Microbiological evaluation of peracetic acid for disinfection of acrylic resins

Avaliação microbiológica de ácido peracético na desinfecção de resinas acrílicas

Abstract

Purpose: The aim of this study was to assess the efficacy of peracetic acid (PAA) for the disinfection of dental acrylic resins experimentally contaminated with *Candida albicans, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Methods: Fifteen materials were used for each type of resin (thermosetting, self-curing and microwave-curing). Each material was placed in a test tube containing culture medium with a suspension of each microorganism and then incubated. The materials were rinsed and transferred to other tubes containing 50 mL of water for 5 min, 0.2% peracetic acid for 5 min or glutaraldehyde for 30 min. The materials were placed in the culture agar and incubated. Microbial growth was determined by colony counting after plating.

Results: *Candida albicans* growth was inhibited by peracetic acid and glutaraldehyde treatments. The number of colonies on resins treated with saline was greater than 10^5 CFU/mL. In resins infected with *E. coli, S. aureus* and *P. aeruginosa* the colony growth was not inhibited by saline and peracetic acid, but it was totally inhibited by glutaraldehyde.

Conclusion: Surface disinfection using peracetic acid effectively inhibited *C. albicans* growth on all acrylic resins.

Key words: Peracetic acid; acrylic resins; *Candida albicans*

Resumo

Objetivo: O objetivo deste estudo foi avaliar a eficácia do ácido peracético (PAA) na desinfecção de resinas acrílicas dentais experimentalmente contaminadas com *Candida albicans, Escherichia coli, Staphylococcus aureus* e *Pseudomonas aeruginosa*.

Metodologia: Quinze corpos de prova (CP) foram utilizados para cada tipo de resina (termopolimerizáveis, autopolimerizáveis e ativados por energia de micro-ondas). Cada CP foi colocado em um tubo teste com meio de cultura contendo uma suspensão de cada microrganismo e incubado. Posteriormente, os CP foram lavados e transferidos para outros tubos contendo 50 mL de água por 5 minutos, em 0,2% de ácido peracético por 5 min ou em glutaraldeído por 30 minutos, plaqueados em ágar de cultura e incubados. O crescimento microbiano foi determinado por contagem de colônias após o plaqueamento.

Resultado: O crescimento de *Candida albicans* foi inibido nos tratamentos com ácido peracético e glutaraldeído. O número de colônias nas resinas tratadas com solução salina foi superior a 10^5 UFC/mL. Nas resinas infectadas com *E. coli, S. aureus* e *P. aeruginosa*, o crescimento das colônias não foi inibido nas resinas tratadas com salina e ácido peracético, mas foi totalmente inibida pelo glutaraldeído.

Conclusão: A desinfecção com ácido peracético inibiu efetivamente o crescimento de *C. albicans* em todas as resinas acrílicas.

Palavras-chave: Ácido peracético; resinas acrílicas; *Candida albicans*

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Introduction

Peracetic acid (PAA) is a chemical agent that originates from the mixture of hydrogen peroxide and acetic acid. The decomposition of a solution containing PAA has water, acetic acid and oxygen as by-products, all of which are biodegradable and atoxic. The PAA solution can be used as high-level disinfectant, and its action is similar to that of hydrogen peroxide, i.e., it promotes protein denaturation, impairment of cell wall permeability, oxidation of sulphydryl groups and of sulphur bonds in proteins, enzymes and other metabolites (1).

PAA is characterised by a very rapid action and broad-spectrum antimicrobial activity, which inactivates Gram-positive and Gram-negative bacteria, as well as fungi (2). PAA has been used since 1955 as a disinfectant or sterilant, mainly in the food industry and in suture threads. It has also been employed for the disinfection of plastic insulators and medical and dental equipment (3). Acrylic resins are polymers (plastics) used for the manufacture of prostheses, dental braces and intraocclusal devices. Acrylic resins are thermosensitive materials, and as they cannot be submitted to high-temperature disinfection techniques, the use of chemical disinfectants is necessary (4).

Acrylic resin items are considered semi-critical articles because they get in contact with the patient's healthy mucosa and must be submitted to high-level disinfection or sterilisation. The 1% sodium hypochlorite and 2% glutaraldehyde have been widely recommended for disinfection of acrylic resin items; however, these chemical agents are not ideal: 1% sodium hypochlorite is a bleaching agent and may interfere with the aesthetics of the prostheses whereas 2% glutaraldehyde releases toxic vapours, irritants and allergens, which cause eye, nose, and throat irritation, allergy, contact dermatitis, asthma and rhinitis (1,5-6).

The use of devices made of acrylic resins favours the growth of microorganisms, and is associated with diseases such as angular cheilitis, median rhomboid glossitis and denture-induced stomatitis. This infection has a multifactorial aetiology and can be caused by denture trauma, poor oral health, poor denture hygiene, continuous denture wearing, *Candida albicans* infection, and hypersensitiveness to denture materials. Thus, every intraoral device should be disinfected before placement in the patient's mouth. As PAA is biodegradable and does not produce toxic compounds, it seems to be ideal for the disinfection of acrylic resins (7).

