

Antimicrobial Efficacy of Chemical Disinfectants on Contaminated Full Metal Crowns

Iara Augusta ORSI¹
Camilo Andrés VILLABONA²
Eliana KAMEOKA³
Marly Christiënne C.G. FERREIRA⁴
Izabel Yoko ITO⁵
Marta Estela SARAIVA⁶

¹Department of Dental Materials and Prosthodontics, Ribeirão Preto Dental School, University of São Paulo, Ribeirão Preto, SP, Brazil

²Dental School, University of Santo Tomas de Aquino, Bucaramanga, Santander, Colombia and Department of Dental Materials and Prosthodontics, Ribeirão Preto Dental School, University of São Paulo, Ribeirão Preto, SP, Brazil

³Private Practice, Ribeirão Preto, SP, Brazil

⁴Private Practice, Franca, SP, Brazil

⁵Department of Clinical Analysis, Toxicology and Bromatology, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil

⁶Dental School, National University of Tucumán, Argentina

Prosthetic restorations that have been tried in the patient's mouth are potential sources of infection. In order to avoid cross-infection, protocols for infection control should be established in dental office and laboratory. This study evaluated the antimicrobial efficacy of disinfectants on full metal crowns contaminated with microorganisms. Full crowns cast in a Ni-Cr alloy were assigned to one control group (n=6) and 5 experimental groups (n=18). The crowns were placed in flat-bottom glass balloons and were autoclaved. A microbial suspension of each type of strain - *S. aureus*, *P. aeruginosa*, *S. mutans*, *E. faecalis* and *C. albicans*- was aseptically added to each experimental group, the crowns being allowed for contamination during 30 min. The contaminated specimens were placed into recipients with the chemical disinfectants (1% and 2% sodium hypochlorite and 2% glutaraldehyde) for 5, 10 and 15 min. Thereafter, the crowns were placed into tubes containing different broths and incubated at 35°C. The control specimens were contaminated, immersed in distilled water for 20 min and cultured in Thioglycollate broth at 35°C. Microbial growth assay was performed by qualitative visual examination after 48 h, 7 and 12 days. Microbial growth was noticed only in the control group. In the experimental groups, turbidity of the broths was not observed, regardless of the strains and immersion intervals, thus indicating absence of microbial growth. In conclusion, all chemical disinfectants were effective in preventing microbial growth onto full metal crowns.

Key Words: microorganisms, cross-infection, disinfection, denture partial fixed, disinfectants.

INTRODUCTION

Blood and saliva-contaminated dental prostheses, appliances and other items brought into the dental office and prosthetic laboratory may carry high concentrations of pathogen microorganisms (1), which may be alive for extended periods even outside their human host.

Regarding the prosthodontic treatment of debilitated and immune-compromised individuals, and

the likelihood of these patients to transmit or acquire infectious diseases, dental prosthetic procedures should not involve potential sources of infection (2-3). The use effective infection control policies in the dental office and laboratory might prevent cross-contamination that could extend to dentists, dental office staff, technicians and patients.

Proper use of disinfectants is important for prevention of cross-infection when the materials do not

tolerate thermal methods (4-7). The choice for a specific disinfectant depends on a number of factors: toxicity to the patient and/or staff, potential damage to the surface, stability, degree of microbial killing required, and ability to kill microorganisms rapidly (8).

Disinfection efficacy is affected by the prior cleaning of the object, organic load (1,9,10), type and level of microbial contamination (1,7,10,11), exposure time to the germicide, object nature, temperature and pH of the disinfection process (1,10) and biocide resistance (12). A chemical germicide with at least an intermediate level of activity (tuberculocidal hospital disinfectant) is appropriate for denture disinfection (13). The most indicated chemical agents are hypochlorite and glutaraldehyde.

Sodium hypochlorite (NaOCl) is the most important type of hypochlorite and has a broad antimicrobial spectrum (14). It is bactericidal, virucidal, tuberculocidal (6) and sporicidal, although spores are more resistant than vegetative cells (15). However, as hypochlorite activity is greatly reduced in the presence of organic matter (6,10,14,15), it is important to use this agent on clean surfaces (6,10).

Glutaraldehyde is a saturated dialdehyde with a powerful antimicrobial agent (10,15) and the most widely used high-level disinfectant. Glutaraldehyde is active in the presence of organic matter (16); its low surface tension permits its penetration through blood and/or exudates to reach surfaces and facilitates rinsing (17). Gélinas and Goulet (18) evaluated the effect of organic matter on disinfectant activity and found that while glutaraldehyde kept its disinfecting activity after contact with high concentrations of organic matter, NaOCl did not tolerate the presence of organic matter.

