



An In-Vitro Evaluation of Alternative Disinfection Methods of Acrylic resin and Thermoplastic Resin Denture Base Materials

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ABSTRACT

Objectives: This study was designed to evaluate the antimicrobial effect of alternative disinfection methods of heat cured acrylic resin and thermoplastic resin denture base materials and to investigate the porosity resulting from disinfection by these methods. **Material and Methods:** Disc specimens of heat cured acrylic resin and thermoplastic resin were fabricated and divided into four groups for each denture base material according to the disinfection method. The specimens were contaminated in vitro by standardized suspensions of *Candida albicans* (*C. albicans*). The following test agents were used: distilled water (as a control group), 5% sodium hypochlorite (NaOCl), 100% white vinegar and 650 W microwave (MW) energy. After the disinfection procedure, the number of viable microbial cells was counted in CFU/ml. Porosities of acrylic resin and thermoplastic resin specimens were evaluated with Scanning Electron Microscope (SEM) after one month of daily exposure to disinfection. **Results:** ANOVA test showed that, there was a statistical significant difference among the tested treatment agents against *C. albicans* in each denture base material ($P \leq 0.05$). Post Hoc test showed a statistical significant difference between the control samples and the other disinfected samples in the microbial count, however, there was no statistical significant difference among the tested methods of disinfection ($P > 0.05$). Regarding porosity area percentage, there was a statistical significant difference among the tested methods of disinfection ($P \leq 0.05$), where, 100% white vinegar showed the highest porosities followed by NaOCl then the MW disinfection and the least is the control group. Regarding comparison between the two denture base materials, thermoplastic resin showed statistically significant lower microbial adherence as well as lower porosity area percentage than heat cured acrylic resin ($P \leq 0.05$). **Conclusion:** All the tested disinfection methods shown to be efficient against *C. albicans*. Thermoplastic resin demonstrated lower microbial adherence than heat cured acrylic resin and lower porosity area percentages.

KEYWORDS

Candida albicans,
disinfection, denture base.

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INTRODUCTION

The number of elderly people worldwide has been increased by increasing lifetime⁽¹⁾. This condition resulted in a high prevalence of edentulism and complete denture wearers⁽²⁾. Polymethyl methacrylate (PMMA) acrylic resin has a long, clinically established history for being utilized as denture base material; it has some advantages like ease of manipulation, repair and polish, low cost and acceptable esthetic properties.

However, polymerization shrinkage, poor wear resistance, inadequate mechanical properties, microbial adhesion, and residual monomer content are the main limitations of the material^(3,4). Continuous research focusing on PMMA properties improvement has led to the emergence of new processing techniques and alternative polymeric materials known as thermoplastic resins. These materials exhibit high creep and solvent resistance, excellent wear characteristics and high fatigue endurance. In addition, they have very little or almost no free monomer; therefore, they offer another option for allergic patients^(5,6).

Denture stomatitis is common recurring problem of the denture wearers. The etiology of the disease includes infection, trauma and probably a defect in the host defense mechanism⁽⁷⁾. Complete dentures acts as a reservoir for microbial colonization, particularly *C.albicans*, on the porous surface of the acrylic resin⁽⁸⁾. This microbial colonization is one of the main etiological factors associated with denture stomatitis⁽⁹⁾. *C. albicans* has been shown to be the most common important opportunistic pathogen causing infection in the oral cavity and to be able to colonize acrylic materials⁽¹⁰⁾. The effective removal of denture plaque by brushing requires a certain degree of manual dexterity which is commonly compromised in the elderly. In addition, the irregularities and porosities present on the acrylic resin surface may also contribute to penetration of microorganisms into dentures, making it difficult to clean them by brushing⁽¹¹⁾.

Ideal denture care products should remove inorganic/organic deposits and stains; have cost-effective, bactericidal, and fungicidal properties; and be easy to handle for the human health and harmless for the denture materials⁽¹²⁾. Various types of disinfectants were used in dental practice like glutaraldehyde, formaldehyde, sodium hypochlorite, hydrogen peroxide, chlorhexidine, vinegar and mixture of different chemicals⁽¹³⁻¹⁵⁾. Sodium hypochlorite solutions are very effective in various concentrations against microorganism; however, the concentration is a factor that should be considered to prevent any adverse effects on the materials of the prosthetic devices⁽¹⁶⁾. The vinegar is a sour and astringent liquid consisting mainly of acetic acid. This is product is cheap, easily found in the market, and seems to have antimicrobial potential^(15, 17).

