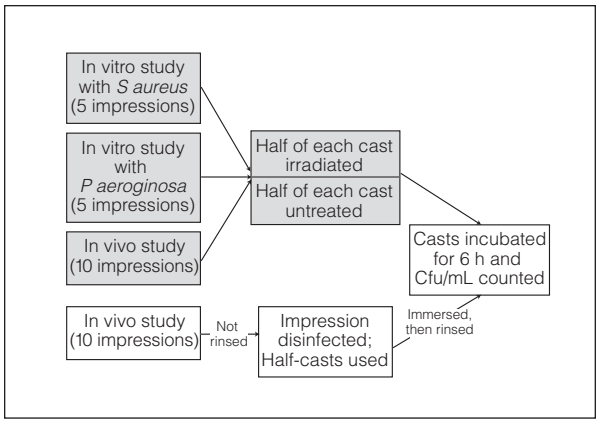

Fig 1 Design of the experiment.

Microwave Irradiation Study

This part of the study consisted of an in vitro and an in vivo experiment. In the in vitro experiment, 10 impressions were made of a disinfected (70% ethanol) acrylic resin model of a maxilla. Of these, 5 were contaminated with 1 mL of a suspension of *Staphylococcus aureus* (American Type Culture Collection 6538) and 5 with *Pseudomonas aeruginosa* (Culture Collection, University of Göteborg 2080); both organisms were selected and suspensions prepared according to the European Standard EN 1040,[20] with turbidity corresponding to MacFarland standard 1 (approximately 3×10^8 colony-forming units/mL [cfu/mL]). After 7 minutes, the bacterial suspension was gently shaken off the impressions to remove excess liquid, and the casts were poured in type III gypsum (Kerr Dental Hydrocal). In the in vivo experiment, impressions were made of 10 different consenting subjects and poured without rinsing within 30 minutes.

All impressions were made with an irreversible hydrocolloid (Blueprint Cremix, Dentsply/De Trey). Sterile water was used for both the impression material and the gypsum. The irreversible hydrocolloid and the gypsum powder were dispensed from commercial packages and were not disinfected before use. The impression and casting procedures were made in accordance with the recommendations of the manufacturers.

All casts were cut transversely with a sterile wax knife before the gypsum had fully set to facilitate subsequent division. The casts were removed from the impressions approximately 30 minutes after pouring and broken in two. One half of each cast was irradiated in a microwave, and the other half was left untreated as a control. The microwave irradiation was performed in a household Samsung microwave oven type ck 99 s, set at 900 W and 2,450 MHz frequency, for a total of 5 minutes. To ensure that the casts were adequately irradiated on all surfaces, they were first exposed for 2.5 minutes and subsequently turned upside down and irradiated again for the same amount of time.

Chemical Disinfection Study

With 1 exception, 10 irreversible hydrocolloid impressions were obtained from the same test subjects who were used for the microwave experiment. The impressions were immersed in a freshly prepared aqueous 0.07% sodium hypochlorite (NaOCl) (Klorin, De-No-Fa) solution at pH 10 for 3 minutes. They were then rinsed in 250 mL of sterile water before the casts were poured. This part of the procedure was in accordance with a previously validated study, which established the minimum concentration and exposure time of NaOCl at the optimum pH to effect adequate disinfection of irreversible hydrocolloid impression.[21] The set gypsum casts were broken in 2, as described above. In conformity with the microwave irradiation study, only one half of each cast was used for the bacteriologic procedures.

Table 1 Results (cfu/mL) of In Vitro Microwave Irradiation Experiments

Bacteria type/ sample no.	Untreated	Microwave irradiated
S aureus		
1	3.3×10^6	0
2	2.8×10^6	0
3	1.4×10^6	0
4	8.6×10^5	0
5	3.1×10^6	0
Median	2.8×10^6	0
P aeruginosa		
1	2.4×10^6	0
2	3.3×10^6	0
3	3.3×10^6	0
4	6.7×10^6	0
5	6.6×10^6	0
Median	3.3×10^6	0

Samples were infected with either *S aureus* (ATCC 6538) or *P aeruginosa* (CCUG 2080). Bacteria count was determined using sample found in Bacto tryptic soy broth after incubating the casts for 6 hours.

