Eur. J. Prosthodont. Rest. Dent., Vol.17, No. 3, pp 121-125
Printed in Great Britain

doi:10.1922/EJPRD_794Marchini05

Evaluation of the Antimicrobial Activity and Dimensional Alterations of Alginate Impression Disinfectants

Ana Paula Nocentini Semensato*, Sonia Khouri Crosariol† and Leonardo Marchini‡

Abstract - *This paper offers a quantitative evaluation of the antimicrobial efficacy of eight different disinfection procedures for irreversible hydrocolloid impressions and the dimensional changes induced by them. Samples were collected immediately after impressions, after the disinfection procedures and over casts and analyzed for bacterial growth. Control, enzyme solutions, acetic acid and ultraviolet irradiation samples showed bacterial growth. Chlorhexidine and 1% sodium hypochlorite presented adequate antimicrobial activity, while 2% sodium hypochlorite solution showed the best results. Dimensional changes were similar to those of the controls in all the tested agents. The results indicated 2% hypochlorite was the most appropriate disinfectant tested.*

KEY WORDS: Irreversible hydrocolloid, antimicrobial activity, disinfection, dimensional alteration, sodium hypochlorite, chlorhexidine.

INTRODUCTION

The transference of oral microorganisms from the patient's mouth to gypsum casts by impressions is a well-documented fact [1-3]. Contaminated casts can bring pathogenic microorganisms into dental laboratories, spreading potential infections to dental technicians. A survey of dental laboratories in the UK [4] found that cross-infection control procedures in dental laboratories are variable. Irreversible hydrocolloid is a widely used impression material, thanks mainly to its advantages of easy handling, comfort for the patient, and relatively low cost. However, this material must be disinfected rapidly to prevent dimensional changes. Moreover, some disinfectants can modify the surface of stone casts of disinfected irreversible hydrocolloid impressions [5-7].

A variety of disinfectants have been recommended for irreversible hydrocolloid impressions, mostly chemical solutions [8,9]. Larsen et al. [10] reported an unsuccessful experiment using ultraviolet irradiation to disinfect dental impressions. The disinfectants used most frequently are sodium hypochlorites, iodophors and synthetic phenols.

The immersion of irreversible hydrocolloid impressions in disinfectant solutions for long periods can, in theory, lead to dimensional changes of the resulting stone casts, mainly due to imbibition phenomena. However, there is evidence [11,12] that chemical disinfectants in the form of sprays do not cause significant dimensional changes in the resulting gypsum casts.

Some commonly used disinfectants and cleanser solutions have not been subjected to standardized tests to compare the antimicrobial activity and possible dimensional alterations induced by them. Enzymatic solutions are commonly used to predecontaminate instruments. These solutions, which contain amylase, lipase and protease, detergent and isopropanol, are cleansers rather than disinfectants, which serve to reduce the organic matter (bioburden) from impressions and are not preevaluated for microbial control in alginate impressions.

This study involved a quantitative evaluation of the antimicrobial activity of six chemical disinfectants and one physical disinfectant and measurements of the dimensional changes they caused in the resulting stone casts. All the agents that were tested were potentially safe and low cost.

METHODS

This study was designed in two parts. The first involved a quantitative approach to evaluate antimicrobial activity of seven disinfectant agents used with irreversible hydrocolloid impressions. The second part consisted of evaluating the dimensional changes produced on the resulting casts by the most effective disinfectants identified in the first part.

Evaluation of the antimicrobial activity of disinfectant agents

Twenty 18 to 25-year-old male and female dentate volunteers were selected to participate in the study. The protocol used in this study was previously approved by the ethics committee of the University of Vale do Paraíba (H261/CEP/2006).

Nine irreversible hydrocolloid impressions of the upper arch were taken from each patient at 4-hour intervals between impressions to allow for recolonization of the patient's mouth. All the materials used to obtain alginate

* DDS, MSc
† BMD, MSc
‡ DDS, MSD, PhD

impressions (except the irreversible hydrocolloid powder) were sterilized before making the impressions. The irreversible hydrocolloid was handled according to the manufacturer's instructions.