In addition to the chemicals used for denture cleansing, microwave irradiating the dentures has also been used as an inexpensive alternative for denture disinfection⁽¹⁸⁾. MW irradiation has been studied to detect its effectiveness and its influence on physical properties of complete denture materials. Some studies have demonstrated the effectiveness of microwave irradiation as an alternative method for disinfection of denture base acrylic resins⁽¹⁹⁻²¹⁾. In addition, denture microwave disinfection was as effective topical antifungal therapy for treating denture stomatitis^(22,23). The influence of MW irradiation on physical properties of denture materials was investigated by researches⁽²⁴⁻²⁶⁾. However, microwaving may negatively affect denture resins, liners or teeth due to the material heating after irradiation⁽²⁷⁾.

The surface topography of the denture has been shown to greatly influence adhesion and subsequent retention of microorganisms⁽¹²⁾. Several studies have linked the surface characteristics of denture acrylics to the amount of *Candida* biofilm adhesion⁽²⁸⁻³¹⁾. It has been reported that imperfections on the denture surface contribute to an increase in the adhesion of *Candida*, which becomes imbedded

within these imperfections⁽³²⁾. The presence of surface and subsurface porosities may compromise the physical, aesthetic and hygienic properties of a processed denture base⁽³³⁾. Porosity in denture bases weakens the prosthesis due to accumulation of internal stresses. It can also lead to distortion and warpage of resin denture bases⁽³⁴⁾.

Therefore, this study was designed to investigate the effect of different denture cleaners on the *C.albicans* concentration values and porosity of heat cured acrylic resin and thermoplastic resin denture base materials. The null hypothesis of this study was that the application of different denture cleaners or the type of denture base materials would not affect the Candida concentration values and porosity.

MATERIAL AND METHOD

Fabrication of specimens: Two different denture base materials were used; heat cured acrylic resin (Acron Duo, Associated Dental Products Ltd., Kemdent, Purton, Swindon, Wiltshire, UK) and thermoplastic resin (Vertex ThermoSens Rigid, Vertex-Dental B.V., Zeist, Netherland). A total of one hundred and sixty identical disc specimens (25 mm in diameter and 2mm in thickness) were processed. Eighty were made from heat-cured acrylic resin and the other eighty were fabricated from thermoplastic injection molded resin. All specimens were produced in molds prepared by insertion of stainless steel rings into the metal dental flask filled with type III dental stone. After complete stone setting, the stainless steel rings removed and the mould was ready for fabrication of tested discs. Each denture base material was proportioned, mixed and processed according to each manufacturer's instructions. Then the flasks were allowed to bench cool and the specimens were removed. Eighty discs were chosen for the adherence microbiological test (forty for each denture base material) and the other eighty for the porosity evaluation (forty for each denture base material).

Microbiological evaluation

Microorganisms: Standard strain of *C. albicans* (RCMB 05036) was used. The microorganisms were grown on Muller Hinton Agar plates (Oxoid, Basingstoke, UK). After 24h, the colonies were suspended in tubes containing 5ml of brain heart infusion (BHI) broth (Oxoid). The final concentration of cells was 10^6 CFU/ml (Colony Forming Unit/ml). The cell suspension in each tube was adjusted to match 0.5 McFarland scale (1.5×10^8 CFU/ml). Eighty sterile disc specimens (forty acrylic resin and forty thermoplastic resin) were placed into the tubes containing the microorganism culture and incubated for 72 hours at 37°C to allow the fungi to grow and contaminate the acrylic resin and thermoplastic resin specimens.

Disinfection treatment of the specimens: Following incubation, the specimens were exposed to following four treatments (ten discs from each denture base material for each treatment); **group 1:** 250 ml distilled water for 15 min (as a control group), **group 2:** 5 % sodium hypochlorite for 5 min, **group 3:** 100% white vinegar for 10 min and **group 4:** microwave (MW) oven 650 W at full power (100 % potency) for 3 min (the specimen was immersed in a beaker containing 200 ml water).