Several studies have demonstrated the effectiveness of disinfectants against different microorganisms (5,8,9,14). However, there is little research about disinfectant efficacy on contaminated prostheses and appliances.

The disinfection of metallic prostheses has concerned dental researchers for over 50 years, since Morden et al. (19), in 1956, evaluated the effects of different antiseptic cleaning solutions on cobalt-chromium (Co-Cr) alloys. Mc Gowan et al. (20) investigated the optimal immersion time and NaOCl concentration for total asepsis of bacterial-contaminated nickel-chromium (Ni-Cr) and Co-Cr alloys.

Dental professionals, especially prosthodontists, should know the chemical solutions indicated for

disinfection of oral appliances and prosthetic pieces, their efficacy against different microbial strains and the time interval required for these products to yield proper disinfection.

The purpose of this study was to assess the antimicrobial efficacy of 3 chemical disinfectants on the disinfection of full metal crowns contaminated with 5 microbial strains after 3 immersion periods. The first hypothesis tested was that there is difference among the chemical disinfectants, as regards their efficacy against the test organisms. The second hypothesis was that high-level disinfection of the specimens is achievable at the 3 immersion periods evaluated.

MATERIAL AND METHODS

Specimen Preparation

A Co-Cr die reproducing a mandibular hemiarch with a first molar prepared for full-crown was obtained. Preliminary impressions were made with individual impression trays of chemically activated acrylic resin (Jet; Clássico Ind. e Com. Ltda., São Paulo, SP, Brazil) and polyether impression material (Impregum; ESPE Dental AG, Seefeld, Germany). The impressions were poured with improved hard plaster (Durone; Dentsply Ind. and Trade Ltd., Petrópolis, RJ, Brazil) and stone cast were obtained.

A full crown was waxed up on one of the stone casts and was cast in Ni-Cr alloy (Verabond II; Aalba Dent Inc, Cordelia, CA, USA). After trimming and finishing (airborne-particle abrasion and polishing), the full metal crown was seated on the Co-Cr die and an impression was made with a silicone impression material (Optosil-Xantopren; Haeraus-Kulzer, Wehrheim, Germany). After polymerization, the silicone impression was removed and the crown was taken from the Co-Cr die. The silicone impression served as a matrix for fabricating resin crowns with standardized characteristics.

A chemically activated acrylic resin (Duralay; Reliance Dental Mfg. Co., Worth, IL, USA) was prepared according to the manufacturer's instructions. The silicone impression was completely filled with the resin mixture and then adapted on the Co-Cr die, previously lubricated with a thin layer of petroleum jelly. After complete polymerization of the resin, the silicone impression was removed and an acrylic resin crown was obtained. This sequence was repeated several times to obtain the sample size required for the study. All resin

excesses were trimmed away from the margins of the acrylic crowns with #1508 tungsten burs (Edenta AG, Hauptstrasse, Switzerland) at a low-speed, the acrylic resin crowns were seated on the stone cast and cervical adaptation was provided with wax.

Crowns were cast in Ni-Cr alloy (Verabond II; Aalba Dent Inc.) and were thoroughly cleaned both internally and externally with aluminum oxide particles air-blasting for removal of investment residues and consequently leave the rough surface. A total of 96 full Ni-Cr crowns were fabricated, corresponding to 6 controls and 90 experimental specimens.

Full metal crowns were chosen as specimens in an attempt to simulate clinical situation. Internally, the occlusal and cervical surfaces of crowns exhibit a rough topography with peaks and grooves that form niches, in which microorganisms can be lodged, hindering the disinfection process. The crowns were not polished because there is a greater likelihood of microbial adherence in rough surfaces, showing the efficacy of disinfectants.

The full crowns were placed in flat-bottom glass balloons and were autoclaved. Five microbial strains were used as markers/indicators of the efficacy of the chemical disinfectants: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus mutans*, *Enterococcus faecalis* and *Candida albicans*. These microorganisms are usually employed for controlling and monitoring the action of disinfectants in specific culture media (Table 1). For each type of strain, 25 mL of microbial suspension were prepared. The suspensions were adjusted to match the turbidity of the 2.0 McFarland scale for the yeast (*C. albicans*), and the 0.5 McFarland scale for the other microbial strains.

Experimental Groups

A microbial suspension of each type of strain was added aseptically to one of the 5 balloons containing 18 specimens each. The specimens underwent 30-min incubation and, at each 5 min, the balloons were slightly agitated to provide homogenization.