Bacteriologic Procedures

Where applicable, the bacteriologic procedures were performed in compliance with European Standard EN 1040.[20] All casts were submerged in Bacto tryptic soy broth (TSB) (Difco) and incubated aerobically at 37°C for 6 hours. TSB aliquots, undiluted and diluted 10^{-2} and 10^{-3}, were then prepared from the cultures of each of the disinfected or uncontaminated casts. Similarly, TSB dilutions of 10^{-4}, 10^{-5}, and 10^{-6} were prepared from the cultures of each of the contaminated casts. For each dilution, the TSB was plated in triplicate on tryptone soy agar (TSA) (Difco) plates. All dilutions were made with tryptone sodium chloride solution (Becton, Dickinson). The inoculated plates were incubated aerobically at 37°C for 18 hours. After incubation of the TSA plates, the cfu/mL for each cast was calculated as a weighted mean.[20]

The bacteriologic procedures were tested with 6 positive and 9 negative controls. For each control sample, a gypsum cast from an impression of the disinfected acrylic resin model was poured and split in 2. With the positive controls, one half of a cast was contaminated with 0.5 mL of the *S aureus* suspension. The object was to test whether the method used for bacterial recovery could detect the test bacteria. With the negative controls, half of a noncontaminated cast was used. The object was to test possible bacterial contamination of the irreversible hydrocolloid impression material, gypsum powder, and the aseptic procedures. These casts were immersed in TSB and incubated, the cultures were plated, and cfu/mL was calculated as described above.

Aseptic Procedures

Throughout the study, an effort was made to work aseptically. A standard barrier technique was used with nonsterile vinyl gloves (Optima, Nitritex) and TopDent face mask (DAB Dental Gruppen). All laboratory procedures except plating were carried out in a flow bench. In the in vitro experiments, impressions were made of an acrylic resin model (see above), which was disinfected with 70% ethanol immediately before taking impressions. The impression trays (President Tray, Coltene/Whaledent), spatulas, and mixing bowls used for the impression and casting procedures were also disinfected with 70% ethanol immediately before each usage. Tweezers and wax knives were flame sterilized.

Statistical Methods

The results were analyzed using one-tailed Wilcoxon signed-rank test, with a chosen alpha level of .05.

Results

In the in vitro experiment, the median cfu/mL count of the TSB of the untreated casts from impressions inoculated with *S aureus* was 2.8×10^6 (Table 1). The corresponding count pertaining to casts from impressions inoculated with *P aeruginosa* was 3.3×10^6 (Table 1). For both in vitro experiments, after microwave irradiation, the median cfu/mL count was 0. The differences between the untreated casts and the irradiated ones were statistically significant ($Z = -2.023$, $P = .031$).

In the in vivo microwave study, the median count of the TSB of the untreated casts was 2.0×10^6 cfu/mL. After microwave irradiation, the median count was 0 cfu/mL (Table 2). This difference was statistically significant ($Z = -2.666$, $P = .002$). With regard to 1 of the test subjects, 34 colonies were observed. In the in vivo chemical disinfection study, the count varied greatly: from 0 cfu/mL to 4.9×10^5 cfu/mL. The median count was 4.4×10^4 cfu/mL (Table 3), indicating a median 2-log reduction in viable counts.

The median count for the positive control was 5.1×10^7 cfu/mL (range 1.6×10^7 to 1.6×10^8 cfu/mL), and for the negative control the median was 0 cfu/mL (range 0 to 7.0×10^4 cfu/mL). The high and consistent (1 log range) cfu/mL values of the positive controls indicate that the cultivation protocol used was adequate. With regard to the negative control, in 1 case scant colonies were observed in 1 of the plates with the 1:1,000 TSB dilution. This was most likely a spurious result, because the other 8 plates for this cast showed no growth. The negative control showed that neither the gypsum nor the impression material were contaminated by bacteria.

