Journal of Advanced Zoology

ISSN: 0253-7214

Volume ***44*** *Issue* ***S-1 Year 2023*** *Page* ***37:43***

An in Vitro Comparative Evaluation of the Effect of Three Disinfectants and Three Time Intervals in Controlling the Growth of *Candida Albicans* on Heat Polymerized Acrylic Resin

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Article History

Received: 07 May2023
Revised: 18 August 2023
Accepted:21August 2023

Abstract

Various disinfection modalities are available for dental materials; however, acrylic resins are heat sensitive materials and so cannot be autoclaved. Chemical disinfection has immerged as a safe alternative that is least likely to cause severely detrimental changes to the physical properties of the acrylic resin and be simultaneously potent enough to control the growth of Candida albicans which is the most common cause of denture stomatitis and other inflammatory conditions of the oral mucosa following denture us. Recent research has suggested use of Peracetic Acid on acrylic resins, however the potential of the acid at low concentrations in comparison with commonly available commercial chlorhexidine has not been examined at time intervals that simulate clinical, laboratory and household constraints. Therefore, this study was designed to evaluate the effect of 01% and 0.25% peracetic acid in comparison with 2% chlorhexidine at 5-minute, 10 minute and 8-hour immersion in the chemical solutions of acrylic resin specimens contaminated with Candida Albicans. The percentage elimination of C. albicans with 0.1% peracetic acid and 0.25% peracetic acid was 100% after immersion in disinfectant at every time point. While, the percentage elimination of C. albicans with ICPA Hexidine mouthwash was 60%, 70% and 100% after immersion in disinfectant for 5miutes, 10minutes and 8 hours respectively. The difference in reduction of Candida Albicans as compared to control group was significant ($p<0.05$) in ICPA Hexidine mouthwash, 0.1% peracetic acid and 0.25% peracetic acid.Acrylic resin samples subjected to 0.1% and 0.25% peracetic acid depicted higher levels of disinfection in comparison with ICPA Hexidine (2% chlorhexidine) following all three intervals of time. It can therefore be concluded that peracetic acid even at low concentrations is capable of controlling the proliferation of Candida albicans even at short immersion time intervals.

Keywords: *Peracetic Acid, Disinfection, PMMA*

1. Introduction

It is in the nature of every dental surgeon to provide the best care for every patient. A part and parcel of effective treatment planning is training the patient in maintaining the restorative treatment provided, not only for the maintenance of their own health but also limit the possibility of disease

transmission. This leads to a need for training the patient in keeping up with hygiene care of restorations and prostheses like complete and partial removable dentures. These prostheses are usually, fabricated with heat polymerized acrylic resin due to its cost effectiveness and ease of availability. Even though these are biocompatible dental materials that bode well in the oral cavity, a lack of cleaning effort on behalf of the patient or inaccurate fit of the dentures, among other predisposing factors, may result in a plethora of undesired microbial proliferation. Among all the microorganisms that may invade the oral mucosa or form biofilms on the prostheses themselves (Monteiro et al., 2014) *Candida albicans* is the most commonly isolated strain of opportunistic pathogenic yeast. It causes a wide array of symptomatic manifestations like denture stomatitis and oral candidiasis. The acrylic resin prostheses provide a surface for the formation and propagation of the *Candidal* hyphae. The oral environment; particularly in the nutrition deficient geriatric demographic, or among those that maybe immune-compromised; boosts the progression of disease, if left unchecked.

In addition, when a patient walks into a dentist's practice for any kind of treatment, the various procedures involved not only expose the patient to various contaminants but also render the dental practitioner and eventually the laboratory staff, susceptible to opportunistic cross-infections. These contaminants/microorganisms found flourishing on the surface of prostheses and inside the oral cavity of an affected patient are then transmitted, to the clinical and laboratory equipment and personnel. There may further be inter-transmission between successive patients if stipulated disinfection procedures are not followed.