After disinfection of the specimens, each specimen was first washed with saline and the excess saline was removed with a gentle compression of sterile gauze. Discs were then transferred to plates of BHI agar, left for 10 minutes and then removed gently. The plates were incubated at 37°C for 72 hours. Then, the numbers of colony forming units (CFUs) were counted. Assays were independently performed in triplicates and data were recorded as means and standard deviations.

Porosity evaluation:

Forty disc specimens of each denture base material were assigned for porosity evaluation, after daily exposure to the four disinfection methods (ten discs for each disinfection method) for one

month. All specimens were first coated with gold by sputtering deposition in Gold Sputter Coater (SPI – Module, Canada) at low vacuum mode for 2-3 minutes as a conductive layer. Scanning electron microscope (Joel JSM-5500 LV, Japan) at the Regional Center of Mycology and Biotechnology, Cairo, Egypt, was used to scan the specimens for porosity evaluation by taking 5 snapshots for each specimen at predetermined points (center, mid-right, mid-left, mid-upper and mid-lower). Each point was photographed using its camera at a fixed magnification power for all specimens and points (300x). The images were displayed directly on the computer screen (Fig 1-4). Porosities area percentage was measured with reference to a standard measuring frame for each image. In each disc, total area of surface porosities in the five snapshots were calculated and expressed in percentage form. Mean values were obtained for the all specimens in each group.

Data were collected, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when their distribution found parametric while qualitative data were presented as number and percentages. The comparison between two independent groups with quantitative data and parametric distribution was done by using Independent t-test. The comparison between more than two independent groups with quantitative data and parametric distribution was done by using One Way Analysis of Variance (ANOVA) followed by post hoc analysis using LSD test. The confidence interval was set to 95% and the margin of error accepted was set to 5%. The significance level was set at $P \leq 0.05$.

RESULTS

The effect of disinfection method on acrylic resin and thermoplastic resin disc samples against *C. albicans* were demonstrated as the mean viable cells (CFU) after disinfection (Table 1). The results showed that all the disinfection methods have

high antimicrobial effect in comparison to control group for both types of denture bases. 100% white vinegar showed the highest antimicrobial effect followed by sodium hypochlorite then the microwave disinfection. Statistical analysis using one-way ANOVA test showed that there was highly significant difference between the tested treatments against *C. albicans* in each group of denture base material ($P \leq 0.05$). However, Post Hoc LSD test, intergroup comparisons of microbial count, showed no statistical significant difference between 100% white vinegar, sodium hypochlorite and microwave disinfection, but there is a highly significant difference in microbial count between these methods of disinfection and distilled water (control) group.

Statistical comparison between *C. albicans* adherence on acrylic resin and thermoplastic resin disc samples showed that, thermoplastic resin has statistically significant lower candidal count than acrylic resin after all the tested methods of disinfection and for the control group (Table 2).

Inspection of porosities in % form of acrylic resin and thermoplastic resin disc samples after daily treatment for a month revealed that, 100% white vinegar showed the highest porosities followed by sodium hypochlorite then the microwave disinfection and the least is the control group (Fig. 1-4). Statistical analysis using one-way ANOVA test showed that there was highly significant difference between the tested sample discs for each denture base material ($P \leq 0.05$). Post Hoc LSD test, intergroup comparisons of porosity %, showed highly significant difference between each method of disinfection, also highly significant difference between the disinfected samples and control samples (Table 3).

Statistical comparison between porosities % for acrylic resin and thermoplastic resin disc samples showed that thermoplastic resin has a statistically significant lower porosities % than acrylic resin after all the treatment methods and also for the control samples ($P \leq 0.05$) (Table 4)

Table 4: Porosity area percentage comparison between heat cured acrylic resin and thermoplastic resin disc samples after each disinfection method:

Types of denture base & treatment	Porosities area percentage	Test value	P-value
Thermoplastic resin (control)	0.2% ± 0	0.000	0.000
Acryl heat cured group (control)	0.5% ± 1.12		
Thermoplastic resin by 5% NaOCl	3.3% ± 3.21	13.164	0.000
Acryl heat cured group by 5% NaOCl	7.1% ± 5.6		
Thermoplastic resin by 100% white vinegar	6% ± 4.8	7.678	0.001
Acryl heat cured group by 100% white vinegar	9% ± 7.3		
Thermoplastic resin by MW	1.35% ± 2.3	18.734	0.000
Acryl heat cured group by MW	5% ± 3.7		

