10.5005/jp-journals-10024-1731

ORIGINAL RESEARCH

Comparison of Antimicrobial Activity between Chemical Disinfectants on Contaminated Orthodontic Pliers

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ABSTRACT

Aim: To compare the antimicrobial activity of the chemical substances—70% isopropyl alcohol, 2% glutaraldehyde (GTA) and 0.25% peracetic acid (PAA) in disinfecting orthodontic pliers contaminated *in vitro* with *Streptococcus mutans*, *Staphylococcus aureus* and *Candida albicans*.

Materials and methods: Distal end cutter pliers were divided into five groups: group 1 (negative control—sterilized pliers), group 2 (positive control—sterilized plier, subsequently contaminated), group 3 (disinfected with 70% isopropyl alcohol, friction method), group 4 (disinfected with 2% GTA, immersion method for 30 minutes), group 5 (disinfected with 0.25% peracetic acid (PAA), immersion method for 10 minutes). After the pliers were treated with one disinfectant and submitted to microbiological evaluation (by counting colony forming units), they were submitted to the same cleansing, sterilizing and contaminating processes, and were used in the following groups (crossover and washout study). The two-factor analysis of variance (ANOVA) test, followed by the Tukey test, was used to compare the groups.

Results: The results showed that there was no statistically significant difference between the three tested disinfectants.

Conclusion: Although there were no statistically significant differences between the disinfectants, the chemical agents 2% glutaraldehyde and 0.25% PAA were effective in inhibiting the growth of the three microorganisms tested; however, 70% isopropyl alcohol was unable to completely eliminate *S. aureus*.

Clinical significance: The chemical substances 2% glutaraldehyde and 0.25% PAA completely eliminated the microorganisms tested.

Keywords: Disinfection, Glutaraldehyde, Infection control, Isopropyl alcohol, Laboratory research, Microbiology, Orthodontics, Peracetic acid.

How to cite this article: Carvalho MRA, dos Santos da Silva MA, de Sousa Brito CAR, Campelo V, Kuga MC, Tonetto MR, De Jesus Tavarez RR, Bandéca MC, Pinzan-Vercelino CRM. Comparison of Antimicrobial Activity between Chemical Disinfectants on Contaminated Orthodontic Pliers. J Contemp Dent Pract 2015;16(8):619-623.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Effective infection control actions are important to prevent cross-contamination at dentistry practice. Some specialties, such as orthodontics, involve the use of materials that are considered semicritical items, since these instruments come into contact, but do not penetrate, with the intact mucosa.[1] This is the case of the orthodontic pliers that are routinely used. Inspite of not penetrating into mucosa, when pliers come into contact with the oral cavity, they become contaminated with the microorganisms existing in the patients' mouths, so infection control is extremely necessary.[2-4]

The autoclave sterilization is the better way to kill and permanently inactivate the microorganisms, however, chemical disinfectants can be used to disinfect orthodontic pliers, since it is classified as a semicritical material.[2,5] Several types of chemical products are used to disinfect dental materials and instruments, among

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them the most frequent are 70% isopropyl alcohol and glutaraldehyde (GTA). However, recently, peracetic acid (PAA) has been suggested as an alternative to avoid cross-infection.[6,7]

Isopropyl alcohol (70%) is considered a mid-level disinfectant, and is used as a disinfectant of surfaces and instruments. Alcohol acts by precipitating nucleic acids, denaturing proteins and dissolving fat, thereby exerting its antimicrobial action.[8]

Two percent GTA is a high-level disinfectant, used in the processing of medical/dental thermosensitive equipments, and is effective against a great variety of microorganisms.[2,7,9] It is active in the presence of organic matter and can act both as a disinfecting (30 minutes) and sterilizing (10 hours) agent. Its disadvantage is the toxicity, potentially causing skin and mucosal irritation, ulceration and burns; it may also cause irritation to the eyes and to the respiratory system.

