

Use of 0.25% and 0.025% peracetic acid as disinfectant agent for chemically activated acrylic resin: an *in vitro* study

Uso do ácido peracético a 0,25% e a 0,025% como agente desinfetante de resina acrílica ativada quimicamente: estudo *in vitro*

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ABSTRACT

Objective

This *in vitro* study evaluated the disinfection action of peracetic acid on chemically activated acrylic resin.

Methods

Sixty chemically activated acrylic resin specimens were contaminated with *Candida albicans* (30) and *Bacillus subtilis* (30) for 15 minutes. Next, specimens were divided into Control Group (Group 0) and Test Group (T) for each studied microorganism. The antimicrobial effect of Proxitane® Alfa (Thech Desinfecção, São Paulo, Brazil), containing 0.25% and 0.025% concentrations of peracetic acid was verified after 1, 3, 5 and 10 minutes of exposure. The specimens were transferred to saline solution for 5 minutes, homogenized and aliquots of 100µL were plated on BHI and Sabouraud Dextrose agar. After incubation at 37°C/24h, the number of CFU/mL recovered from each specimen was obtained.

Results

The 0.025% peracetic acid was effective against *B. subtilis* only after 10 minutes and against *C. albicans* after 3 minutes of exposure. At 0.25% concentration, the solution showed fungicidal and bactericidal efficacy after 1 minute of exposure.

Conclusion

The 0.25% peracetic acid was shown to be efficient for disinfection of chemically activated acrylic resins.

Indexing terms: Acrylic resin. Complete denture. Disinfection. Peracetic acid.

RESUMO

Objetivo

Avaliar *in vitro* a ação desinfetante do ácido peracético sobre resina acrílica quimicamente ativada.

Métodos

Sessenta corpos de prova em resina acrílica quimicamente ativada foram contaminados em suspensão de *Candida albicans* (n=30) e *Bacillus subtilis* (n=30) por 15 minutos. A seguir, os corpos de prova foram divididos em grupo Controle (Grupo 0), com 6 espécimes e Grupo teste composto por 24 espécimes para cada microrganismo estudado. Proxitane® Alfa (Thech Desinfecção, São Paulo, Brasil) ácido peracético foi testado nas concentrações de 0,25% e 0.025%, após 1, 3, 5 e 10 minutos de exposição. Após, cada corpo de prova foi transferido para solução fisiológica por 5 minutos, homogeneizados e alíquotas de 100 µL foram semeadas em duplicata, em BHI e Sabouraud Dextrose ágar. Após incubação a 37°C / 24 horas, determinou-se o número de UFC/ml recuperado de cada espécime.

Resultados

Na concentração de 0,025%, o ácido peracético mostrou efeito frente a *Bacillus subtilis* apenas após 10 minutos e para *Candida albicans*, após 3 minutos. Na concentração de 0,25%, a solução mostrou efeito fungicida e bactericida após apenas 1 minuto de exposição.

Conclusão

O ácido peracético a 0,25% demonstrou-se eficaz na desinfecção de resina acrílica quimicamente ativada.

Termos de indexação: Resinas acrílicas. Prótese total. Desinfecção. Ácido peracético.

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INTRODUCTION

The acrylic resins widely used in dental prosthetic devices are heat sensitive materials that cannot be sterilized by autoclaving or in ovens, and must be disinfected, or preferably sterilized by chemical or mechanical agents. According to Anusavice¹, acrylic resin has liquid sorption capacity and when it comes into contact with the oral cavity, it is capable of absorbing and adsorbing saliva and blood, thereby becoming a vehicle of cross contamination.

The disinfection of prosthetic devices is an important stage in preventing cross contamination among patients, dentists and laboratory technicians²⁻³ because a series of not sterilized materials and instruments are used in denture manufacture⁴⁻⁶.

Denture immersion in chemical products has been shown to be more efficient than mechanical brushing⁷⁻⁹. Thus, many studies have recommended efficient chemical solutions, such as glutaraldehyde, sodium hypochlorite and chlorhexidine⁹⁻¹². However, it is highlighted in the literature that the use of these solutions is not recommended for acrylic resins disinfection. Glutaraldehyde is not safe due to its toxicity³, and sodium hypochlorite is hardly effective as antimicrobial agent, and at residual concentration¹³, may affect the surface roughness of acrylic resin causing degradation of the product¹⁴. In contrast, chlorhexidine is considered an effective disinfectant agent, however it may cause staining¹⁴ and dimensional alteration of acrylic resin¹⁵.