As soon as the impressions were made, the first samples were collected from the impression with a sterile swab. These samples were collected from the right first molar and adjacent palatal area, using standardized movement and time (3 seconds for each sample). The second samples were collected after disinfection of the impressions (from the left first molar and adjacent palatal area) and a third sampling was done by applying swabs on the surface of the resulting casts (in the second right premolar and adjacent palatal area), which were produced by pouring type IV gysum into the impressions.

One untreated impression was kept as a control. Six impressions from each volunteer were immersed and gently swirled in different chemical solutions (Table 1) for 10 seconds, kept for 10 minutes in a closed plastic bag and then washed with sterile water.

The other two impressions were subjected to 250nm ultraviolet irradiation. One of these impressions received ultraviolet irradiation for 30 min immediately after removal from the mouth. The other one was also irradiated for 30 min, after 10 seconds of immersion and gentle shaking in a 5% enzymatic solution.

After collecting the samples, the swabs were immersed immediately in 5ml of brain and heart infusion (BHI) broth for 24 hours at 37°C, after which 0.5 ml from each BHI tube were spread on blood-agar dishes to determine the colony forming units (CFU). The latter procedure was undertaken to visualize the presence of bacteria that do not grow sufficiently to be visible in BHI broth (MacFarland scale 0.5).

The efficacy of the disinfectants was than compared by the Two Ratio Equality Test at a significance level of 7%, using the Minitab software package. This significance level was chosen due to a statistical analysis considering sample size and test reliability.

Dimensional changes generated by the most effective disinfectants

A stainless steel die was made (Figure 1) to evaluate the dimensional changes in the casts. Thirty-six irreversible hydrocolloid impressions were made from the metal die and divided into three groups. One group of 12 impressions which served as the control, was not disinfected. The other two groups were treated with two disinfectants that had previously been identified as effective: chlorhexidine and 2% sodium hypochlorite.

After disinfection, type IV gypsum was poured immediately into the impressions. Using a 0.01mm resolution pachymeter, the dimensions of the casts were recorded. All the dimensions presented in Figure 1 were measured in triplicate and the mean value was used for comparison. The measurements of the casts were then compared with the metal die by the T test with a significance level of 7%, using the software package Minitab. This significance level was chosen due to a statistical analysis considering sample size and test reliability.

Table 1. *Chemical disinfectants used in this study – trade names, manufacturers and locations*

Disinfectant	*Trade name*	*Manufacturer/City/Country*
Chlorhexidine 0.2%	Sommacare	LM-Farma/São José dosCampos/Brazil
Enzymatic solution 1%	Lifezyme	Lifemed/São Paulo/Brazil
Enzymatic solution 5%	Enzi-Tec Plus	LM-Farma/São José dosCampos/Brazil
Sodium hypochlorite 1%	LM Clor	LM-Farma/São José dosCampos/Brazil
Sodium hypochlorite 2%	Clorox	Clorosul/Gravataí/Brazil
Acetic acid 4%	Na mesa	Agrin/Jundiaí/Brazil

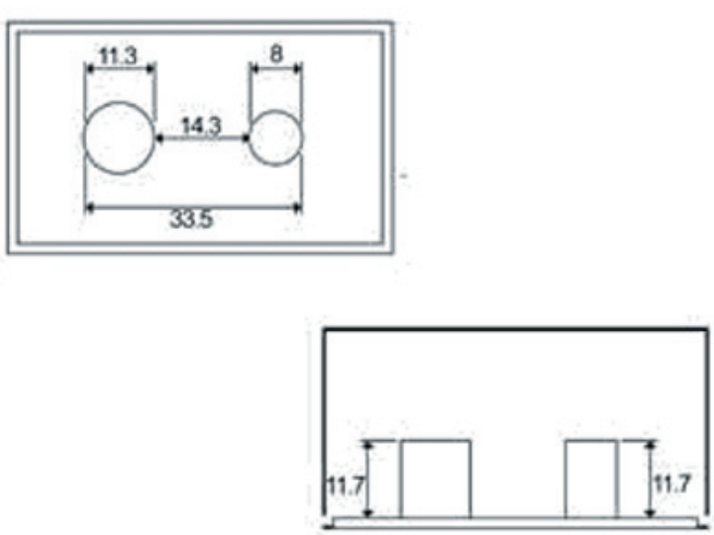

Figure 1. *Schematic presentation of the metal die. Distances are shown in millimeters (mm).*

RESULTS

Evaluation of the disinfectants' antimicrobial activity

All the BHI broth tubes inoculated with the initial samples (immediately after removing impressions from mouth) revealed bacterial growth.