Peracetic acid has been used to disinfect and sterilize critical and semicritical medical and hospital instruments and equipments.[7,10] Its advantages are that it is sporicidal at low concentrations and in contact with organic matter; it performs disinfection in 10 minutes and sterilization within 30 minutes to 1 hour. Moreover, it is biodegradable, nontoxic and noncorrosive.[7,11-13] Its disadvantages are high instability and high cost.[10,14]

Dental professionals need information regard the chemical solutions indicated as disinfectant agents. To date, there is scarce information regarding the use of PAA as a disinfecting agent in dentistry.[6,11,12] Since the orthodontic pliers are materials considered to be semicritical, and can be treated using disinfectant agents, this study was designed. The aim of this experiment was to test the following null hypothesis: there is no difference in antimicrobial activity of 70% isopropyl alcohol, 2% glutaraldehyde and 0.25% PAA, in disinfecting orthodontic pliers contaminated with the microorganisms *Streptococcus mutans*, *Staphylococcus aureus* and *Candida albicans*.

MATERIALS AND METHODS

Three reference strains were used to test the antimicrobial activity: *S. mutans* (American type culture collection—ATCC 25175), *S. aureus* (ATCC 25923) and *C. albicans* (ATCC 10231).

Staphylococcus aureus were seeded onto mannitol salt agar (Difco laboratories, Sparks, MD, USA); *S. mutans* were seeded onto mitis salivarius agar (Difco Laboratories, Sparks, MD, USA), with 15% sucrose and 3.3 mg/ml bacitracin; and *C. albicans* were seeded onto sabouraud dextrose agar (Difco Laboratories, Detroit, MI, USA), with 50 mg/ml chloramphenicol.

To obtain pure cultures, each microorganism strain was cultivated on its specific medium in petri dishes and was incubated at 37°C for 48 hours. After the incubation period, a platinum handle was used to retrieve one isolated colony of the microorganism which was inoculated into an individual brain heart infusion (BHI) broth (Acumédia, Baltimore, Maryland, EUA), incubated at 37°C for 24 hours (*S. aureus*) or 48 hours (*S. mutans* and *C. albicans*).

The disinfectants tested were: 70% isopropyl alcohol (Quality, Vic Plasma Indústria e Comércio Ltda., Taguatinga, SP, Brazil), 2% GTA (Glutaron, Indústria Farmacêutica Rioquímica Ltda., São José do Rio Preto, SP, Brazil) and 0.25% PAA (Proxitane® Alfa, Peróxido do Brasil, Curitiba, PR, Brazil).

Distal end cutter pliers (Orthometric, Marília, SP, Brazil) were divided into five groups: group 1 (negative control)—sterilized pliers, group 2 (positive control)—sterilized plier, subsequently contaminated, group 3: disinfected with 70% isopropyl alcohol, friction method, group 4—disinfected with 2% GTA, immersion method for 30 minutes, group 5—disinfected with 0.25% PAA, immersion method for 10 minutes (Table 1).

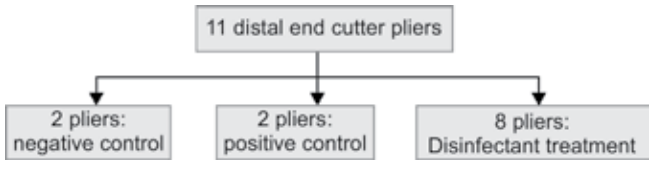
After the orthodontic pliers were infected, treated with one disinfectant and submitted to microbiological evaluation, they were submitted to the same cleansing, sterilizing and contaminating processes, and were used in the following groups (cross-over study, 7 days of wash out, Flow Chart 1).

Initially, 11 pliers were sterilized in autoclave for 15 minutes at 121°C and 1 atm of pressure. After this, two pliers were randomly chosen to comprise the negative control group (group 1), and were immediately submitted to microbiological evaluation since they were not

Table 1: Groups description

Groups	Protocol
1 (negative control)	No contamination and sterilized in autoclave for 15 minutes at 121°C and 1 atm of pressure
2 (positive control)	Contaminated and without disinfection treatment
3 (70% isopropyl alcohol)	Friction in three phases, intercalated with natural drying periods, for a total of 10 minutes
4 (2% glutaraldehyde—GTA)	Immersion for 30 minutes
5 (0.25% peracetic acid—PAA)	Immersion for 10 minutes

Flow Chart 1: Study design: crossover study; 7 days of washout. Nine experiments were conducted; each of them tested one microorganism strain with one disinfectant



contaminated and would not undergo any of the disinfecting treatments. The pliers of group 1 were not infected, aiming to check the asepsis during the experiment. The remaining nine pliers were contaminated *in vitro* with the tested microorganisms.