Dental prostheses are considered semi-critical articles (Chassot et al., 2006). Thus, a need exists to establish a definitive protocol for the disinfection of prosthetic materials, when handled by the patient or by a dental professional (Salvia et al., 2013). Even though sterilization maybe ideal for preventing cross contamination, it may not be ideal for every instrument or material used in the clinical or laboratory setting particularly those that may respond detrimentally towards heat or radiation. The deterioration of heat cured acrylic resins when subjected to microwave radiation or sterilization by steam autoclaving has been established by various authors (Keller & Lautenschalger, 1985).

Patients are educated to use abrasives of different sorts and even brush their dentures daily. Even though mechanical cleaning of acrylic resin denture bases may remove physical debris and superficial staining, it produces an increased surface roughness. It maybe advocated in the absence of any other means of disinfection, but it also provides the microorganism a wider surface area to proliferate (Salvia et al., 2013). Chemical disinfection by immersion into solutions may not be considered as effective as autoclave or microwave sterilization, it is still potent enough to contain the growth and further spread of microorganisms. Different solutions at various concentrations are available along with research backed evidence which can be used to disinfect several dental materials. However, the physical properties of the prosthetic materials maybe negatively affected when they are subjected to the chemicals. There could be an increase in surface roughness, change in color stability and various other unrequited dimensional changes. Even the residual remnants of the disinfection solution may not be biocompatible, ecofriendly or easily disposable. There are several options present like formaldehyde, glutaraldehyde, sodium hypochlorite, chlorhexidine and even common household chemicals like vinegar etc. The aldehyde group of disinfectants; though strong, are highly toxic. They cause irritation to the oral and facial tissues (Chassot et al., 2006). Hydrogen peroxide and chlorhexidine do show mild anti-fungal effects against *Candida*. The bleaching effect of sodium hypochlorite when used for longer durations is unfavorable. Sterilization of denture base acrylic resins via microwave radiation has been considered but there occur irreversible changes in physical properties of the resins (Keller & Lautenschalger, 1985; Ekren & Ozkomur, 2016).

The main aim, therefore, is to achieve disinfection of the acrylic resins at minimal concentrations of the chemicals with immersion for a minimal duration of time. Recent research suggests the use of Peracetic Acid viz. widely used in hospitals (endoscopes, UV lenses) as well as food industry (Ekren & Ozkomur, 2016; Ceretta et al., 2008). It is a safe, nontoxic disinfectant used in wine making to disinfect tanks and drains. Produced using tetraacetylethylenediamine and alkaline hydrogen peroxide, it is a potent antibacterial, antifungal and antiprotozoal agent, at low concentrations. The remnants are acetic acid and oxygen after 24 hours and these can be drained via sewage, thus

requiring no superfluous biosafety precaution for disposal (Chassot et al., 2006; Ekren & Ozkomur, 2016; Reis et al., 2012).

Besides being non-toxic it is also considered non-allergenic. This is of utmost importance since acrylic resins have absorptive ability where liquids are concerned, and any disinfectant maybe absorbed into the resin surface. The worrisome consequence would be the release of any harmful residues into the oral cavity (Chassot et al., 2006). US Food and Drug Administration (FDA), Centers for Disease Control and Prevention (CDC) and the Association for Professionals in Infection Control and Epidemiology (APIC) are all agencies that recommend the use of Peracetic acid instead of glutaraldehyde (Ekren & Ozkomur, 2016).

The need for a disinfectant that shows maximum disinfection at very low concentrations and requires immersion of the material for minimum time is significant in the time restrained clinical and laboratory routine. In addition, the disinfectant solution should also be non-aggressive towards the material if the immersion duration is longer as is the situation wherein geriatric patients soak their dentures overnight (8 hours). Any dimensional changes or changes in esthetic or biological properties of the heat polymerized polymethymethacrylate resins would nullify the objective of a biosafe disinfection procedure.

