

Resistance to disinfection of a polymicrobial association contaminating the surface of elastomeric dental impressions

Giovanni M. Giammanco¹, Dario Melilli², Antonio Rallo², Sonia Pecorella²,
Caterina Mammì¹, Giuseppe Pizzo²

¹Department of Health Promotion Sciences, Section of Microbiology, University of Palermo, Italy;

²Department of Oral Sciences, University of Palermo, Italy

SUMMARY

The aim of this study was to evaluate the ability to resist disinfection of a polymicrobial association contaminating the surface of dental impressions obtained with two different elastomers: a polyether (Impregum) and an addition-polymerized silicone (Elite). Impressions were contaminated with a mixture of three biofilm-forming microorganisms (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*) and disinfected immediately after contamination, or after microbial layers were allowed to develop during a six-hour storage. Two commercial disinfectants were tested: MD 520 containing 0.5% glutaraldehyde and Sterigum Powder without glutaraldehyde. Residual contamination was recovered by mechanical rinsing immediately after disinfection and after a six-hour storage of disinfected impressions, and assessed by colony counting. Both disinfectants tested were shown to be effective in reducing the microbial presence on the impression materials, achieving at least a 10² reduction of microbial counts compared to water rinsing. However, Sterigum was generally less effective on the Elite elastomer and could not grant disinfection on six-hour aged *P. aeruginosa* and *C. albicans* microbial layers. The results of this study suggest that the materials used for the impressions influence the efficacy of disinfection. Disinfectants should be tested according to conditions encountered in everyday clinical practice and the need for immediate disinfection of impressions should be clearly indicated by manufacturers.

KEY WORDS: Polyether, Addition-polymerized silicone, Glutaraldehyde, Quaternary ammonium compounds, Disinfection

Received October 28, 2008

Accepted December 16, 2008

INTRODUCTION

Dental impressions that have been exposed to infected saliva and blood provide a significant source for cross-contamination. In fact, infectious microorganisms from the oral cavity can survive on the impression surface and be transferred to

the stone casts. Handling of both impressions and stone casts can potentially transmit infectious diseases to dental staff and technicians (Mitchell *et al.*, 1997; Sofou *et al.*, 2002; Muller-Bolla *et al.*, 2004; Al-Jabrah *et al.*, 2007; Mehtar *et al.*, 2007). Therefore, the routine disinfection of impressions has become an important infection control practice in dental health care settings (BDA, 2003; Kohn *et al.*, 2004).

Although the recommendations of dental advisory bodies for the implementation of disinfection procedures for impressions have undergone considerable modifications over time, no universally agreed disinfection regimen has yet been recognized (ADA, 1996; BDA, 2003; Muller-Bolla *et al.*, 2004). According to the current guidelines

Corresponding author

Giovanni M. Giammanco

Dipartimento di Scienze per la Promozione della Salute
"G. D'Alessandro", Sezione di Microbiologia

Università di Palermo

Via del Vespro 133

90127 Palermo, Italy

E-mail: g.m.giammanco@unipa.it

from the US Centers for Disease Control and Prevention (CDC), a chemical germicide which has at least an intermediate-level of activity (i.e., a hospital disinfectant with a tuberculocidal claim) is appropriate for impression disinfection and, recently, disinfectant solutions containing low concentrations of glutaraldehyde, a high-level disinfectant, have been marketed for this use (Kohn *et al.*, 2004).

The impressions taken by the dentist are frequently sent to distant dental laboratories to be moulded into various types of dental stone or plaster. In this case, the impressions are commonly not disinfected by the dentist, but just rinsed with running water, on the assumption that impressions will be disinfected by the dental technician when received (Jagger *et al.*, 1995; Muller-Bolla *et al.*, 2004). Unfortunately, almost half of the laboratory directors report that they received inadequate instructions with regard to disinfection techniques (Jagger *et al.*, 1995).