After the 30-min contamination period, the microbial suspensions were aseptically removed from the balloons, and the crowns were placed onto the surface of sterile Petri plates with filter paper. After 3 min, the crowns were taken to different Petri plates with filter paper to allow for complete removal of humidity. After complete drying (15 min), the crowns contaminated with each type of strain (n=18) were assigned to 3 labeled glass beakers and the chemical disinfectants were dosed. One type of chemical solution was poured in each glass beaker (Table 2).

After 5 min of immersion, 2 crowns *per* solution were taken and each of them was placed into a labeled test tube (20 x 200 mm) containing 25 mL of Letheen Broth culture medium (Difco Laboratories, Detroit, MI, USA). After 10 min of immersion, other 2 crowns were taken and placed into test individual tubes, and after 15 min both crowns remaining in the glass beaker were taken and placed into individual test tubes. This protocol was strictly followed for all the tested chemical disinfectants and strains, except for *S. mutans*, incubated in different culture media. For *S. mutans*, 2 crowns were taken from the glass beaker at 5-, 10- and 15-min intervals, one of them being placed into a test tube (20 x 200 mm) containing 25 mL of Tryptic Soy Broth (Difco Laboratories) and the other into a screw-cap test tube containing Thioglycollate broth (Difco Laboratories).

Table 1. Source, type, and culture media of 5 strains of microorganisms used to assess the antimicrobial activity of the tested chemical disinfectants.

Microorganisms	Type	Culture media
<i>S. aureus</i> (ATCC 6538)	Gram-positive coccus	Mueller Hinton Broth (MHB) Difco Laboratories, Detroit, MI, USA
<i>P. aeruginosa</i> (ATCC 2327)	Gram negative bacillus	Mueller Hinton Broth (MHB) Difco Laboratories
<i>S. mutans</i> (ATCC 25175)	Gram-positive coccus	Tryptic Soy Broth (TSB), Difco Laboratories
<i>E. faecalis</i> (ATCC 10541)	Gram-positive coccus	Tryptic Soy Broth (TSB), Difco Laboratories
<i>C. albicans</i> (ATCC 1023)	Yeast	Mueller Hinton Broth (MHB) Difco Laboratories

*ATCC = American Type Culture Collection.

The tubes were incubated at 35°C.

Control Group

The 6 crowns of the control group were aseptically and individually placed into sterilized test tubes. A microbial suspension of the 5 strains was poured into one of the test tubes. Next, a broth with the inoculum of each microbial strain was poured into each one of the remaining 5 tubes.

After drying, the 6 control crowns were placed into a glass beaker, in which sterilized distilled water was poured and left undisturbed for 20 min. Afterwards, 3 crowns were placed into test tubes containing Thioglycollate broth and incubated at 35°C.

Microbial Growth Assay

The first analysis was performed 48 h after incubation and the second after 7 days. Thereafter, the tubes were removed from the stove and left at room temperature for 12 days. At the end of this period, the third analysis was accomplished. Three examiners who assessed the appearance of the culture media performed visual examination. Turbidity of the broths indicated microbial growth, which was recorded as positive.

RESULTS

Microbial growth was observed only in the control group. In the experimental group, the 3 chemical disinfectants destroyed all microbial strains at the 3 periods of disinfection evaluated (5, 10 or 15 min), as no turbidity of the media was detected.

DISCUSSION

This study investigated the chemical disinfection of full metal crowns contaminated with different microbial strains after 3 periods of exposure to 3 biocidal solutions.

To assess the antimicrobial efficacy of the chemical disinfectants, one selected 3 pathogenic microbial strains commonly used for investigations on biocidal activity (*S. aureus*, *P. aeruginosa* and *E. faecalis*), *C. albicans* (predominant over other yeasts in the oral cavity) and *S. mutans* (a bacterial strain usually found in oral flora).

P. aeruginosa grows in poor nutritional conditions and are able to produce a biofilm within a short period of time (5). It has been shown that *P. aeruginosa* is considerably resistant to biocide agents and are remarkably more resistant to these substances than *S. aureus* (7). Staphylococci and enterococci are very important pathogens due to their potential to be transmitted between patients, especially when adequate disinfection procedures are not practiced and a cross-infection policy is not routinely adopted (6). In general, enterococci are known to be able to cope with adverse physical environments (6), and tend to be less sensitive than staphylococci and streptococci (11). Fungi such as *C. albicans* produce biocide-resistant spores (2).

The disinfectants evaluated in this study - 1 and 2% NaOCl and 2% alkaline glutaraldehyde are chemical solutions advised for disinfection of prosthetic appliances. The findings of this study revealed that there was no difference regarding the biocidal efficacy of the different chemical solutions against the test organisms, all the microbial strains prevented the growth

Table 2. Chemical disinfectants, formulation and manufacturer.