Contamination of the nine pliers was achieved by total immersion of the instruments' active part for 5 minutes in BHI broth prepared with *S. aureus* or *C. albicans*. The pliers spontaneously dried, forming a thin layer of biofilm. For contamination with *S. mutans*, microaerophilia were applied (candle jar method) for 48 hours.

The surface of the active part of one plier (group 2), randomly selected from the nine contaminated instruments, was rubbed for 30 seconds with a sterile swab moistened in sterile saline solution, to test its contamination. Following friction, the swab was inserted into a test tube containing 2 ml of 0.9% saline solution and agitated for 30 seconds. From this suspension (non-diluted solution—NDS), serial dilutions were prepared and an aliquot of each dilution was seeded in duplicates into specific culture medium plates, and incubated for 48 hours for subsequent counting of colony forming units (CFU).

The remaining eight pliers received disinfection treatment according to each experimental group. After treatment, each plier was placed in a 400 ml sterile beaker. Collections were then made of the active part of the pliers with a swab moistened in saline solution for subsequent microbiologic evaluation, as described for group 2. Two previously trained operators conducted the disinfectant procedures.

The CFU counting was performed by two examiners that were blinded (they did not have knowledge of which group they were evaluating). The numeric variable CFU/ml was logarithmically transformed (log_{10}) and statically analyzed using two-factor analysis of variance (ANOVA), followed by the post-hoc Tukey test. The level of significance was set at 5%. The data were evaluated using SPSS for Windows 17.0 software (IBM Corporation, New York, NY, USA, 2007).

The pliers were considered 'decontaminated' when the number of colonies was below the lower detection threshold (10 CFU = 2.0).

RESULTS

No statistically significant difference was observed between the three experimental treatment groups. Nevertheless, statistically significant differences were detected between the five treatments groups (Table 2).

A multiple comparison of the five groups performed for *S. mutans* and *C. albicans* showed that these microorganisms were eliminated after treatment in groups 1, 3, 4 and 5, indicating decontamination of all pliers. In group 2, however, the mean log of CFU/ml was 6.04 for *C. albicans* and 6.47 for *S. mutans*, indicating substantial contamination of the pliers. A multiple comparison of the five groups performed for *S. aureus* showed that pliers were decontaminated in groups 1, 4 and 5. Group 2 showed substantial contamination (Table 3).

No statistically significant difference was observed between groups 1, 3, 4 and 5. Nevertheless, the mean CFU/ml for group 3 was 2.70, i.e. greater than the means found for groups 1, 4 and 5, and also greater than 2.0, indicating the presence of contamination in 25% of the pliers in this group.

DISCUSSION

It is evident that the daily dental practice necessitates the use of effective disinfectants to control cross-infection. New disinfecting products have been recently introduced to the market, with good results in medicine practice.[7,15] Therefore, it is important to evaluate them in dentistry also.

The microorganisms tested in this study are often used to control and monitor the action of disinfectants in specific culture media.[2,7,11,16-18] These microorganisms were effective in contaminating the pliers tested in this study, as showed in group 4. This group was designed with the aim of verifying the existence of pliers' contamination.

The results of the present study revealed that there were no statistically significant differences regarding the antimicrobial efficacy of the three chemical solutions for

Table 2: Log_{10} (CFU/ml) analysis by two-factor ANOVA

SV	*SS*	*DF*	*MS*	*F-value*	*p-value*
Microorganisms	0.548	2	0.274	1.13	0.330
3 experimental groups	0.88	2	0.441	1591.5	0.212
3 microorganisms × 3 experimental treatment groups	1.76	4	0.441	1.591	0.188
5 treatment groups	147.61	4	36.803	151.5	0.000
Error	17.532	72	0.243		

SV: Source of variation; SS: Sum of squares; DF: Degree of freedom; MS: Mean square; ANOVA test value (F), ANOVA test significance (p)