Thus, the use of Peracetic acid is being investigated as a feasible alternative to promote an effective disinfectant and sterilizing action without affecting the physical-chemical properties of acrylic resin or compromising the individual's health. 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. showed that the use of 0.2% Peracetic acid for disinfection does not significantly alter the sorption, solubility and microhardness properties of heat polymerized and chemically activated acrylic resins.

The efficacy of 0.2% Peracetic acid for the purpose of disinfection at 5, 10 and 30 minute durations has been assessed for the control of *Candida albicans* on acrylic resins. It has proved to be effective. However, the actual level of potency of Peracetic acid in comparison with commercially available mouthwashes such as Chlorhexidine, has not been researched. Chlorhexidine has good biocompatibility and low cytotoxicity (Hashizume et al., 2015). It is easily available for the patient at the local pharmacy as well. Counting the number of colonies forming units of *Candida albicans,* that remain following the immersion disinfection, gives us sound evidence of the efficacy of each chemical solution at their respective concentrations i.e., 0.25% and 0.1% Peracetic acid and ICPA chlorhexidine mouthwash. These were also compared with a control which is distilled water. Due to the fact that even diluted acids can be corrosive (Reis et al., 2012), concentrations as low as 0.1% of Peracetic need to be evaluated for disinfection ability to eliminate the possibility of damage to resins or oral tissues. The time intervals i.e., 5 minutes, 10 minutes and 8 hours were chosen to simulate both clinical/laboratory and at home disinfection durations.

There would be no difference in the efficacy of 0.1% Peracetic acid, 0.25% Peracetic acid and 2% Chlorhexidine in controlling the growth of *Candida albicans* on heat cured polymethymethacrylate.

2. Materials And Methods

One hundred and fifty denture base acrylic resin specimens with the same dimensions (10×10×2 mm) were obtained by means of wax patterns. Finishing and polishing was done using fine grit sandpaper and wet rag wheel with pumice slurry. The surface roughness of a material used for a removable prosthesis is relevant to adhesion of *Candida albicans*.[4] In order to eliminate a bias based on roughness of each acrylic sample, the strips were analyzed to measure surface roughness using Profilometer (SINSIL International). Mean roughness value for all samples after polishing, were kept at 2.5±0.253 µm (Keller & Leutenschalger, 1985).

The specimens were sterilized in Ultraviolet light (wavelength: 250nm) chamber for 5 mins. on both sides using a sterile forceps and placed into a presterilized ziplock bag. Two to three colonies of *Candida albicans* grown on blood agar were taken and inoculated onto Sabouraud dextrose broth for 24 hours and the density of the broth was matched to 1 McFarland unit (10^6 CFU/ml). 50 ml of each

solution was used i.e Peracetic acid (0.1% and 0.25%), 2% ICPA Hexidine mouthwash and water as control.

The sterile acrylic resin specimens of various groups were immersed, with labeled surfaces facing down, in Petri dishes containing 20 ml of Sabouraud dextrose broth containing *Candida albicans*. These were incubated for 48 hours at 37° C in incubator. Then the inoculated specimens were washed under tap water to simulate the patients' routine denture cleaning procedure.

The specimens were then immersed in Petri dishes containing 50 ml of the denture cleansers and control agent. They were stored for 5mins, 10 mins and 8 hours (to mimic the overnight soaking of dentures in cleansers by the patient) at room temperature. After this the specimens were washed under running tap water, swabbed, fixed with methanol, stained with crystal violet, dried and examined under the microscope. *Candida albicans* cells adherent to acrylic resin specimens were counted under the microscope (×40). The entire surface of the slide was examined and counted. Each field was counted and totaled. The number of cells adherent to the test samples were compared to those adherents to the control.