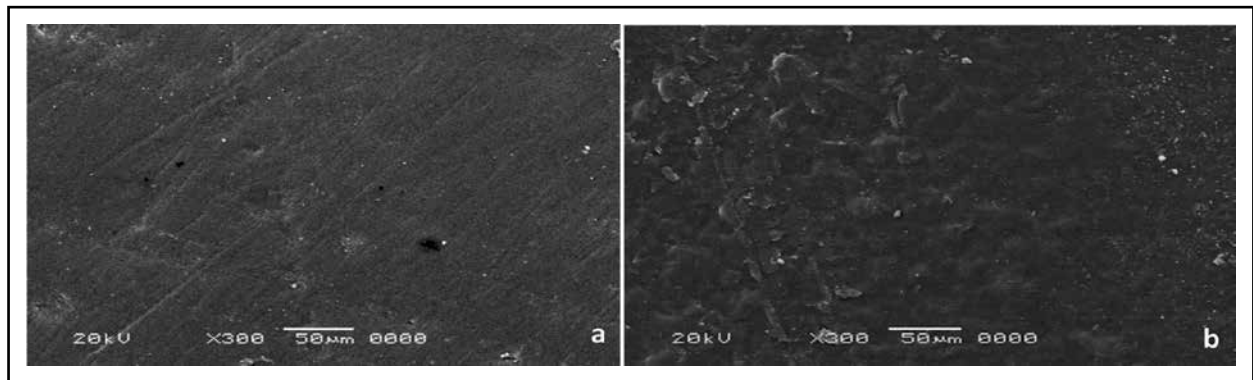


Fig. (1) A scanning photomicrograph of acrylic resin disc (a) and thermoplastic resin disc (b) after treatment with distilled water (control).

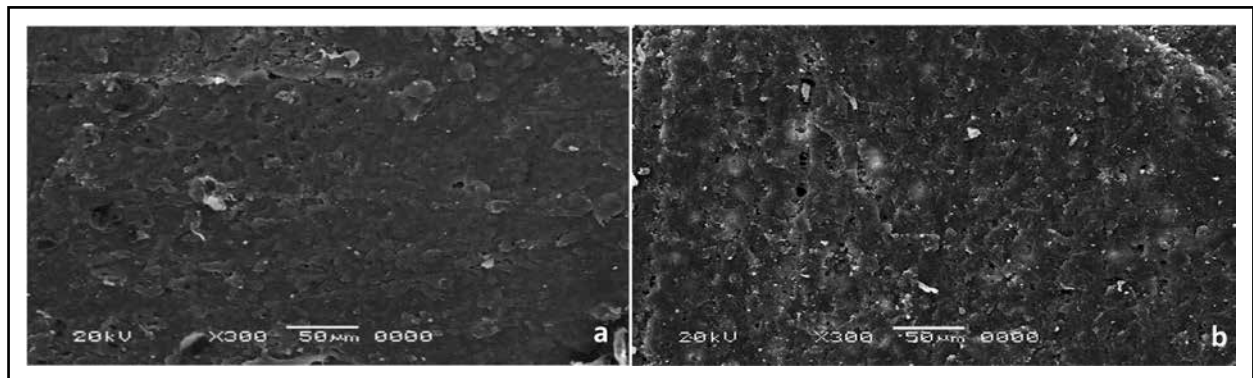


Fig. (2) A scanning photomicrograph of acrylic resin disc (a) and thermoplastic resin disc (b) after treatment with 5% NaOCl.