Chemical disinfectants	Principle ingredients	Manufacturer
Milton solution	1% NaOCl	Chemical Biodynamic and Pharmaceutics Ltd., Ibiborã, PR, Brazil
NaOCl	2% NaOCl	Da Terra, Ribeirão Preto, SP, Brazil
Cidex	Glutaraldehyde solution: 2.4% glutaraldehyde and water - q.s.p. Activator powder: 51.5% sodium bicarbonate, 40.45% sodium hydroxymethane sulfinate, 8.0% sodium orthophosphate and green D&C dye	Johnson & Johnson, Dental Products Division, East Windsor, NJ, USA

of microbial strains within the shorter exposure period (5 min).

The microbicidal activity of chlorine is largely attributable to undissociated hypochlorous acid (HOCl), not chlorine (10). The antimicrobial efficacy of hypochlorite is decreased in the presence of organic material and/or biofilm (1) because this material reacts with the available chlorine and reduces the disinfectant-efficiency (13). In contrast with these statements, the outcomes of this study showed that all microbial strains from the experimental groups in which 1% and 2% NaOCl were used as chemical biocides were killed, thus indicating that neither of the tested chlorine-releasing disinfectants was inactivated by the presence of organic material. It seems reasonable to assume that the thin biofilm layer formed on metal crown surface did not prevent the chlorine action against the microorganisms. In the present study, the full metal crowns were not washed in tap water after contamination with the test organisms. Instead, the microbial suspension was allowed drying on the surface of the crowns, thereby forming a thin biofilm layer.

Glutaraldehyde has a broad spectrum of activity, covering gram-negative and gram-positive organisms, viruses, fungi and mycobacteria (12). A complex relationship exists between the parameters of concentration, temperature and pH for this agent (16). Glutaraldehyde is more active at alkaline than acidic pHs (7). As the external pH is altered from acidic to alkaline, more reactive sites will be formed at cell surface, leading to a more rapid bactericidal effect. The enhanced biocidal activity of glutaraldehyde in alkaline solutions is ascribed to an effect on its molecule in relation to polymerization, the outer layers of the microbial cell or a combination of both. The 2% glutaraldehyde disinfectant (Cidex) tested in this study becomes an alkaline solution (pH 8.2 to 9.2) after activation, which have been reported to yield higher antimicrobial activity than the acidic solutions. A temperature increase reduces or abolishes the difference in activity of acid and alkaline glutaraldehyde (16).

It has been stated that a surface disinfectant must kill target organisms within 10 min to be deemed effective (9,16). The rationale behind including a 5-min immersion period in the methodology of the current study was to assess whether there is a margin of safety when disinfectants are used for 10 min. The obtained results showed that all strains were destroyed within 5 min. These findings are in accordance with those of previous studies (8,20) in which 2% NaOCl and

alkaline glutaraldehyde, respectively, yielded effective disinfection within 5 min.

Because immersion of the crowns in the chemical biocides for 5 min was effective in preventing the growth of all microbial strains, it may be speculated a 10-min immersion in the disinfectants would yield an adequate margin of safety. It must also be considered that having full metal crowns with nonporous surfaces helps preventing the growth of microbial and renders the disinfection process much easier. Moreover, the antimicrobial efficacy of biocides is further increased when the prosthetic appliances are subjected to cleaning procedures before chemical disinfection because the presence of organic substances may invalidate or limit the disinfection process (10). Therefore, a 10-min immersion period seems to be adequate for disinfection of prosthetic crowns.

Further research should be done to evaluate the tested disinfecting solutions at different pHs and temperatures, and also to test more highly resistant organisms such as *Mycobacterium bovis* (tuberculocidal test) and *Bacillus subtilis* (sporicidal test).

On the basis of the results obtained and within the limitations of an *in vitro* study, the following conclusions may be drawn: 1. The 3 chemical disinfectants evaluated, 1% and 2% NaOCl and 2% alkaline glutaraldehyde were effective in preventing the growth of microbial strains used as test organisms; 2. High-level disinfection of the contaminated full metal crowns was obtained at the 3 immersion periods (5, 10 and 15 min).