The impressions are usually enclosed inside plastic bags during transportation, thus allowing moisture conditions that are ideal for microbial survival and proliferation. Under such conditions, microbes tend to attach to surfaces and quickly form microcolonies in an extracellular polymeric matrix providing the structure for the development of a biofilm. The materials used for the impressions could influence the ability of microorganisms to adhere and aggregate depending on their surface characteristics. Moreover, the colonization of the surface of impressions by aggregated populations of microbes originating from the oral cavity could protect microbial pathogens from disinfection. In fact, surface-associated bacteria are much harder to treat with antimicrobials probably due to reduced access of the disinfectant to the cells within aggregate populations, chemical or enzymatic interactions with extracellular material decreasing or neutralizing the activity of the product, and altered growth rate of adherent microorganisms (Donlan and Costerton, 2002). The activity of disinfectants is currently tested *in vitro* separately on single microorganisms either in suspension or freshly contaminating flat sample surfaces according to the approved methods published by the European Committee for Standardization (ECS, 2001; ECS, 2003). The antimicrobial efficacy of products aimed at the disinfection of impressions, howev-

er, would be better tested under conditions representative of everyday clinical practice.

The aim of the present study was to compare the efficacy of two commercially available disinfection products, one containing 0.5% glutaraldehyde, and the other containing quaternary ammonium compounds, for disinfection by immersion of impressions contaminated with a mixture of three biofilm-forming microorganisms, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. The ability of the disinfectants to remove bacterial contamination was assessed on two different elastomeric impression materials, a polyether and an addition-polymerized silicone. The resistance to disinfection of the polymicrobial association was tested both immediately after impression contamination and after a six-hour storage of contaminated impressions inside plastic bags, corresponding to the conditions encountered in everyday clinical practice. The ability of the tested microorganisms that had survived following immediate disinfection to recolonize impression materials during a six-hour storage was also evaluated.

MATERIALS AND METHODS

An artificial dental arch was used as a model for the impressions. Small resin impression trays fitted with two occlusal stops allowed the correct positioning and the standardization of the thickness of the impression material. Both the artificial dental arches and the impression trays were disinfected before use by immersion in 1% NaClO for 15 minutes. Trays were then rinsed thoroughly with sterile saline solution to eliminate any residual NaClO that could interfere with the curing of the impression materials. Impressions of the artificial arch were taken with two different elastomers: a medium viscosity polyether (Impregum Penta Soft, 3M ESPE, Seefeld, Germany) and an addition-polymerized silicone rubber (Elite Mono Maxi, Zhermack, Badia Polesine, Italy), both used according to the manufacturer's instructions. The correct setting time of the impression materials was determined by a cyclo-viscosimeter (Cyclovisco-E, Brabender, Duisburg, Germany). A 2 cm-long specimen was taken from each impression with a sterile scalpel and immersed in 20 mL of a microbial suspension obtained by mixing inoc-

ula of *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538 and *Candida albicans* ATCC 10231. The strains were chosen among those suggested by the European Committee for Standardization (ECS) for the evaluation of disinfectants and antiseptics and the inocula were prepared at predetermined optical densities (OD) (10^8 cells per mL) according to the ECS guidelines (ECS, 2001). The sterile phosphate buffered saline solution (PBS) used for the microbial suspensions was supplemented with 40 M glucose, corresponding to the mean glucose levels in human saliva (Gough *et al.*, 1996). The contamination was prolonged for 6 minutes, corresponding to the suggested setting time of the impression materials when used *in vivo*. After contamination, the impressions were rinsed for two minutes under a moderate flow of tap water.

Two commercially available disinfectant products were tested: MD 520 (Dürr, Bietigheim-Bissingen, Germany), a high-level disinfectant containing 0.5% glutaraldehyde plus an amino derivative, and Sterigum Powder (Zhermack), an intermediate-level disinfectant containing quaternary ammonium compounds and without glutaraldehyde. Disinfection was performed by immersion in accordance with the times suggested by the manufacturers (5 minutes for MD 520 and 3 minutes for Sterigum). The impressions underwent immediate disinfection and delayed disinfection (protocol a and b, respectively), the second being performed after a six-hour storage of the contaminated impressions in sterile plastic bags at room temperature. Disinfection of the impressions was always followed by a 10 second rinse with a moderate flow of tap water.