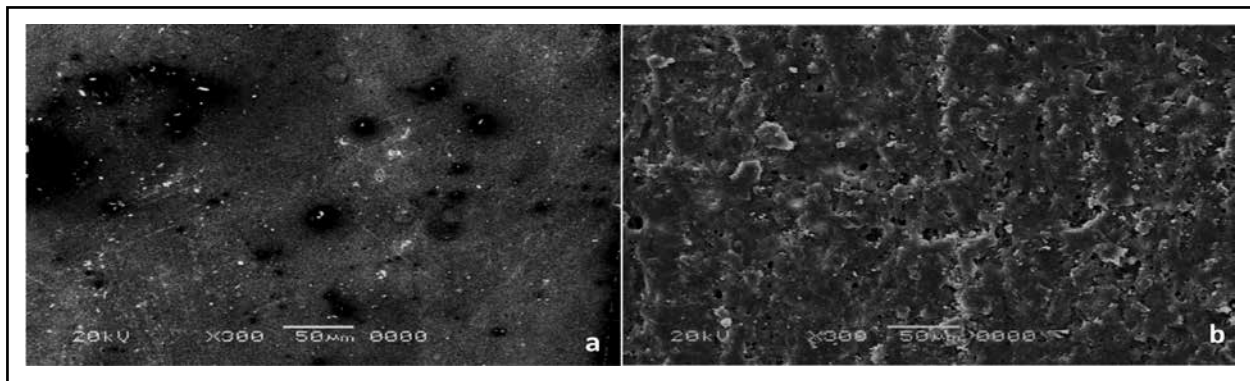


Fig. (3) A scanning photomicrograph of acrylic resin disc (a) and thermoplastic resin disc (b) after treatment with 100% white vinegar

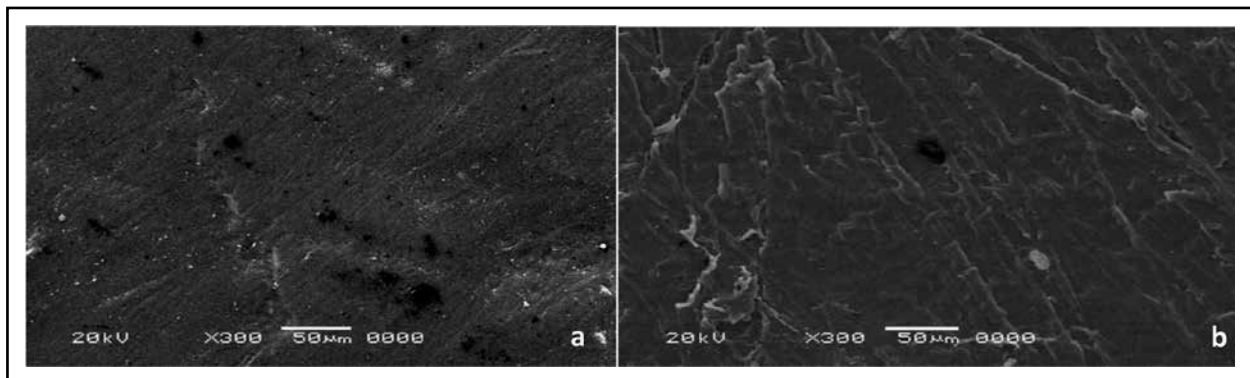


Fig. (4) A scanning photomicrograph of acrylic resin disc (a) and thermoplastic resin disc (b) after treatment with MW.

DISCUSSION

Poor oral health can negatively affect the mental, social, and general systemic health and physical well-being of denture wearers. Denture care is an important process that conduces to oral health and denture longevity⁽³⁵⁾. Unclean dentures and poor oral hygiene are usually predisposing factors for *Candida* associated denture stomatitis⁽³⁶⁾. *C. albicans* adhesion to resin materials is promoted by oral environment temperature and the acquired pellicle formed over dentures. Ribeiro et al⁽³⁷⁾. Found *Candida* spp. (65.5%) more than *Strep. Mutans* and *Staph. aureus* on dentures. *C. albicans* is a well-known etiologic agent at denture stomatitis. This inflammatory disorder affects approximately 60% of denture wearers and causes inflammation of the oral mucosa in close contact with the denture⁽³⁸⁾. For this reason, *C. albicans* was chosen to determine

the better disinfection method for denture base materials.

Denture cleaning methods with soaking the dentures in a solution of some chemical agents have advantages of being simple to use and with efficient disinfection more than mechanical cleaning⁽³⁹⁾. However it is an important criterion that the cleaners have no adverse effect on the physical and mechanical properties of denture base materials and artificial teeth while effective on removing the organic and inorganic deposits; bactericidal and fungicidal⁽⁴⁰⁾.

Therefore, in the present study, different chemical disinfectants were evaluated on two different denture base material; heat cured acrylic resin which is the most popular denture base material and thermoplastic resin which is biocompatible material with unique physical and esthetic properties⁽⁴¹⁾.