The use of peracetic acid is being investigated as a feasible alternative of disinfectant solution. This product is of promoting to promote an effective disinfectant and sterilizing action without affecting the physical-chemical properties of acrylic resin¹⁸ or compromising the individual's health. For McDonell & Russell¹⁶, peracetic acid is considered a more powerful biocide than hydrogen peroxide, as it is sporicidal¹⁷, bactericidal, virucidal and fungicidal properties at low concentrations (<0.3%). Peracetic acid probably denatures proteins and enzymes and increases the cell wall permeability by rupturing the hydrated sulphate (-SH) and sulphur (S-S) bond. Thus, peracetic acid is effective against a wide variety of microorganisms¹⁸, oxidizing vital components for the survival of viruses, bacteria, fungi and spores.

It is necessary to provide a clean and cross contamination-free⁹⁻¹⁰ denture to the patient which does not compromise the individual's health. Due to the importance of this subject, this research was developed to

verify *in vitro* the efficacy of the use of peracetic acid as a disinfectant agent for chemically activated acrylic resin, in different time intervals and concentrations.

METHODS

To conduct this research, 60 test specimens were manufactured of chemically activated acrylic resin powder and liquid (Jet® Artigos Odontológicos Clássico Ltda., São Paulo, Brazil), which are replicas of an old mandibular complete denture¹⁹ made with the use of a laboratory silicone matrix (Labormass - Ruthbras®, São Paulo, Brazil) and pressed in a muffle (Vipi-STG®, São Paulo, Brazil). The purpose of the technical procedure performed for each test specimen was to simulate the conditions normally used for polishing a mandibular complete denture. All the parts were polished with fresh pumice stone and water solution. The tests specimens were not decontaminated with any disinfectant solution since the purpose was to evaluate the efficacy of peracetic acid in microbial reduction. For this aim, the test specimens were contaminated with a suspension of *Candida albicans* and *Bacillus subtilis* at the concentration of 15X10⁸ microorganisms/ml (Factor No. 5 Mc Farland's Nephelometric Scale, Nefelobac®, São Paulo, Brazil) in 100 ml of sterilized physiological solution. The investigation was conducted to observe the behavior of the disinfectant against a fungus commonly found as contaminant on acrylic resin, as well as a spore-producing microorganism, due to the high resistance of spores to chemical agents.

Test specimen manufacture

Four measures of extra hard laboratory silicone (Labormass - Ruthbras®, São Paulo, Brazil) was mixed with catalyzer to model the internal part of the denture, covering around 2 mm of the outer edge. After the silicone was cured, the edges were trimmed with a stiletto and "V" shaped depressions were made in the lateral platform. After this, the silicone that would come into contact with the second part to be molded was smeared with vaseline. Afterwards, the external part of the denture was molded, so that the silicone would penetrate into the "V" shaped depressions.

The first molded part (which corresponds to the internal part of the denture) was included in the base of the muffle with 120g of common plaster (Asfer®, São Paulo, Brazil). After crystallization, the plaster was isolated with a separating liquid. Next, the second molded part (which

corresponds to the external part of the denture) was fitted into the first part that had already been included. The base was fitted into the counter-muffle and the 4 screws were fastened. The plaster was prepared using 180g of common plaster (Asfer®, São Paulo, Brazil) and poured in through the counter-muffle orifice. In the beginning, circular movements were made with the muffle so that the plaster would penetrate into all the crevices. Then, with the muffle supported on the bench, it was completely filled with plaster. After plaster crystallization, the 4 screws were removed, the base separated from the counter-muffle, and the plaster was isolated with separating liquid.

Chemically activated acrylic resin (Jet® Artigos Odontológicos Clássico Ltda., São Paulo, Brazil) was mixed in accordance with the manufacturer's instructions. For each test specimen, 18 parts of powder (14g) to 7.2 (plastic cup for measuring monomer) of liquid (10 ml) of acrylic resin were measured. The parts of the muffle that would come into contact with the resin were previously smeared with vaseline. After preparing the mixture, when the resin reached the sticky stage, it was pressed into the muffle. Then the muffle was closed, the screws were fastened, and when the resin reached the plastic stage, it was pressed at 1000 kgF/F (Vipi Delta hydraulic press, Delta®, São Paulo, Brazil). After completed the polymerization time (30 minutes), the 4 screws were removed, the muffle was opened and the clone removed. The excesses were removed and the test specimen was polished and finished. The same procedure was used to make the other test specimens. After manufacture, the test specimens were polished (Polidor VH® Equipamentos, Araraquara, Brazil).