Residual microbial contamination of the impressions was assessed by recovering contaminating cells in 20 mL of sterile PBS by vigorous mechanical shaking (8 hits per sec. for 30 seconds) with a Stomacher® mixer (Seward, Thetford, Norfolk, UK). The residual contamination from the impressions undergoing disinfection immediately after contamination was recovered just after disinfection (immediate recovery; protocol a1) and after a six-hour storage at room temperature in sterile plastic bags (delayed recovery; protocol a2). Microbial recovery from impressions undergoing delayed disinfection was performed immediately after disinfection (pro-

ocol b). Positive control impressions were contaminated as previously described.

Contaminating organisms were recovered without prior disinfection, after a moderate flow of tap water was applied for two minutes. For all the tested conditions, 100 µL of the PBS wash buffer and of 1:10 and 1:100 serial dilutions were plated on Mueller-Hinton agar and incubated at 37°C. The plates were inspected for the presence of colony forming units (CFU) after 48 hours of incubation at 37°C. The peculiar characteristics of the colonies of the three strains used for the tests made it easy to distinguish them from each other.

All the tests were performed three times and all protocols were tested in duplicate.

RESULTS

The results of the disinfection tests are summarized in Figure 1. When MD 520 disinfectant was used in each of the three tested conditions (immediate disinfection with immediate and delayed recovery: protocols a1 and a2; delayed disinfection: protocol b), average colony counts from the rinsing solutions ranged from 0 to 15 CFU/ml and a reduction of the microbial contamination with respect to controls >3 logs for bacteria and >2 logs for *C. albicans* was achieved, irrespective of the impression material used.

The tests with Sterigum disinfectant generally produced lower reductions of microbial counts with respect to MD 520 and complete elimination of the microbial contamination was never achieved. Moreover, when the Elite impressions were disinfected with Sterigum, only a ≤ 2 log reduction of microbial counts was generally observed.

Six hours after disinfection, residual bacterial contamination had disappeared in MD 520 treated impressions and little or no increases (<1 log) in microbial counts were observed in Sterigum treated impressions. However, *C. albicans* numbers increased by >1 log on the Impregum elastomer following Sterigum disinfection and the yeast recolonized the Elite elastomer to an average of 15 CFU/ml after apparently complete disinfection with MD 520.

Delayed disinfection (protocol b) obtained results comparable to those of immediate disinfection (protocols a1 and a2) only when the disinfectants