In this study, distilled water was determined as control since it is indicated for complete denture overnight immersions⁽³¹⁾. Also sodium hypochlorite disinfectant was evaluated as there are several studies reported satisfactory results for the antimicrobial activity of NaOCl solutions with different concentrations on denture base materials^(42,43). However, NaOCl has some disadvantages on denture base materials such as discoloration and an increase in surface roughness, depending on the concentration and immersion time^(35,44). NaOCl concentration of 5% used in this study has been recommended by several studies to dissolve mucin, organic substances and shows antimicrobial activity by the action of hydroxyl ions and chlorination^(36,45).

Also white vinegar disinfection was investigated although it is not frequently used in dentistry as a disinfectant, but it is considered as a promising alternative disinfectant in several areas because of its low toxicity and low cost. White vinegar was frequently used in 50% and 100% concentrations to disinfect tooth brushes and acrylic resins⁽¹⁾. Several studies found that 100% concentration white vinegar is more effective against *C. albicans* than 50% concentration^(1,42). For this reason white vinegar with 100% concentration was investigated.

MW disinfection was reported to be effective and quick which may be a significant advantage for some patients, and it is suitable for disinfection of acrylic resins^(1,46). Some authors suggested denture MW disinfection in water because bubbles released by boiling water help removing microorganisms from the surface^(47,48). While others have recommended denture disinfection with steam heat in the MW oven⁽⁴⁹⁾. In this study, MW irradiation was used at 650w for 3 minutes and resin specimens were put into MW oven with 200 ml water.

The results of this study showed that all the disinfection methods have high antimicrobial effect in comparison to distilled water for both types of denture bases. 100% white vinegar showed the

highest antimicrobial effect followed by sodium hypochlorite then the microwave disinfection however the difference between these methods of disinfection was statistically non-significant. This results were in agreement with a previous study who reported that, the white vinegar showed effective antimicrobial activity against *C. albicans* and *Staphylococcus aureus* in 100% concentration for acrylic resins as 1% NaOCl and 2% glutaraldehyde⁽⁴²⁾. Also it has been reported that, both 50% and 100% concentrations of white vinegar used for 10min were found to be considerably effective for *C. albicans* for both heat-cured acrylic resin and autopolymerized acrylic resin and white vinegar 100% was the most effective method for *C. albicans*⁽¹⁾. On the contrary, previous study investigated the effectiveness of 50% white vinegar for toothbrush disinfection and it was found to be effective for *Staphylococcus aureus*, *Streptococcus mutans*, and *Streptococcus pyogenes*, but not for *C. albicans*⁽⁵⁰⁾.

The results of the present study agreed with a previous study which concluded that using sodium hypochlorite for 5 min sterilized contaminated dentures. However, Webb et al⁽⁵⁹⁾, have demonstrated that MW was more effective in denture disinfection than 0.02% and 0.0125% NaOCl⁽⁵¹⁾.

Many authors reported that microwave denture disinfection eliminated the mycelial forms of *Candida* from the dentures of patients with denture stomatitis^(46, 52). Also, Silva et al⁽⁵³⁾ evaluated the effectiveness of microwave irradiation on the disinfection of simulated complete dentures. Dentures were individually immersed in 200 ml of water and submitted to microwave irradiation at 650 W for 6 min. They concluded that this irradiation produced sterilization of complete dentures contaminated with *Staphylococcus aureus* and *C. albicans* and disinfection of those contaminated with *Pseudomonas aeruginosa* and *Bacillus subtilis*. Previous studies showed that microwave irradiation (650 W) for 3 min resulted in sterilization of all dentures contaminated with

all *Candida* species whereas, only a significant decrease in *Candida* species resulted after microwave irradiation for 2 min^(48,54). Silva et al⁽¹⁹⁾, inoculated samples of acrylic resin with species of *Candida* and later immersed in 100 ml of sterile water and irradiated with microwave at 650 W for 3 min and they found that, microwave irradiation was an effective method for disinfection of the acrylic resins inoculated with *C. albicans*, *C. dubliniensis* and *C. tropicalis*. Senna et al⁽⁵⁵⁾ suggested adding denture cleanser to microwave disinfection regimen to reduce the irradiation time and the exposure of dentures to high temperatures.