Table 2 shows the bacterial growth of the second group of samples in BHI broth (collected after disinfection of the impressions). All the control samples were found to be positive, as were the samples treated with 4% acetic acid, 1% enzymatic solution, UV irradiation and 5% enzymatic solution. The samples treated with 2% sodium hypochlorite showed the best results. Compared to the other materials, 2% sodium hypochlorite proved statistically more efficient ($p>0.001$), except when compared with 1% sodium hypochlorite ($p=0.185$).

The third group of samples (collected from casts) showed a bacterial growth pattern similar to that of the second group, as indicated in Table 3. Again, the samples treated with 2% percent sodium hypochlorite showed the best results. Compared with the other disinfectants, 2% sodium hypochlorite was statistically more efficient ($p>0.001$) except when compared with chlorhexidine 0.2% ($p=0.337$).

Tables 4 and 5 show the CFU counts in the blood-agar dishes from impressions and casts, respectively. All the dishes with colonies contained more than $1x10^5$ colonies. The results are similar to those presented in Tables 2 and 3, demonstrating the higher efficacy of 2% sodium hypochlorite than the other disinfectants tested in this experiment ($p>0.001$) with the exception of the 1% sodium hypochlorite ($p=0.151$ and $p=0.376$, respectively)

Dimensional changes caused by the most effective disinfectants

Tables 6 to 8 compare the dimensions of the casts after their treatment with the most effective disinfectants and those of the control groups against the dimensions of the metal die.

DISCUSSION

The first part of this experiment demonstrated the bacterial contamination of irreversible hydrocolloid impressions, since all the samples taken from impressions showed bacterial growth. Likewise, all the samples collected from the non-disinfected gypsum casts (control) also showed bacterial growth. These results are congruous with most [1-3], albeit not all [13,14] of the literature, reinforcing the need for disinfecting dental impressions before they are sent to dental laboratories.

Table 2. *Bacterial growth of second samples in BHI broth (collected after impression disinfection) for each disinfectant.*

	Clear		*Initial turbidity*		*Turbidity*	
	n	%	*N*	%	*n*	%
Control group	0	0	0	0	20	100
Chlorhexidine 0.2%	11	55	5	25	4	20
Enzymatic solution 5% and UV	2	10	0	0	18	90
Enzymatic solution 1%	0	0	0	0	20	100
Enzymatic solution 5%	0	0	0	0	20	100
Sodium hypochlorite 1%	15	75	0	0	5	25
Sodium hypochlorite 2%	18	90	2	10	0	0
30 minutes under UV	0	0	0	0	20	100
Acetic acid 4%	0	0	0	0	20	100

Table 3. *Bacterial growth of third samples in BHI broth (collected from casts) for each disinfectant.*

	Clear		*Initial turbidity*		*Turbidity*	
	n	%	*N*	%	*n*	%
Control group	0	0	0	0	20	100
Chlorhexidine 0.2%	10	50	9	45	1	5
Enzymatic solution 5% and UV	2	10	11	55	7	35
Enzymatic solution 1%	0	0	0	0.0	20	100
Enzymatic solution 5%	0	0	0	0.0	20	100
Sodium hypochlorite 1%	7	35	0	0.0	13	65
Sodium hypochlorite 2%	13	65	7	35	0	0
30 minutes under UV	0	0	0	0.0	20	100
Acetic acid 4%	0	0	0	0.0	20	100

Table 4. *CFU count in blood-agar dishes from impressions for each disinfectant.*

	No Growth		*CFU >1x10^5*	
	n	*%*	*n*	*%*
Control group	0	0	20	100
Chlorhexidine 0.2%	13	65	7	35
Enzymatic solution 5% and UV	6	30	14	70
Enzymatic solution 1%	0	0	20	100
Enzymatic solution 5%	0	0	20	100
Sodium hypochlorite 1%	16	80	4	20
Sodium hypochlorite 2%	19	95	1	5
30 minutes under UV	0	0	20	100
Acetic acid 4%	0	0	20	100