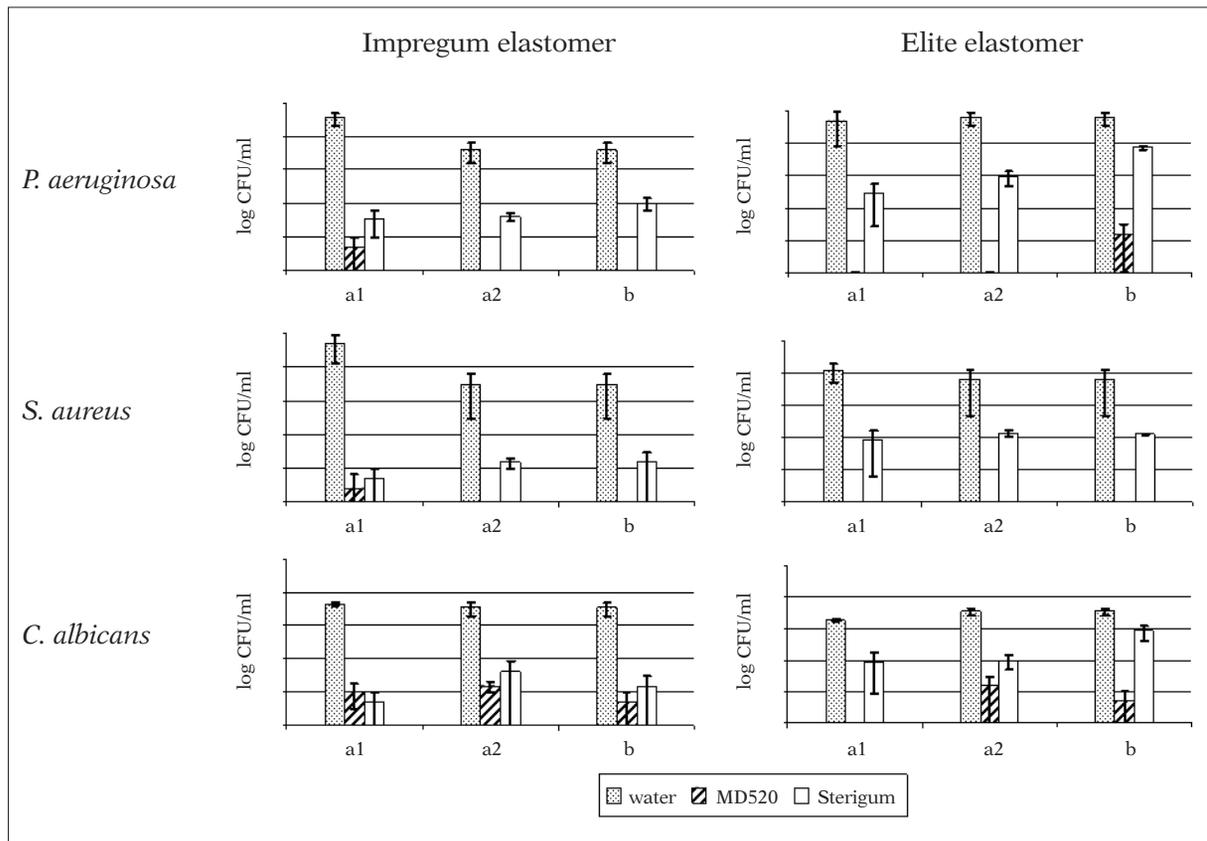


FIGURE 1 - Quantitative surface test for the evaluation of bactericidal and fungicidal activity of MD520 and Sterigum on impressions obtained with Impregum and Elite, after contamination with a suspension of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans*. Results are expressed in log CFU/ml averaging the colony counts obtained in three separate experiments under duplicate conditions. Vertical lines represent standard deviation. a1 protocol: contamination, rinsing, immediate disinfection, rinsing, immediate recovery, plating. a2 protocol: contamination, rinsing, immediate disinfection, rinsing, delayed recovery after six hours of storage in plastic bags, plating. b protocol: contamination, rinsing, delayed disinfection after six hours of storage in plastic bags, rinsing, immediate recovery, plating. water = control impressions rinsed in tap water. Adherent microorganisms have been recovered either immediately after contamination (protocol a1) or after six-hour storage in plastic bags (protocol a2 and b).

were used on Impregum impressions. On the Elite elastomer, protocol b did not allow MD 520 to completely eliminate *P. aeruginosa* and *C. albicans* and induced low reductions (~ 1 log) in the colony counts of the same two microorganisms when Sterigum was used.

It is noteworthy that while a spontaneous reduction of ≥ 1 log in bacterial contamination after trivial water rinsing was observed for both *P. aeruginosa* and *S. aureus* on the Impregum surface after six hours, little or no reduction was observed under the same conditions on the Elite impressions. On the contrary, although *C. albicans* colony counts were lower compared to bacteria, no spontaneous reduction and a slight increase in

its counts were observed after six hours in plastic bags when recovered from Impregum and Elite impressions, respectively.