Table 3: ANOVA and Tukey test results, mean Log CFU/ml for the three microorganisms in the five treatment groups

Groups	*S. mutans*	*S. aureus*	*C. albicans*
1 (negative control)	1.99^b	1.99^b	1.99^b
2 (positive control)	6.47^a	6.47^a	6.04^a
3 (70% isopropyl alcohol)	1.99^b	2.70^b	1.99^b
4 (2% GTA)	1.99^b	1.99^b	1.99^b
5 (0.25% PAA)	1.99^b	1.99^b	1.99^b
ANOVA: (p-value)	0.00	0.00	0.00

Different letters represent statistically significant differences (Tukey test); Mean < 2.0 (log_{10} of 100)—decontaminated pliers

the microorganisms tested, accepting the null hypothesis. Nevertheless, only GTA and the PAA prevented the growth of all the microbial strains after the disinfecting treatments. Wichelhaus et al and Orsi et al evaluated the effectiveness of GTA, and their results confirmed those of the present study, showing that this disinfectant eliminated the tested microorganisms.[2,9]

The reason for choosing PAA in the present research is that studies testing this disinfectant are scarce in the dental literature. Moreover, it is effective against all microorganisms (including spores), and it is a safe material for patients, professionals and the environment.[10] It is also nontoxic and nonallergenic at low concentrations, such as that used in the present study. The results obtained in the present study for PAA confirm its effectiveness as a disinfectant, corroborating the results of other studies.[6,11,12,14,19] Marques et al demonstrated the high efficiency of PAA, but their results showed that *S. aureus* adhered to glass and stainless steel were not completely removed.[19] It is important to highlight that the authors applied PAA for 30 seconds and not for 10 minutes that is recommended by the fabricant for disinfection procedure.[19]

Even though no statistically significant difference was observed between the three disinfectants tested, contamination by *S. aureus* was observed in two pliers after treatment with 70% isopropyl alcohol. This fact should be considered of clinical relevancy, since the existence of even a single bacterial colony may cause cross-infection. Reddy et al showed that the alcohol proved to be an inferior disinfectant when compared with glutaraldehyde, formaldehyde and leversept.[20]

Staphylococcus aureus was the most resistant microorganism of this experiment, corroborating the results obtained by Marques et al.[19] This microorganism, responsible for causing infection in hospitals, is one of the most resistant pathogenic bacteria for humans, and is capable of surviving during months on dry surfaces at temperatures over 60°C.

It should be noted that no viruses or spores were used in the present study, but only fungi and bacteria in the vegetative state. Therefore, the results obtained do not warranty that disinfection of orthodontic pliers using chemical substances between patient sessions is a safe procedure.

CONCLUSION

The results showed that there were no statistically significant differences between the disinfectants. The disinfectants 2% glutaraldehyde and 0.25% PAA were effective in inhibiting the growth of the three microorganisms tested while 70% isopropyl alcohol was unable to completely eliminate *S. aureus*. Since only fungi and bacteria were tested, the results obtained do not warranty that disinfection of orthodontic pliers using chemical substances between patient sessions is safe.

CLINICAL SIGNIFICANCE

The results suggested that 0.25% PAA was equivalent to 2% glutaraldehyde in disinfecting orthodontic pliers. While 0.25% PAA and 2% glutaraldehyde protocols completely eradicated the microorganisms tested, 70% isopropyl alcohol was unable to completely eliminate *S. aureus*.

ACKNOWLEDGMENTS

This work was supported by the Fundação de Amparo à Pesquisa e Desenvolvimento Científico do Maranhão (FAPEMA), Brazil. The authors Maria Reggiani Azevedo Carvalho, Marcos André Silva and Célia Regina Maio Pinzan-Vercelino are grateful to FAPEMA. The authors are also thankful for the support of the Microbiology Research Laboratory, Federal University of Piauí (Universidade Federal do Piauí, UFPI).