3. Results and Discussion

In all the groups the growth (CFU) of *Candida albicans* was compared before immersing the acrylic blocks into the disinfectants and the growth was comparable in all the groups (p>0/05), establishing baseline comparability in all the groups. (Table 1)

Table 1: Baseline CFU count in all the groups

Disinfectant	Baseline CFU Count										
	Test Specimens										Mean
	1	2	3	4	5	6	7	8	9	10	
Control	225	230	254	243	256	212	232	226	200	253	233
0.1% Peracetic Acid	222	227	251	240	253	209	229	223	197	198	225
0.25% Peracetic Acid	230	235	259	248	261	217	237	231	205	206	233
ICPA Hexidine mouthwash	218	223	247	236	249	205	225	219	193	194	221

In the control group the CFU count kept on increasing as the time elapsed. In the IPCA Hexidine mouthwash arm the CFU count gradually decreased as the immersion time increased. In Peracetic acid arm across both the strengths the CFU count was zero even after immersion time of 5 minutes. Mean change in CFU count at different time points across all the groups is reflected in Table 2.

Table 2: Mean change in CFU count at different time points across all the groups

Disinfectant	CFU count (Mean ± SD)			
	Baseline	5 minutes after immersion in disinfectants	10 minutes after immersion in disinfectants	8 hours after immersion in disinfectants
Control	233 ± 18.63	266.1 ± 18.63	U	U
0.1% Peracetic Acid	225 ± 19.68	0±0	0±0	0±0
0.25% Peracetic Acid	233 ± 19.68	0±0	0±0	0±0
ICPA Hexidine mouthwash	221 ± 19.68	57.89 ± 69.42	18.40 ± 30.52	0±0

There was statistically significant difference seen between elimination effects of difference disinfectants. Both strengths of Peracetic acid, 0.1% and 0.25%, showed statistically significant elimination as compared to control at all the time points. As compared to ICPA Hexidine mouthwash, both strengths of Peracetic acid, 0.1% and 0.25%, showed statistically significant elimination after 5 and 10 minutes of elimination, however after 8 hours of immersion, elimination was comparable between ICPA Hexidine mouthwash and Peracetic acid 0.1% and 0.25%. (Table 3)

Table 3: Statistical comparisons between mean elimination observed in different arms

Timepoints	p-value (paired t-test)	Significance

		0.1% Peracetic Acid	0.25% Peracetic Acid	
Control	5 minutes	0.0001	0.0001	Significant
	10 minutes	0.0001	0.0001	Significant
	8 hours	0.0001	0.0001	Significant
ICPA Hexidine mouthwash	5 minutes	0.0034	0.0034	Significant
	10 minutes	0.0112	0.0112	Significant
	8 hours	0.8324	0.8324	Not significant

The percentage elimination of C. albicans with 0.1% Peracetic acid and 0.25% Peracetic acid was 100% after immersion in disinfectant at every time point. While, the percentage elimination of C. albicans with ICPA Hexidine mouthwash was 60%, 70% and 100% after immersion in disinfectant for 5 miutes, 10 minutes and 8 hours respectively. Thus, Peracetic acid even at the lower concentration of 0.1% is superior in terms of disinfectant property as compared to control and ICPA Hexidine mouthwash even after 5 and 10 minutes of immersion.

Biofilm is defined as "complex communities of microbial cells, irreversibly attached to surfaces or interfaces or to each other and embedded in extracellular polymeric substances (EPS) of their own origin that have an altered gene expression and growth rates compared to their planktonic counterparts." (Donlan & Costerton, 2002) In the 1970s, it was established that biofilms are a natural and relevant phenomenon present in the environment with strong adherence to both living and nonliving material and have high resistance towards disinfection.