Microbiological analysis

For this study, strains of *Candida albicans* ATCC 10231 (Ca) and *Bacillus subtilis* ATCC 19659 (Bs), both obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) were used.

Of the total of 60 test specimens manufactured, 30 were immersed in 100 ml of *Candida albicans* (Ca) suspension and 30 in *Bacillus subtilis* (Bs) suspension, at the concentration of 15×10^8 cells/ml for 5 minutes. Six test specimens contaminated with each microorganism were used as positive control, and were transferred to physiological solution. The remaining 48 specimens were immersed in 0.25% and 0.025% Proxitane® Alfa Sterilization solutions (Thech Desinfecção®, São Paulo, Brazil) for time intervals of 1, 3, 5 and 10 minutes. After the immersion time, each test specimen was submerged in sterile saline solution for 5 minutes and homogenized. Aliquots of 100 µL of controls and tests were plated on agar BHI (Brain Heart Infusion) for B.

subtilis and on agar Sabouraud for *C. albicans* and incubated at 37°C for 24 hours. The colony forming units (CFU/ml) were determined and the percentage of microorganism inhibition by peracetic acid was determined. The experiments were carried out in a blind fashion in duplicate.

Statistical analysis was performed using ANOVA followed by the Tukey test using the SAS 8.2 statistical program (SAS Institute, Cary, NC, USA). The level of significance was 5%.

RESULTS

The results demonstrated that on 0.025% peracetic acid concentration, growth of *C. albicans* until 1 minute as well as *B. subtilis* until 5 minutes of disinfectant exposure was observed. After these periods, no microbial growth was observed (Table 1). In contrast, no growth was observed in the specimens contaminated with strains of *C. albicans* and *B. subtilis* by immersion in 0.25% peracetic acid in all the studied time intervals.

ANOVA statistical analysis followed by the Tukey test showed no statistically significant difference between the studied groups ($p > 0.05\%$).

Table 2 summarizes the average percentage of the obtained results. 100% of both studied microorganisms were eliminated when exposed in 0,25% peracetic acid. On the other hand, at 0,025% peracetic acid, 91.5% of the *C. albicans* strains and 68,65% of *B. subtilis* strains were eliminated.

Table 1. Colony forming unit (CFU) counts in samples obtained in duplicate, according to the groups contaminated with strains of *Candida albicans* and *Bacillus subtilis* immersed in 0.025% Proxitane® Alfa (Thech Desinfecção, São Paulo, Brazil) at the time interval from 1 to 10 minutes.

Strains	Test specimen	Control group		Contaminated groups Immersion time (minutes)							
				1		3		5		10	
<i>C. albicans</i> (CFU)	1	U	U	0	0	0	0	0	0	0	0
	2	U	U	48	65	0	0	0	0	0	0
	3	U	U	0	0	0	0	0	0	0	0
	4	U	U	0	0	0	0	0	0	0	0
<i>B. subtilis</i> (CFU)	5	U	U	85	86	0	0	0	0	0	0
	6	U	U	0	0	0	0	0	0	0	0
	1	U	U	210	179	96	90	17	0	0	0
	2	U	U	85	90	0	0	0	0	0	0
	3	U	U	79	64	0	0	0	0	0	0
	4	U	U	109	103	0	0	0	0	0	0
	5	U	U	201	201	0	0	0	0	0	0
	6	U	U	453	264	0	0	0	0	0	0

U = uncountable

Table 2. Percentage of *C. albicans* and *B. subtilis* colonies elimination on 0.25% and 0,025% peracetic acid (Proxitane® Alfa, Thech Desinfecção, São Paulo, Brazil) at different time of exposure.

Type of microorganism	Proxitane® Alfa at 0.25% (Thech Desinfecção, São Paulo, Brazil)				Proxitane®Alfa at 0.025% (Thech Desinfecção, São Paulo, Brazil)					
	Time of contact				Time of contact					
	1 min	3 min	5 min	10min	1 min	3 min	5 min	10 min	Média	
C. albicans	100%	100%	100%	100%	66%	100%	100%	100%	91,5%	
B. subtilis	100%	100%	100%	100%	0%	83%	91.6%	100%	68,65%	

DISCUSSION

The development of disinfectant chemical solutions that are capable of maintaining dentures free of plaque with a daily immersion of 15 or 30 minutes, and that do not affect the color or surface of acrylic resin dentures is recommended. In this respect, Thamlikitkul et al.¹⁸ showed that the use of 0.2% peracetic acid for disinfection did not significantly alter the sorption, solubility and microhardness properties of heat polymerized and chemically activated acrylic resins. Based on this, the present *in vitro* study aimed to evaluate the microbial effect of peracetic acid in two concentrations, after different time intervals.