REFERENCES

1. Eklund KJ. Infection control. Dent Clin North Am 2003;47 (4):697-708.
2. Wichelhaus A, Bader R, Sander FG, Krieger D, Mertens T. Effective disinfection of orthodontic pliers. J Orofac Orthop 2006;67(5):316-336.
3. McCarthy GM, Mamandras AH, MacDonald JK. Infection control in the orthodontic office in Canadá. Am J Orthod Dentofacial Orthop 1997;112(3):275-281.
4. Woo J, Anderson R, Maguire B, Gerbert B. Compliance with infection control procedures among California orthodontists. Am J Orthod Dentofacial Orthop 1992;102(1):68-75.
5. Vendrell RJ, Hayden CL, Taloumis LJ. Effect of steam versus dry-heat sterilization on the wear of orthodontic ligature-cutting pliers. Am J Orthod Dentofacial Orthop 2002;121(5):467-471.
6. Montebugnoli L, Chersoni S, Prati C, Dolci G. A between-patient disinfection method control water line contamination and biofilm inside dental units. J Hosp Infect 2004;56(4): 297-304.
7. Vizcaino-Alcaide MJ, Herruzo-Cabrera R, Fernandez-Aceñero MJ. Comparison of the disinfectant efficacy of Perafase and 2% glutaraldehyde in in vitro tests. J Hosp Infect 2003;53(2):124-128.
8. Jeong EK, Bae JE, Kim IS. Inactivation of influenza A virus H1N1 by disinfection process. Am J Infect Control 2010; 38(5):354-360.
9. Orsi IA, Villabona CA, Kameoka E, Ferreira MC, Ito IY, Saravia ME. Antimicrobial efficacy of chemical disinfectants on contaminated full metal crowns. Braz Dent J 2010;21(3):241-246.
10. Kitis M. Disinfection of wastewater with peracetic acid: a review. Environ Int 2004;30(1):47-55.

11. Ceretta R, Paula MM, Angioletto E, Méier MM, Mitellstadt FG, Pich CT, Junior SA, Angioletto E. Evaluation of the effectiveness of peracetic acid in the sterilization of dental equipament. Indian J Med Microbiol 2008;26(2):117-122.
12. Chassot AL, Poisi MI, Samuel SM. In vivo and in vitro evaluation of the efficacy of a peracetic acid-based disinfectant for decontamination of acrylic resins. Braz Dent J 2006;17(2): 117-121.
13. Loukili NH, Becker H, Harno J, Bientz M, Meunier O. Effect of peracetic acid and aldehyde disinfectants on biofilm. J Hosp Infect 2004;58(2):151-154.
14. Kunigk L, Gomes DR, Forte F, Vidal KP, Gomes LF, Sousa PF. The influence of temperature on the decomposition kinetics of peracetic acid in solutions. Braz J Chem Eng 2001;18(2): 217-220.
15. Rauh J, Despang F, Baas J, Liebers C, Pruss A, Gelinsky M, Gunther KP, Stiehler M. Comparative biomechanical and microstructural analysis of native versus peracetic acid-ethanol treated cancellous bone graft. Biomed Res Int 2014;2014: 784702.
16. Yildirim-Bicer AZ, Peker I, Celik I. In vitro antifungal evaluation of seven different disinfectants on acrylic resins. Biomed Res Int 2014;2014:519098.
17. Pereira CA, Romeiro RL, Costa AC, Machado AK, Junqueira JC, Jorge AO. Susceptibility of Candida albicans, Staphylococcus aureus, and Streptococcus mutans biofilms to photodynamic inactivation: an in vitro study. Lasers Med Sci 2011;26(3):341-348.
18. Lynam PA, Babb JR, Fraise AP. Comparison of the mycobactericidal activity of 2% alkaline glutaraldehyde and 'Nucidex'(0,35% acid peracetic). J Hosp Infect 1995;30(3):237-240.
19. Marques SC, Rezende LG, Alves JAF, Silva BC, Alves E, Abreu LR, Piccoli RH. Formation of biofilm by Staphylococcus aureus on stainless steel and glass surfaces and its resistance to some selected chemical sanitizers. Braz J Microbiol 2007; 38(3):538-543.
20. Reddy RV, Tanveer K, Sharma KD, Kokkula N, Suresh PL, Sudhakar M. Evaluation of effectiveness of chemical disinfectants in reducing bacterial growth on orthodontic instruments. J Contemp Dent Pract 2013;14(6):1039-1043.