In the present study, the comparison between *C. albicans* adherence on acrylic resin and thermoplastic resin disc samples showed that thermoplastic resin has a statistically significant ($P \leq 0.05$) lower *C. albicans* count than acrylic resin for the control group and after treatment with the other disinfection methods. This could be explained on the basis that the adherence of *C. albicans* is influenced by, among other factors, the denture base material; in which the chemical composition of base material is important in determining the ability of pathogenic yeast cells to attach and form biofilms^(56,57).

The results of the present study was in agreement with recent studies which reported that, thermoplastic denture base material has lesser biofilm development and candidal count as compared to heat cure acrylic resin^(58,59). They explained this might be caused by the porosity of heat-cured acrylic resin, which easily triggers the piling of food debris and microorganisms inside⁽⁵⁹⁾.

In this study, surface porosity was analyzed by means of SEM of samples. SEM image analysis allows the determination of porosity as well as the porosity distribution in high magnification images of a certain sample area. Results were obtained and analyzed on the basis of occurrence of porosities in material. Areas of surface pores were expressed in percentage form.

Statistical comparison of porosity in % form showed statistically significant difference

between all disinfection methods of acrylic resin and thermoplastic resin disc samples after daily treatment for a month. White vinegar showed the highest porosity area percentage followed by sodium hypochlorite then the microwave disinfection then and the least one is the control group. These findings might be due to the influence of the low pH in the vinegar solution, a chemical bonding of the polymer structure, and the ability to absorb the fluid. The heat-cured acrylic resin and thermoplastic resin material had properties that could absorb liquids. In turn, this caused hydrolysis of the polymer chains in an aqueous environment resulting in an unstable polymer. The results of this study were relevant to the "corrosive wear" theory, which states that an acid solution is corrosive, so it may lead to chemical degradation^(60,61).

The results of this study was in accordance of other studies, which reported that 5% NaOCl solutions may cause structural changes in the polymer matrix of resins. Resin plasticizers leach out when coming in contact with chlorine-containing solutions. Thus consequently induce the deterioration on the surface layer and increase the roughness^(36,62).

Also it was conducted that, disinfection by MW raises the internal temperature of the resin and structural alterations could possibly occur during this process. It was shown that resin with a high level of residual monomer has an increase in the number of pores, which is related to monomer vaporization⁽⁶³⁾.

Statistical comparison of porosity area percentage between heat cure acrylic resin and thermoplastic resin revealed that, thermoplastic resin has lower porosity % than heat cured acrylic resin for all the treatment groups. This can be explained that porosity has been defined as a complex phenomenon that depends on the type of material, inclusion technique and polymerization method. Porosity has also been attributed to the presence of residual monomer, shrinkage and monomer evaporation during the polymerization process, insufficient incorporation

of the powder into the liquid and the inclusion of air during the mixture of the material. Moreover, the occurrence of porosity also depends on the speed of polymerization and heat dissipation during the polymerization process⁽⁶³⁾. Acrylic resin is an amorphous polymer, where the molecular structure was arranged in irregular manner, so that the bond lengths and angles are also irregular, this influences the surface roughness and permeability⁽⁵⁹⁾. While thermoplastic resin is a crystalline polymer, where the structure of its constituent molecules are arranged regularly based on the length and angle of the bond and also has a strong hydrogen bonds in its chemical structure resulting to its low permeability, resistant to chemical solvents and high temperature condition⁽⁶⁴⁾.

It has been found that, there is a correlation between porosity and microorganism adherence ;porosity favors microorganism adherence, making it difficult to remove them by mechanical cleaning methods, favoring calculus deposition and adherence of other substances⁽⁶³⁾. The surface irregularities on denture base materials may act as a reservoir of infection and increase the possibility of hosting microorganisms even after the cleaning of dentures. Rough and porous surfaces make easier the penetration of bacterial and fungal cells on the denture base resins^(36,58,59,63).

CONCLUSION

Within the limitations of the present in-vitro study, all the tested disinfection methods shown to be efficient against *C. albicans*. However 100% white vinegar disc specimens showed higher porosity followed by sodium hypochlorite then microwave specimens. Thermoplastic resin demonstrated lower microbial adherence than heat cured acrylic resin and lower porosity area percentages.

Further studies are recommended to investigate the long-term use of disinfectant solutions and its effect on the mechanical properties of denture base material and artificial teeth.

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