RESUMO

Restaurações protéticas provadas na cavidade bucal dos pacientes são fontes potenciais de infecção. Para evitar infecção cruzada, protocolos de controle de infecção devem ser estabelecidos no consultório e laboratório odontológicos. Este estudo avaliou a eficácia antimicrobiana de desinfetantes químicos em coroas metálicas contaminadas com microorganismos. Coroas totais fundidas com liga de Ni-Cr foram divididas em grupo controle (n=6) e 5 grupos experimentais (n=18). As coroas foram colocadas em balões de vidro e esterilizadas em autoclave. A suspensão microbiana de cada tipo de cepa (*S. aureus*, *P. aeruginosa*, *S. mutans*, *E. faecalis* e *C. albicans*) foi assepticamente adicionada a cada grupo experimental, e as coroas foram deixadas contaminar por 30 min. Os corpos-de-prova contaminados foram colocados em recipientes com os desinfetantes químicos (hipoclorito de sódio 1% e 2% e glutaraldeído) por 5, 10 e 15 min. A seguir, as coroas foram colocadas em tubos contendo diferentes meios de cultura e incubadas a 35°C. Os corpos-de-prova do grupo controle foram contaminados, imersos em água destilada por 20 min e a seguir colocados em tubos de ensaio com meio de cultura Thioglycollate e incubados a 35°C. A análise do crescimento

microbiano foi realizada pelo exame visual qualitativo após 48 h, 7 e 12 dias. Houve crescimento microbiano apenas no grupo controle. No grupo experimental não foi observada turvação dos meios de cultura, independentemente das cepas e períodos de imersão. Conclui-se que todos desinfetantes químicos foram eficazes para prevenir o crescimento microbiano.

REFERENCES

1. Rutala WA, Weber DJ. Uses of inorganic hypochlorite (bleach) in health-care facilities. *Clin Microbiol Rev* 1997;10:597-610.
2. Kwok WM, Ralph WJ. The use of chemical disinfectants in dental prosthetics. *Aust Dent J* 1984;29:180-183.
3. Connor C. Cross-contamination control in prosthodontic practice. *Int J Prosthodont* 1991;4:337-344.
4. Spaun GO, Goers TA, Pierce RA, Cassera MA, Scovil S, Swanstrom LL. Use of flexible endoscopes for NOTES: sterilization or high-level disinfection? *Surg Endosc* 2010; 24:1581-1588.
5. Takeo Y, Oie S, Kamiya A, Konishi H, Nakazawa T. Efficacy of disinfectants against biofilm cells of *Pseudomonas aeruginosa*. *Microbios* 1994;79:19-26.
6. Fraiese AP. Choosing disinfectants. *J Hosp Infect* 1999;43:255-264.
7. Mc Donnell G, Russell AD. Antiseptics and disinfectants: activity, action and resistance. *Clin Microbiol Rev* 1999;12:147-179.
8. Angelillo IF, Bianco A, Nobile CG, Pavia M. Evaluation of the efficacy of glutaraldehyde and peroxygen for disinfection of dental instruments. *Lett Appl Microbiol* 1998;27:292-296.
9. Lado EA, Anekal S, Stout FW. Efficacy of various surface disinfectants on an irregular surface. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1993;75:439-442.
10. Rutala WA. APIC Guideline for selection and use of disinfectants. *Am J Infect Control* 1996;24:313-342.
11. Russell AD. Bacterial resistance to disinfectants: present knowledge and future problems. *J Hosp Infect* 1999;43:557-568.
12. Fraiese AP. Susceptibility of antibiotic-resistant cocci to biocides. *J Appl Microbiol* 2002;92:158S-162S.
13. Wood PR. Cross infection control in dentistry. A practical illustrated guide. Mosby Year Book, 1992.
14. Maillard JY, Hann AC, Baubet V, Perrin R. Efficacy and mechanisms of action of sodium hypochlorite on *Pseudomonas aeruginosa* PAO1 phage F116. *J Appl Microbiol* 1998;85:925-932.
15. Russell AD. Bacterial spores and chemical sporicidal agents. *Clin Microbiol Rev* 1990;3:99-119.
16. Gorman SP, Scott EM, Russell AD. A review: antimicrobial activity, uses and mechanism of action of glutaraldehyde. *J Appl Bacteriol* 1980;48:161-190.
17. Molinari JA, Runnells RR. Role of disinfectants in infection control. *Dent Clin North Am* 1991;35:323-337.
18. Gélinas P, Goulet J. Neutralization of the activity of eight disinfectants by organic matter. *J Appl Bacteriol* 1983;54:243-247.
19. Morden JFC, Lammie GA, Osborne J. Effect of various denture cleansing solutions on chrome cobalt alloys. *Dent Pract Dent Record* 1956;6:304-310.
20. Mc Gowan MJ, Shimoda LM, Woolsey GD. Effects of sodium hypochlorite on denture base metals during immersion for short-term sterilization. *J Prosthet Dent* 1988;60:212-218.

Accepted June 11, 2010