For the Prosthodontist dental and denture plaque are two clinically important biofilms. This relevance stems from the fact that as soon as the prosthesis is placed in the oral cavity, a layer of 'acquired pellicle' coats their surface (Baier and Glantz, 1978; Edgerton et al., 1993) which provides adhesion receptors specific for microorganisms like *Streptococcus* spp. and *Candida albicans*. The denture fitting surface covered with plaque is a highly acidic surface that supports the growth of *Candida* spp. The plaque growth here is also promoted by the lack of salivary flow, increased availability of nutrients, irregular surface topography essentially indicating a greater need for maintaining the hygiene of the dentures. This makes it essential to reduce continual plaque accumulation since several oral mucosal inflammatory disorders like denture stomatitis are a result of the presence of denture plaque (Catalan et al., 1987; Abelson, 1981). It affects the quality of life of the patient as diet and lifestyle choices become limited. The subsequent treatments and procedures further increase physical, psychological and financial liability upon the patient.

Denture cleansing tablets produce both a mechanical and chemical cleansing of the denture base. They are easy to use, require less brushing, remove organic debris and have low abrasive tendency. However, even though effervescing denture cleansers decrease microbial load on the denture surface, they do not cause complete sterilization (Glass et al., 2011). If the soak time and temperature was increased, hardness and esthetics of the acrylic resin deteriorate.

Denture cleansers should therefore, target the physical removal of the microorganisms from the surface of the dentures with minimal damage to the acrylic resin, as well as have a chemical inactivation effect for maintaining good denture hygiene. However, acrylic resins have an inherent liquid sorption property due to the high internal energy and polarity of the carboxyl groups (Chassot et al., 2006). This further complicates chemical immersion disinfection. Several chemical disinfectant solutions have been researched for denture cleansing; like glutaraldehyde, formaldehyde, sodium hypochlorite, chlorhexidine etc. Of these, the aldehyde solutions are strong disinfectants but have severe cytotoxic effects since any residues of their toxic products may be transferred from the denture surface (sorption) to the saliva and thus to the oral tissues. They cause contact dermatitis, asthma, allergy, irritation to the skin eyes and nose as they release toxic vapors. The use of glutaraldehyde therefore requires a well-ventilated space, use of mask and gloves and a thorough washing of the denture base before use. Sodium hypochlorite though commonly recommended as a denture cleansing agent has the drawback of being a bleaching agent. Its prolonged use causes esthetic deterioration of the prostheses. Chlorhexidine and hydrogen peroxide have shown a mild effect in containing the growth of *Candida albicans* (Ekren & Ozkomur, 2016). Chlorhexidine is a proven broad spectrum

antimicrobial drug. This is a result of its molecules being positively charged whereas the most surfaces in oral cavity as well as bacteria are negatively charged. Since oppositely charged ions attract, the molecules of chlorhexidine bind well with theses surface structures. Once bound, it induces cell death by altering the osmotic balance leading to precipitation of cytoplasm. It is safe and does not cause the production of resistant microorganisms (Kaplowitz & Cortell, 2005).

A relatively newer chemical disinfectant in the form of Peracetic Acid (PAA) has emerged to be a forerunner in the denture disinfection scenario. It remains unaffected in the presence of protein residues or organic matter. It acts by oxidizing the cell membranes of the microorganisms which causes cell lysis due to elimination of the basic structure of the cell. It inactivates both aerobic and anaerobic microorganisms (Stopiglia et al., 2011). The non-dissociated forms of weak acids pass freely through the cell membranes and if the pH of the cytoplasm is greater than that of the medium, the acid dissociates. It releases a proton and acidifies the cytoplasm causing death of the microorganism (Costa et al., 2015). Therefore, Peracetic acid can be used as a disinfectant at greater dilutions. According to a study conducted by da Silva et al in 2015, Peracetic acid does not cause corrosion of the acrylic resin even after prolonged immersion irrespective of the disinfection efficacy. Variations in temperature do not alter the disinfection potential of Peracetic acid significantly (Ekren & Ozkomur, 2016). Upon decomposition, it breaks down into oxygen, water and acetic acid.