Table 2 Results (cfu/mL) of In Vivo Microwave Irradiation Experiment

Patient	Untreated	Microwave irradiated
1	2.2×10^6	0
2	6.3×10^6	0
3	2.8×10^6	3.4×10^1
4	6.9×10^5	0
5	1.8×10^6	0
6	9.4×10^6	0
7	7.3×10^5	0
8	7.2×10^5	0
9	00	
10	4.3×10^6	0
Median	2.0×10^6	0

Results expressed as cfu/mL found in Bacto tryptic soy broth after incubating casts for 6 hours.

Table 3 Results (cfu/mL) of In Vivo Experiment

Patient	Disinfected
1	4.9×10^3
2	1.2×10^3
3	2.8×10^3
4	7.6×10^4
5	8.4×10^1
6	0
7	1.2×10^4
8	1.3×10^3
9	2.9×10^2
10	0
Median	4.4×10^4

Results expressed as cfu/mL found in Bacto tryptic soy broth after disinfecting in vivo impression with freshly prepared aqueous 0.06% NaOCl solution, rinsing it with 250 mL sterile water, and incubating it for 6 hours.

Macroscopically, the surfaces of the casts appeared unaffected by the microwave irradiation. No obvious cracks or porosities were observed.

Discussion

The most important finding in this study was the striking reduction of bacteria on the casts after 5 minutes of microwave irradiation in an ordinary household microwave oven set at 900 W and 2,450 MHz. In both the in vitro and the in vivo experiments, the untreated casts showed TSB counts of 10^6 cfu/mL compared to the irradiated ones, in which no bacteria appeared to survive (Tables 1 and 2). The 6-log reduction of cfu/mL exceeds the requirements of European Standard EN 1040.[20] These requirements are satisfied if the chemical disinfectant tested demonstrates a reduction of 10^5 or more cfu/mL in viable counts. This high-level disinfection also complies with the current infection control guidelines for the dental laboratory.[1]

The above results are all the more convincing in view of the fact that the study was intentionally designed in such a way that a maximum amount of bacteria should be transmitted to the casts. In a clinical setting, irrespective of the microbiologic aspects, rinsing the impression prior to eventual chemical disinfection would be carried out, if for no other reason than to improve the quality of the cast by removing mucin, blood, and loose particles. In so doing, the bacterial count would be reduced significantly, increasing the effect of a subsequent disinfectant.

An investigation[22] of the bactericidal activity of a microwave oven set at 2,450 Mhz, 325 W, 650 W, and 1,400 W on suspensions of various non-sporogenic bacteria, including *S aureus* and *P aeruginosa*, and sporogenic medically important bacteria, showed that the vegetative bacteria were promptly killed in 5 minutes or less. Bacterial spores, on the other hand, were only killed in aqueous suspension when a 1,400-W setting was used for 10 to 20 minutes.[22] Based on this information, the *S aureus* and *P aeruginosa* strains used in the chemical disinfection part of the study were also used in the microwave irradiation part of this study.

The clinical importance of the present results is obvious. Provided this procedure does not harm the gypsum cast, disinfection can be performed quickly, repeatedly, and without the use of toxic, pungent, or allergenic chemicals. In regard to the above provision, microwave irradiation of gypsum casts has been tested previously as to its effect on the strength and hardness of the cast.[23–25] The results indicated an improvement in these qualities, although there was some concern that cracks or porosities in the surface might occur when type IV gypsum casts were exposed to irradiation with a very high wattage (1,450 W).[24]

However, the results of neither the above studies nor the present investigation furnish sufficient information as to all possible effects of the microwave irradiation on gypsum casts. This will be the subject of separate studies in which the effect of single and multiple irradiations on the casts' physical properties will be investigated. Further experiments are also needed to explore the relationship between the number or weight of casts irradiated at one time and the efficacy of disinfection.