Candida albicans is a commensal yeast that colonises the oral cavity and that is found in 30 to 70% of apparently healthy individuals. *C. albicans* is most commonly found in the oral cavity, either as a commensal organism or as a pathogen (8). *C. albicans* can adhere to and colonise the surface of acrylic resin in prostheses and develop biofilm, both in healthy patients and in those with pathological findings. Therefore, the prosthesis acts as a reservoir for fungi, re-infecting the oral mucosa after antifungal treatment in denture wearers with denture stomatitis (9).

The aim of this study was to assess the efficacy of peracetic acid for disinfection of dental acrylic resins experimentally contaminated with *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Methods

The study was approved by the Research Ethics Committee of the School of Dentistry, affiliated with the Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil.

Sampling

The materials consisted of three types of colourless acrylic resins – thermosetting (VIPI CRIL®), microwave-curing (VIPI WAVE®) and self-curing (VIPI FLASH®), all from Dental VIPI Ltda, São Paulo, Brazil. Fifteen materials measuring 30.0 x 10.0 x 3.2 mm (±0.05 mm) were prepared for each type of resin. The resins were proportioned, handled and polymerised according to the manufacturer's instructions. The materials were polished with 400-600 grit wet sandpaper and mechanically polished with pumice stone and calcium carbonate. We chose colourless resins because the visual inspection of their surface is easier.

Microorganisms

The strains used in the experiment were *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 11/05, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 15422. The inocula were prepared in saline solution and were standardized in a spectrophotometer at 530 nm, with absorbance of 0.08-0.1, and later diluted until obtaining 10^4 CFU/mL.

Microbiological test

For contamination, the materials were placed in a test tube containing 10 mL of brain heart infusion (BHI) broth (Oxoid, Cambridge, UK) with a final concentration of 10^3 CFU/mL of each microorganism, and then incubated at 37°C for 24 h. Negative control was obtained by the immersion of the materials in uncontaminated broth. Prior to the treatment, microbial growth was confirmed by the turbidity of the growth medium. All materials were removed from the broth using a pair of sterile tweezers and immersed in sterile 0.89% saline solution for 5 min. The microbiological test was carried out in three groups, with five materials for each type of resin: control group, with immersion of materials in 50 mL of deionised water for 5 min, PAA group, with immersion of materials in 50 mL of deionised water plus 0.2% PAA for 5 min, and positive control, with immersion of materials in 50 mL of deionised water plus glutaraldehyde (Glutaron II, Indústria Farmacêutica Rioquímica Ltda, São José do Rio Preto, SP, Brazil) at the concentration of 2% for 30 min. After that, the resins were washed in 0.89% saline solution for 5 min for removal of disinfectants, immersed in 10 mL of brain heart infusion (BHI) broth, and incubated at 37°C for 24 h. Thereafter, the culture media were plated

in BHI agar, incubated at 37°C for 24 h, and the number of colonies was counted.

Results

The microbiological test showed that contamination by *C. albicans*, *E. coli*, *S. aureus* and *P. aeruginosa* occurred in all materials, regardless of the type of acrylic resin.

The microbiological test revealed that all materials were contaminated by the microorganisms because the culture medium was turbid after incubation. Growth after treatment with disinfectants was confirmed by the presence of colonies on the culture medium.

Candida growth was inhibited by peracetic acid and glutaraldehyde treatments. The number of colonies on resins treated with saline was greater than 10^5 CFU/mL.

In resins infected with *E. coli*, *S. aureus* and *P. aeruginosa* there was colony growth in those resins treated with saline and peracetic acid (count greater than 10^5 CFU/mL), but their growth was totally inhibited by glutaraldehyde.

Discussion

The results of this study demonstrate that immersion in 0.2% PAA for 5 min was effective for the disinfection of the three types of acrylic resins contaminated with *C. albicans,* thus corroborating the results of another study, which confirmed the efficacy of immersion in 0.2% PAA for 5 min for the sterilisation of the same types of resins, contaminated with *Bacillus subtilis* and *Bacillus stearothermophilus* (10). However, the same treatment was not efficient for the inhibition of *E. coli*, *S. aureus* and *P. aeruginosa* cells.

In order to prevent the cross-contamination of laboratory technicians and dental surgeons, 1% sodium hypochlorite and 2% glutaraldehyde are recommended for the disinfection of dental impression materials and dentures before any handling is done (11). However, glutaraldehyde has toxic effects on the skin and mucous membranes (12), whereas regular use of sodium hypochlorite causes bleaching of the denture material overtime, so its use is often disapproved by the patients (2). Therefore, PAA, which was effective for the disinfection of endoscopes (13), may be a suitable alternative substance to disinfect acrylic resins for dental treatment purposes.

Some limitations of the present study are intrinsically related to the type of laboratorial design, which do not reproduce all the complex conditions found in the oral cavity of denture patients with or without denture stomatitis. Therefore, the direct extrapolation of results for the dental clinics is not possible, and the present findings should be considered as an *in vitro* testing of the efficacy of disinfection substances against specific microorganisms.

Conclusions

In summary, the immersion in 0.2% PAA for 5 min inhibits the growth of *C. albicans* on the surface of all materials of acrylic resins analysed. Nevertheless, it is necessary to assess the influence of this method of disinfection on the physical and mechanical properties of these resins before establishing a disinfection protocol in the daily clinics.

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