The results of the present study are consistent with the research conducted by Reis et al in 2012 where they established complete elimination of *Candida albicans* and *Bacillus subtilis* from the surface of acrylic resin specimens after immersion in 0.25% and 0.025% Peracetic acid for 1, 3, 5 and 10 minutes. This potent antimicrobial potential of per acetic acid even at low concentrations is a result of its action on the cell membrane where per acetic acid oxidizes the hydrated sulphate (-SH) and sulphur bonds (S-S). This increases the cell wall permeability following which the acid denatures the proteins and enzymes leading to cell death of the *Candida albicans* colonies adhered to the surface of the heat polymerized acrylic resins.

In accordance with the research conducted by Reis et al (2012), Chassot et al (2006) as well as Bhathal et al, the antimicrobial efficacy of per acetic acid even at low concentrations, exceeds the antimicrobial capability of chlorhexidine. At the cellular level the higher antimicrobial effectiveness of Peracetic could possibly arise from its ability to remain active even in the presence of organic matter and protein residues which may alter the pH. The chlorhexidine molecules in this case may not bind as effectively with the microorganisms in an environment which is anything other than alkaline or neutral.

In addition, Chlorhexidine also causes staining and a change in dimensional properties of the acrylic resins (Reis et al., 2012). Gary et al have also stated that chlorhexidine is known to cause an altered taste perception and increased calculus formation (denture plaque). The effect of Peracetic on the properties of PMMA as studied by Thamlikitkul et al. is insignificant. They derived from their research that 0.2% per acetic acid does not relevantly alter the sorption, solubility and microhardness of heat cured acrylic resins. Therefore, leading us to the conclusion that Peracetic acid even at low concentrations of 0.1% and 0.25% is more effective and safer denture disinfecting agent than 2% chlorhexidine (ICPA).

4. Conclusion

Since this research was In Vitro, the results may differ when the variables of the oral environment are added creating the need for further foray into the subject matter. The presence of saliva and other microorganisms can lead to different results. Only the antifungal potency of per acetic acid and Chlorhexidine against *Candida albicans* has been evaluated. The antimicrobial effects of these disinfecting agents against other microorganisms also need further research. Within the limitations of this study, it can be concluded that 0.1% and 0.25% are efficient disinfectants against *Candida albicans.* Both the concentrations are more potent than 2% chlorhexidine.

Acknowledgements:

I would like to thank Mrs. Sangeeta and Dr. (Mrs.) Lakhani, Department of Microbiology, S.B.K.S. Medical College, Sumandeep Vidyapeeth Deemed to be University; for their support.

Conflict of interest:
The authors declare no conflict of interest.

References:

Abelson, D. C. (1981). Denture plaque and denture cleansers. *The Journal of prosthetic dentistry*, *45*(4), 376-379. https://doi.org/10.1016/0022-3913(81)90094-9

Baier, R. E., & Glantz, P. O. (1978). Characterization of oral in vivo films formed on different types of solid surfaces. *Acta Odontologica Scandinavica*, *36*(5-6), 289-301. https://doi.org/10.3109/00016357809029079

Catalan, A., Herrera, R., & Martinez, A. (1987). Denture plaque and palatal mucosa in denture stomatitis: scanning electron microscopic and microbiologic study. *Journal of Prosthetic Dentistry*, *57*(5), 581-586. https://doi.org/10.1016/0022-3913(87)90341-6

Ceretta, R., Paula, M. M. S., Angioletto, E., Méier, M. M., Mitellstädt, F. G., Pich, C. T., ... & Angioletto, E. (2008). Evaluation of the effectiveness of peracetic acid in the sterilization of dental equipment. *Indian Journal of Medical Microbiology*, *26*(2), 117-122. https://doi.org/10.1016/S0255-0857(21)01925-3

Chassot, A. L. C., Poisl, M. I. P., & Samuel, S. M. W. (2006). In vivo and in vitro evaluation of the efficacy of a peracetic acid-based disinfectant for decontamination of acrylic resins. *Brazilian Dental Journal*, *17*, 117-121. https://doi.org/10.1590/S0103-64402006000200006