In regard to clinical practice, provided these aspects do not present problems, the impressions need not be disinfected at the dental practitioner's office. It can perhaps be argued that irreversible hydrocolloid impressions should be disinfected anyway to preclude cross-contamination during its transport to a person outside the dental clinic.[1] However, then all the environmental and compatibility problems associated with chemical disinfection come into play. The alternative is

adequate packaging and systematic use of standard barrier technique[1] for all who come into contact with impressions and other potentially contaminated dental items. Another practical matter is that casts ought to be trimmed after disinfection to reduce the risk of cross-contamination. Also, the use of metal mounting rings is precluded when casts are microwave irradiated. However, there are articulators that do not use metal mounting rings, and nonmetallic mounting rings can no doubt be produced.

The lowest concentration (0.07%) and shortest exposure time (3 minutes) of NaOCl that satisfy the requirements for adequate disinfection of irreversible hydrocolloid impressions[1,20] were established in a recent study.[21] This corroborates the results of a previous study, in which comparable concentration and exposure time of NaOCl were used.[7] In the current chemical disinfection study, the bacterial contamination was harvested from the entire gypsum casts. Apart from this, the procedures used for the current chemical disinfection study were similar in all respects to the above-mentioned ones.[7,21]

The effect on the bacterial flora of the casts poured in chemically disinfected irreversible hydrocolloid impressions was conspicuously weak, with only a median 2-log reduction of cfu/mL TSB counts (Table 3). This result was not anticipated, given previous studies documenting adequate disinfection using this protocol.[21] However, the conventional experimental methods used to test the effect of chemical disinfection of impressions might explain the discrepancy. In accordance with these, the microbiologic samples were taken from the irreversible hydrocolloid impressions, not from the casts.[7,9,10,18] Furthermore, in vivo impressions are tested microbiologically by harvesting bacteria from the impression of the occlusal surface of a molar.[7,8,19,21] Finally, in some in vitro microbiologic studies, irreversible hydrocolloid impressions are made of metal models that have been contaminated by immersing them in standardized solutions of different test bacteria.[7–9] This would most likely leave a fairly thin, even layer of bacteria on the surface.

The clinical validity of these methods[7–10,18,19] may be open to discussion for a number of reasons: First, disinfection of impression materials hinders possible cross-contamination only at the time the cast is poured. Because casts become contaminated after the prostheses are tried in the patients' mouths, they must be regarded as the major vehicle for cross-contamination. Second, in many studies,[7,8,19,21] the sample to determine bacterial contamination is harvested from the impression of the occlusal surface of a molar by means of a sterile swab. This is probably the least valid test method, because the occlusal surface is the least contaminated surface of the tooth; the gingival area, where plaque gathers, is the most contaminated. In regard to cross-contamination, a method whereby the entire cast is tested for bacterial contamination therefore appears more appropriate. Third, one would expect relatively few bacteria to be transferred to the impression material from bacterially contaminated metal models, with their thinly distributed bacterial layer. This situation hardly simulates the clinical situation, where dental plaque, containing bacteria in great concentration, is likely to adhere to and become embedded in the impression material.

In view of the complexity of the oral bacterial flora, it seems fair to question whether the chosen test bacteria are valid indicators of the efficacy of disinfection of objects contaminated in the oral cavity. The present results indicate that bacteria survive in considerable quantities after NaOCl disinfection of the impression, with a concentration and exposure time as described.[21] However, it cannot be excluded that a 0.525% concentration of NaOCl, as suggested by several authors,[1–3,8] and possibly a longer exposure time to the disinfectant might be more effective.

Conclusion

The results indicate that, in contrast to the presently described chemical disinfection procedure, microwave irradiation of casts for 5 minutes at 900 W gives high-level disinfection that complies with European Standard EN 1,040[20] and the current infection control guidelines for the dental laboratory.[1]