DISCUSSION

The aim of the present study was to evaluate the ability to resist disinfection of a polymicrobial association contaminating the surface of dental impressions obtained with two different elastomeric materials, a polyether (Impregum) and an addition-polymerized silicone (Elite). This study was conducted *in vitro* with special attention to reproduce conditions and procedures observed

in everyday practice. Therefore, the three disinfection protocols tested not only the disinfection procedure suggested by the manufacturer, but also took into account the effect of delayed disinfection and delayed delivery to the laboratory of disinfected impressions. In these two latter conditions, microbial proliferation on porous materials could produce microbe-microbe and microbe-material interactions that can reduce the efficacy of disinfection procedures (Donlan, 2001; Donlan and Costerton, 2002).

From our results, only the disinfectant product containing 0.5% glutaraldehyde (MD 520) guarantees disinfection in the three tested conditions irrespective of the impression material tested, always achieving >3 log reduction of microbial contamination with respect to controls. According to EN 13727 (ECS, 2003), bactericidal activity can be claimed only if the disinfectant or antiseptic products show a reduction of the test organisms >10⁵ after 60 minutes contact at 20°C under clean conditions (0.3 g/l bovine albumine). As the present study aimed to test antimicrobial efficacy under conditions representative of everyday clinical practice, the contact time was reduced to 6 minutes, and reductions of the test organisms >10³ were considered sufficient for effective disinfection.

Commercial products tend to avoid glutaraldehyde due to toxic residuals, but substitute molecules, although less toxic, should provide similar efficacy. In the present study, the product without glutaraldehyde (Sterigum) obtained poorer results overall. The reduction of the microbial contamination was lower (≤ 2 log reduction) when Sterigum was used on the Elite impressions. Under these test conditions, Sterigum also seems to be unable to grant disinfection after prolonged microbial colonization by *P. aeruginosa* and *C. albicans* (protocol b). Some kind of influence by the Elite elastomer on microbial adherence, proliferation and aggregation can therefore be suspected. This is also suggested by the long-term persistence of bacterial species on this elastomer after water rinsing.

The test for the long-term efficacy of immediate disinfection (protocol a2) provided evidence that this procedure can affordably eliminate or reduce the microbial risk and this condition is maintained also until reception of impressions at distant laboratories after 6h storage of the disinfect-

ed materials. However, the increases observed in residual *C. albicans* contamination after storage may indicate a special skill of this microorganism for recolonization of impression materials after disinfection. If the storage of impression is prolonged, such a skill could represent a risk for the vehiculation of this yeast to the dental laboratory.

The test on late disinfection (protocol b) demonstrated comparable efficacy of this protocol with respect to immediate disinfection (protocols a1 and a2) when applied on the Impregum impressions. On the contrary, this protocol could not grant disinfection against prolonged microbial colonization by *P. aeruginosa* and *C. albicans* when Sterigum was used on the Elite elastomer. The two microbial species possibly shelter each other from the aggressive effects of chemicals when given the time to aggregate together on the surface of materials with appropriate characteristics.

Finally, the results obtained with control samples demonstrate that rinsing with water not followed by disinfection, although removing apparent traces of organic material (blood, saliva) from the impression surfaces, is not sufficient to prevent the risk of cross-infection, as suggested by the most recent guidelines (BDA 2003; Kohn *et al.*, 2004).

In conclusion, in the present study the disinfectant solutions did not appear to be equally effective on adhesive microbes. Our results also suggested that the characteristics of the materials used for the impressions might influence delayed disinfection and microbial recolonization. In fact, MD 520 was shown to be effective in reducing the microbial contamination on both impression materials. Sterigum was generally less effective but its performance was worse on the Elite elastomer where it could not grant disinfection against aged layers of *P. aeruginosa* and *C. albicans*. It is most unlikely that significant levels of *P. aeruginosa* would be found in the oral environment and *C. albicans* is not able to cause severe infections in immunocompetent hosts. However, when dental materials promoting microbial adhesion and disinfectants which are less effective on aggregated microbial populations are used, pathogenic organisms could find shelter in a microbial association with *C. albicans* and be vehiculated to the dental laboratory through dental impressions. To avoid potential transmission

of pathogens to dental staff and technicians, the adoption of immediate or delayed disinfection of impressions should be enforced. Moreover, impressions should be disinfected using only compatible disinfecting product that should be explained by the manufacturer's instructions. These should be produced on the basis of laboratory tests, taking into account the conditions encountered in clinical practice. The need for immediate disinfection should also be clearly indicated.