Table 5. *CFU count in blood-agar dishes from casts for each disinfectant.*

	No Growth		*CFU >1x10^5*	
	n	*%*	*N*	*%*
Control group	0	0	20	100
Chlorhexidine 0.2%	12	60	8	40
Enzymatic solution 5% and UV	5	25	15	75
Enzymatic solution 1%	0	0	20	100
Enzymatic solution 5%	0	0	20	100
Sodium hypochlorite 1%	16	80	4	20
Sodium hypochlorite 2%	18	90	2	10
30 minutes under UV	0	0	20	100
Acetic acid 4%	0	0	20	100

Table 6. *Comparison of measurements on control group and metal die dimensions. The asterisk (*) indicates a statistically significant difference.*

	Large Diameter	*Small Diameter*	*Inner Distance*	*Outer Distance*
Average	11.33	7.92	14.21	33.39
SD	0.04	0.09	0.10	0.09
p-value	0.017*	0.008*	0.012*	0.001*

Table 7. *Comparison of measurements on 2% sodium hypochlorite group and metal die dimensions (in millimeters). The asterisk (*) indicates a statistically significant difference.*

	Large Diameter	*Small Diameter*	*Inner Distance*	*Outer Distance*
Average	11.20	7.88	14.23	33.41
SD	0.15	0.14	0.14	0.03
p-value	0.049*	0.010*	0.082	<0.001*

Table 8. *Comparison of measurements on clorhexidine group and metal die dimensions. The asterisk (*) indicates a statistically significant difference.*

	Large Diameter	*Small Diameter*	*Inner Distance*	*Outer Distance*
Average	11.19	7.76	14.25	33.37
SD	0.25	0.20	0.15	0.09
p-value	0.141	0.002*	0.245	<0.001*

However, this study has a quantitative approach and did not identify the bacterial species that were viable on impressions or transferred to casts, nor was the presence of viable viruses evaluated by the methodology employed. These are important issues that could be addressed in future researches, maybe using culture independent approaches[15], which allows identification of bacteria that do not grow in culture media.

For clinical use, disinfectants should also be nontoxic, easy to handle and low-cost. Despite their adequate antimicrobial activity, some commonly used disinfectants such as glutaraldehyde do not meet these requirements [3].

In our attempt to identify an agent that meets the above requirements, several chemical agents not previously tested for disinfection of irreversible hydrocolloid impressions (enzymatic solutions in two different concentrations and acetic acid) were tested in this study, as well as a physical agent (ultraviolet irradiation). These agents are safe, inexpensive and easy to handle. Ultraviolet irradiation offers the additional advantage of not requiring immersion of the impressions in a solution, thus sidestepping its undesirable effects. However, the results of this study showed that these agents failed to eliminate contaminants from dental impressions, since they did not eliminate microbial activity under the experimental conditions used.

More commonly used disinfectants such as chlorhexidine and sodium hypochlorite in either 1% or 2% concentrations showed greater antimicrobial efficacy and caused similar dimensional changes compared to the control group.

With regard to dimensional changes, it should be highlighted that the methodology applied in the current experiment was intended to reproduce normal clinical impressions, attempting to avoid some of the problems inherent in the ADA specifications for testing dimensional accuracy. ADA specifications, albeit easy to be replicated by other investigators, do not reproduce clinical impressions well [16]. Our methodology involved the use of a tray of the kind generally used for irreversible hydrocolloid impressions, and the master die presented regions corresponding to dentate and edentate areas, seeking to reproduce clinical situations. The most important limitation of this approach, however, is the difficulty of comparing our results directly with other investigations. Nevertheless, since our results for the control group and the most effective disinfectants were similar, the clinical effects of these disinfectants on alginate dimensional changes seem to be negligible. This result is congruous with a recent systematic review of the stability of impression materials subjected to chemical disinfection, which also found that disinfection procedures did not generally affect the impressions significantly in the evaluated studies [17].

Whilst it is important to point out that this study involved a limited number of samples for the analysis of both antimicrobial activity and dimensional changes it should also be noted that 2% sodium hypochlorite produced the best results in the disinfection of both impressions and casts samples. The results using 2% sodium hypochlorite allied to the fact that it is also an inexpensive, nontoxic and easily handled substance, suggest that it is an highly appropriate disinfectant for irreversible hydrocolloid impressions.