References

1. Kohn WG, Collins AS, Cleveland JL, et al. Centers for Disease Control and Prevention (CDC). Guidelines for infection control in dental health care settings-2003. MMWR Recomm Rep 2003;52:1–61.
2. Miller CH, Palenik CJ. Infection Control and Management of Hazardous Materials for the Dental Team, ed 2. St Louis: Mosby, 1998:213–215.
3. Miller CH, Palenik CJ. Sterilization, disinfection, and asepsis in dentistry. In: Block SS (ed). Disinfection, Sterilization, and Preservation. Philadelphia: Lippincott Williams & Wilkins, 2001:1049–1068.
4. Martin MV. Infection Control in the Dental Environment. Cambridge: Martin Dunitz, 1991:68–70.
5. Wood PR. Cross-Infection Control in Dentistry: A Practical Illustrated Guide. London: Wolfe Publishing, 1992:159–162.
6. Owen CP, Goolam R. Disinfection of impression materials to prevent viral cross contamination: A review and a protocol. Int J Prosthodont 1993;6:480–494.
7. Beyerle MP, Hensley DM, Bradly DV, Schwartz RS. Immersion disinfection of irreversible hydrocolloid impressions with sodium hypochlorite. Part 1: Microbiology. Int J Prosthodont 1994;7:234–238.
8. Schwartz RS, Bradley DV, Hilton TJ, Kruse SK. Immersion disinfection of irreversible hydrocolloid impressions. Part 1: Microbiology. Int J Prosthodont 1994;7:418–423.

9. Sofou A, Larsen T, Øwall B, Fiehn N-E. In vitro study of transmission of bacteria from contaminated metal models to stone models via impressions. Clin Oral Investig 2002;6:166–170.
10. Jennings KJ, Samaranayake LP. The persistence of microorganisms on impressions materials following disinfection. Int J Prosthodont 1991;4:382–387.
11. Matayas J, Dao N, Caputo AA, Lucatorto FM. Effects of disinfectants on dimensional accuracy of impression materials. J Prosthet Dent 1990;64:25–31.
12. Rueggeberg FA, Beall E, Kelly MT, Schuster GS. Sodium hypochlorite disinfection of irreversible hydrocolloid impression material. J Prosthet Dent 1992;76:628–631.
13. Taylor RL, Wright PS, Maryan C. Disinfection procedures: Their effect on the dimensional accuracy and surface quality of irreversible hydrocolloid impression materials and gypsum casts. Dent Mater 2002;18:103–110.
14. Verran J, McCord JF, Maryan C, Taylor RL. Microbiological hazard analysis in dental technology laboratories. Eur J Prosthodont Restorative Dent 2004;12:115–120.
15. Hudson-Davies SCM, Jones JH, Sarll DW. Cross-infection control in general dental practice: Dentists' behaviour compared with their knowledge and opinions. Br Dent J 1995;178:365–369.
16. Watkinson AC. Disinfection of impressions in UK dental schools. Br Dent J 1988;164:22–23.
17. Blair FM, Wassel RW. A survey of the methods of disinfection of dental impressions used in dental hospitals in the United Kingdom. Br Dent J 1996;180:369–375.
18. Sofou A, Larsen T, Fiehn N-E, Øwall B. Contamination level of alginate impressions arriving at a dental laboratory. Clin Oral Investig 2002;6:161–165.
19. Davis DR, Curtis DA, White JM. Microwave irradiation of contaminated dental casts. Quintessence Int 1989;20:583–585.
20. European Committee for Standardization. EN 1040. Chemical Disinfectants and Antiseptics. Basic Bactericidal Activity (phase 1). Brussels, 1997.
21. Georgescu CE. Validated Chemical Immersion Disinfection of Dental Alginate Impressions Using Sodium Hypochlorite [thesis]. Bergen: University of Bergen, Norway, 2001.
22. Nadjowski L, Dragas AZ, Kotnik V. The killing activity of microwaves on some non-sporogenic and sporogenic medically important bacterial strains. J Hosp Infect 1991;19:239–247.
23. Luebke RJ, Schneider RL. Microwave oven drying of artificial stone. J Prosthet Dent 1985:53:261–265.
24. Luebe RJ, Chan KC. Effect of microwave oven drying on surface hardness of dental gypsum products. J Prosthet Dent 1985;54:431–435.
25. Hersek N, Canay S, Akça K, Ciftci Y. Tensile strength of type IV dental stones dried in a microwave oven. J Prosthet Dent 2002;87:499–502.