ACKNOWLEDGEMENTS

This work was supported in part by the University of Palermo (ex-60% MIUR grants).

No additional grants or contracts were provided by government agencies, non-profit foundations or companies supporting the preparation of the manuscript or the described research.

The Authors have no financial or employment arrangements with a company whose products figure prominently in the study or with any company making a competitive product.

REFERENCES

- AL-JABRAH O., AL-SHUMAILAN Y., AL-RASHDAN M. (2007). Antimicrobial effect of 4 disinfectants on alginate, polyether, and polyvinyl siloxane impression materials. *Int. J. Prosthodont.* **20**, 299-307.
- AMERICAN DENTAL ASSOCIATION (1996). Infection control recommendations for the dental office and the dental laboratory. *J. Am. Dent. Assoc.* **127**, 672-680.
- BRITISH DENTAL ASSOCIATION (BDA) (2003). Infection control in dentistry. Advice sheet A12. Available at: www.bda.org/advice/docs/A12.pdf. Accessed December 9, 2008
- DONLAN R.M. (2001). Biofilm formation: a clinically relevant microbiological process. *Clin. Infect. Dis.* **33**, 1387-1392.
- DONLAN R.M., COSTERTON J.W. (2002). Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev.* **15**, 167-193.
- EUROPEAN COMMITTEE FOR STANDARDIZATION (ECS) (2001). Chemical disinfectants and antiseptics - Quantitative non-porous surface test for the evaluation of bactericidal and/or fungicidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas - Test method and requirements. European Standard EN 13697.
- EUROPEAN COMMITTEE FOR STANDARDIZATION (ECS) (2003). Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants for instruments used in the medical area - Test method and requirements (Phase 2/Step 1). European Standard EN 13727.
- GOUGH H., LUKE G.A., BEELEY J.A., GEDDES D.A. (1996). Human salivary glucose analysis by high-performance ion-exchange chromatography and pulsed amperometric detection. *Arch. Oral. Biol.* **41**, 141-145.
- JAGGER D.C., HUGGETT R., HARRISON A. (1995). Cross-infection control in dental laboratories. *Br. Dent. J.* **179**, 93-96.
- KOHN W.G., HARTE J.A., MALVITZ D.M., COLLINS A.S., CLEVELAND J.L., EKLUND K.J. (2004). Guidelines for infection control in dental health care settings - 2003. *J. Am. Dent. Assoc.* **135**, 33-47.
- MEHTAR S., SHISANA O., MOSALA T., DUNBAR R. (2007). Infection control practices in public dental care services: findings from one South African Province. *J. Hosp. Infect.* **66**, 65-70.
- MITCHELL D.L., HARIRI N.M., DUNCANSON M.G., JR., JACOBSEN N.L., MCCALLUM R.E. (1997). Quantitative study of bacterial colonization of dental casts. *J. Prosthet. Dent.* **78**, 518-521.
- MULLER-BOLLA M., LUPI-PEGURIER L., VELLY A.M., BOLLA M. (2004). A survey of disinfection of irreversible hydrocolloid and silicone impressions in European Union dental schools: epidemiologic study. *Int. J. Prosthodont.* **17**, 165-171.
- SOFUO A., LARSEN T., FIEHN N.E., OWALL B. (2002). Contamination level of alginate impressions arriving at a dental laboratory. *Clin. Oral. Investig.* **6**, 161-165.