ACKNOWLEDGEMENTS

We would like to thank Fernanda Cristina Marques, DDS, and Jane Rezende de Oliveira, DDS, for their help in the pilot study of this research.

MANUFACTURER´S DETAILS

Irreversible hydrocolloid (Jeltrate™, Dentsply, Brazil)
Type IV gysum (Vel-mix™, SDS Kerr, USA)
Ultraviolet irradiator (Quimis MM-80, Veco, Brazil)
Brain and heart infusion (BHI) broth (Difco, France)
Blood-agar (Difco, France)
Minitab software package (Minitab Inc, USA)
Pachymeter (Mitutoyo 500-171-20B, Mitutoyo, Brazil)

ADDRESS FOR CORRESPONDENCE

Leonardo Marchini, DDS, MSD, PhD, Av. Adhemar de Barros, 1136/153, SJCampos SP Brazil 12245-010.
E-mail: leomarchini@directnet.com.br

REFERENCES

1. Leung, R.L. and Schonfeld, S.E. Gypsum casts as a potential source of microbial cross-contamination. *J Prosthet Dent*., 1983; **49**:210-211.
2. Samaranayake, L.P., Hunjan, M. and Jennings, K.J. Carriage of oral flora on irreversible hydrocolloid and elastomeric impression materials. *J Prosthet Dent*., 1991; **65**:244-249.
3. Block, S.S. *Disinfection, sterilization and preservation*. 5th ed. Philadelphia: Lippincott, Williams & Williams; 2001.
4. Jagger, D.C., Huggett, R. and Harrison, A. Cross-infection control in dental laboratories. *Br Dent J*., 1995; **179**:93-96.
5. Boden, J., Likeman, P. and Clark, R. Some effects of disinfecting solutions on the properties of alginate impression material and dental stone. *Eur J Prosthod Restor Dent*., 2001; **9**:131-135.
6. Jagger, D.C., Al Jabra, O., Harrison, A., Vowles, R.W. and McNally, L. The effect of a range of disinfectants on the dimensional accuracy of some impression materials. *Eur J Prosthod Restor Dent*., 2004; **12**: 154-160.
7. Hussain, S.M., Tredwin, C.J., Nesbit, M. and Moles, D.R. The effect of disinfection on irreversible hydrocolloid and type III gypsum casts. *Eur J Prosthod Restor Dent*., 2006; **14**: 50-54.
8. Blair, F.M. and Wassel, R.W. A survey of the methods of disinfection of dental impressions used in dental hospitals in the United Kingdom. *Br Dent J*., 1996; **80**:369-375.
9. Anusavice, K.J. *Phillips' science of dental materials*. 10th ed. Philadelphia: W.B. Saunders; 1996.
10. Larsen, T., Fieh, N.E., Peutzfeldt, A. and Owall, B. Disinfection of dental impressions and occlusal records by ultraviolet radiation. *Eur J Prosthodont Restor Dent*., 2000; **8**:71-74.
11. Tan, H.K., Hooper, P.M., Buttar, I.A. and Wolfaardt, J.F. Effects of disinfecting irreversible hydrocolloid impressions on the resultant gypsum casts: Part II – Dimensional changes. *J Prosthet Dent*., 1993; **70**:532-537.
12. Poulos, J.G. and Antonoff, L.R. Disinfection of impressions. Methods and effects on accuracy. *N Y State Dent J*., 1997; **63**:34-36.
13. Sofou, A., Larsen, T., Fiehn, N.E. and Owall, B. Contamination level of alginate impressions arriving at a dental laboratory. *Clin Oral Investig*., 2002; **6**: 161-165.
14. Sofou, A., Larsen, T., Owall, B. and 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.
15. Paster, B.J., Olsen, I., Aaas, J.A. and Dewhirst, F.E. The breadth of bacterial diversity in the human periodontal pocket and other oral sites. *Peridontol 2000,* 2006: **42**: 80-87.
16. Walker, M.P., Rondeau, M., Petrie, C., Tasca, A, and Williams, K. Surface quality and long-term dimensional stability of current elastomeric impression materials after disinfection. *J Prosthod*., 2007, **16**: 343-351.
17. Kotsiomiti, E., Tzialla, A., Hatjivasiliou, K. Accuracy and stability of impression materials subjected to chemical disinfection - a literature review. *J Oral Rehabil*., 2008, **35**: 291-299.