Costa, S. A. D. S., Paula, O. F. P. D., LEÃO, M. V. P., & SANTOS, S. S. F. D. (2015). Stability of antimicrobial activity of peracetic acid solutions used in the final disinfection process. *Brazilian oral research*, *29*, 1-6. https://doi.org/10.1590/1807-3107BOR-2015.vol29.0038

Donlan, R. M., & Costerton, J. W. (2002). Biofilms: survival mechanisms of clinically relevant microorganisms. *Clinical microbiology reviews*, *15*(2), 167-193. https://doi.org/10.1128/cmr.15.2.167-193.2002

Edgerton, M., Scannapieco, F. A., Reddy, M. S., & Levine, M. J. (1993). Human submandibular-sublingual saliva promotes adhesion of Candida albicans to polymethylmethacrylate. *Infection and immunity*, *61*(6), 2644-2652. https://doi.org/10.1128/iai.61.6.2644-2652.1993

Ekren, O., & Ozkomur, A. (2016). Influence of ozone and paracetic acid disinfection on adhesion of resilient liners to acrylic resin. *The Journal of Advanced Prosthodontics*, *8*(4), 290-295. https://doi.org/10.4047/jap.2016.8.4.290

Glass, R. T., Conrad, R. S., Bullard, J. W., Goodson, L. B., Mehta, N., Lech, S. J., & Loewy, Z. G. (2011). Evaluation of cleansing methods for previously worn prostheses. *Compendium of continuing education in dentistry (Jamesburg, NJ: 1995)*, *32*(3), 68-73.

HASHIZUME, L. N., HOSCHARUK, M. F., & MOREIRA, M. J. S. (2015). Effect of affordable disinfectant solutions on Candida albicans adhered to acrylic resin for dental prosthesis. *RGO-Revista Gaúcha de Odontologia*, *63*, 309-314. https://doi.org/10.1590/1981-86372015000300083011

Kaplowitz, G. J., & Cortell, M. (2005). Chlorhexidine: a multi-functional antimicrobial drug. *Peer reviewed publication. The Academy of Dental Theraputics and Stomatology*.

Keller, J. C., & Lautenschalger, E. P. (1985). Porosity reduction and its associated effect on the diametral tensile strength of activated acrylic resins. *The Journal of prosthetic dentistry*, *53*(3), 374-379. https://doi.org/10.1016/0022-3913(85)90516-5

Monteiro, D. R., Takamiya, A. S., Feresin, L. P., Gorup, L. F., de Camargo, E. R., Delbem, A. C. B., ... & Barbosa, D. B. (2014). Silver colloidal nanoparticle stability: influence on Candida biofilms formed on denture acrylic. *Sabouraudia*, *52*(6), 627-635. https://doi.org/10.1093/mmy/myu021

Reis, L., Zanetti, A. L., Castro Junior, O. V., & Martinez, E. F. (2012). Use of 0.25% and 0.025% peracetic acid as disinfectant agent for chemically activated acrylic resin: an in vitro study. *RGO. Revista Gaúcha de Odontologia (Online)*, *60*(3), 315-320.

Salvia, A. C. R. D., dos Santos Matilde, F., Rosa, F. C. S., Kimpara, E. T., Jorge, A. O. C., Balducci, I., & Koga-Ito, C. Y. (2013). Disinfection protocols to prevent cross-contamination between dental offices and prosthetic laboratories. *Journal of infection and public health*, *6*(5), 377-382. https://doi.org/10.1016/j.jiph.2013.04.011

Stopiglia, C. D. O., Carissimi, M., Scroferneker, M. L., & Fortes, C. B. B. (2011). Microbiological evaluation of peracetic acid for disinfection of acrylic resins. *Revista Odonto Ciência*, *26*, 238-241. https://doi.org/10.1590/S1980-65232011000300008