The present results demonstrated the efficacy of disinfection, as well as, the fungicidal and sporicidal effect of 0.25% peracetic acid after 1 minute of exposure to the product. These findings are also in accordance with Baldry²⁰, which showed the rapid activity of peracetic acid against sporulated bacteria and yeasts after only 1 minute. In one of the first studies published in the literature about peracetic acid, Greenspan & MacKellar²¹ indicated the high bactericidal and fungicidal activity after application of peracetic acid for the effective washing of fruit and vegetables. In contrast, some studies have reported that the fungicidal and sporicidal effects of peracetic acid at the concentration of 0.2% demands at least 5 minutes of exposure to the product^{21,25-26}.

In respect to the effect of diluted peracetic acid, Penna²⁴ affirmed that peracetic acid is bactericidal, fungicidal, virucidal, micobactericidal and sporicidal even at low concentrations (0.001% to 0.2%). The 0.025% concentration of peracetic acid used in the present study is included in this range of sporicidal effect at low concentration tested and proved. Leaper¹⁷ obtained a sporicidal effect at 0.04% at 40°C, and Sagripanti & Bonifacino²⁵ at 0.03%, at 20°C as for 30 minutes. Nevertheless, our *in vitro* results are in disagreement with the records of Greenspan & MacKellar²¹ who demonstrated that the sporicidal effect of peracetic acid was obtained only at the concentration of 0.3%.

In the tests with Proxitane® Alfa (Thech Desinfecção, São Paulo, Brazil) diluted to 0.025%, with regard to time of exposure, it was also possible to observe that peracetic acid demonstrated a fungicidal effect as from 3 minutes and sporicidal effect as from 10 minutes. Its sporicidal effect was equal to the time of exposure in the tests of

Svidzinski et al.²⁶ who also proved that paracetic acid had a sporicidal effect as from the time of 10 minutes, although it had been tested at another concentration: diluted to 0.1%. However, it is not possible to compare with the fungicidal and sporicidal effects obtained by Greenspan & MacKellar²¹ since they observed these effects after 24 hours of exposure to the product. This also occurred with Sagripanti & Bonifacino²⁵ who observed the sporicidal effect of 0.03% peracetic acid only as from a time of contact of 30 minutes.

As regards the toxicity of paracetic acid, the products of its decomposition are considered safe and harmless (acetic acid and oxygen) that decomposed into non toxic products (oxygen and water)²¹. Peracetic acid remains active even in the presence of peroxidases and organic matter²⁴; it is non allergenic and it is considered a slight irritant²². Nevertheless, diluted acids such as acetic acid are corrosive¹⁴. Due to this disadvantage, and to the excellent fungicidal and sporicidal performance in this study of 0.25% peracetic acid, the following dilution of 0,025% of the product was tested in order to reduce its toxicity. The long-term action of 0.2% peracetic acid was evaluated by Muller et al.²² on the dorsal skin, oral and vaginal mucosa of rabbits after 1 year. The histological exams showed no inflammation, no scar formation, and no risk of dysplasias that could detect the carcinogenic action of peracetic acid. Nevertheless, the authors suggested that the permanent use of peracetic acid in the disinfection of hands might possibly lead to the risk of depilation, and it needs to be tested dermatologically. Therefore, dermatological studies are also necessary to assess and compare the risk of toxicity of 0.25% and 0.025% peracetic acid.

In summary, the immersion of chemically activated acrylic resin dentures in 0.25% peracetic acid for 1 minute and in 0.025% for 10 minutes is recommended as well as effective to promote decontamination in order to deliver them in a clean condition to the patient.

CONCLUSION

The *in vitro* obtained results demonstrated the efficacy of disinfection of chemically activated acrylic resin by immersion in peracetic acid, mainly on 0.25% concentration.

Collaborators

L REIS was responsible for setting up the research project, literature study, performing the methodology and writing the article. AL ZANETTI guided the research and participated in writing the article. OV CASTRO JUNIOR was the originator of the research, responsible for the

first part of the methodology in the field of Prosthesis, supervising the practice developed, interpreting the results and preparing the discussion and conclusion, and writing the article. EF MARTINEZ was responsible for the second part of the methodology in the field of Microbiology, supervising the practice developed, interpreting the results and preparing the discussion and conclusion, and writing the article.

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