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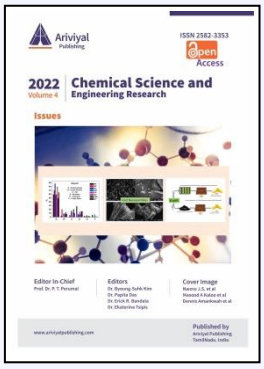

Effect of Chemical Disinfectant Agents on Biofilm and Surface Roughness of Denture Base Material

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Abstract: Denture cleansing is essential to prevent cross contamination. This study aimed to investigate the effect of different disinfecting agents on biofilm and surface roughness of denture base material. Heat cured acrylic resin was used to prepare the tested samples. The disinfectants used are sodium hypochlorite 5% (SHC) - chlorhexidine gluconate 0.12% (CHX) and hydrogen peroxide 6% (HPO). A total of seventy-four discs' shape was prepared. The microorganisms isolated by a swab from the oral cavity and allowed to grow in their selected culture. The samples were divided into three main groups according to disinfectant agents. All samples were placed in the incubator at 37°C for 24h to allow growth of the microorganism. All groups were disinfected by immersion in disinfectant for 10 minutes. The surface roughness (Ra-µm) was analyzed with a surface roughness profilometer. SHC groups showed no signs of microorganism growth (*St. viridans* - *S. aureus* - *C. albicans*). HPO showed growth of *St. viridans* while no signs of *C. albicans* and *S. aureus* growth. The CHX showed no signs of microorganism growth (*St. viridans* - *S. aureus* - *C. albicans*). Regards to surface roughness SHC group showed no significant differences compared to distilled water (DW) and dry samples (P<0.180 and P<0.176 respectively). While HPO groups displayed higher surface roughness with significant differences (P<0.000, SHC P<0.003, and CHX P<0.018). The CHX groups showed also higher surface roughness compared to DW, Dry and SHC samples (P<0.000), as well as with HPO P<0.018). The microscope graphs of SHC showed the no clear effect on surface. The HPO and CHX showed a clear effect on surface roughness. 5% SHC recommended to be used as disinfectant agent rather than HPO and CHX agents.

Keywords: Chemical Disinfectants; Surface Roughness; Denture Base

1. Introduction

The dynamic development of new multidisciplinary areas has a direct impact over the possible treatments and the rehabilitation of the dental function. Teeth rehabilitation with removable denture prosthesis is an established form of treating both partial dentition and complete edentulous patients. The developments in recent decades with dental implants dominate the current dental research, not only medical contraindications but also a negative attitude toward implants and economic limitation are the major disadvantages for their universal applicability, so the rehabilitation with dental prostheses still makes up a significant portion of everyday clinical practice.[1]

The Poly methyl methacrylate (PMMA) material revolutionized the preparation techniques used so far for fabrication of dentures. PMMA is an acrylic resin usually used with a long tradition for prosthetic purposes. It can be classified as a chemically or thermally polymerized material depending on the factors that initiate the reaction. For dental prosthesis, thermally polymerized materials are used and the heat can be generated by hot water bath or microwave energy.[1]

Although dentistry has developed new materials and techniques used in rehabilitation of edentulous patients, PMMA resins have dominated the denture base market for over 80 years. However, PMMA resins have certain disadvantages, such as porosity, water sorption, and may deteriorate and decrease their efficacy overtime.[1]

Accumulation of biofilm as a consequence of poor denture hygiene, which in turn leads to the onset of several systemic and oral infections.[2] The continuous presence of biofilm formed by fungi and bacteria in such denture wearers causes an inflammatory condition called denture stomatitis. Cultures and smears of denture plaque validate a higher concentration of *Candida species*, especially *Candida albicans*.[3,4] The oral flora of denture wearers with healthy palatal mucosa primarily have bacteria such as *Streptococci, Staphylococci, Actinomyces, Lactobacilli*, and Gram-negative *Cocci*, but very few Gram negative rods and yeast.[5]

Denture cleansing is necessary for the removal of biofilm from the dentures which can be achieved mechanically by manual brushing, chemically involving wide varieties of chemical agents, and by combination of both.[2,3]

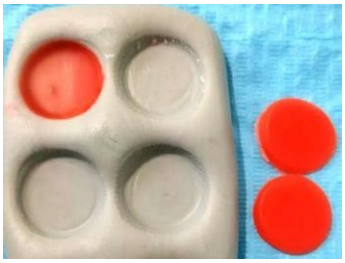

Fig. 1. Putty former filled with base plate wax.

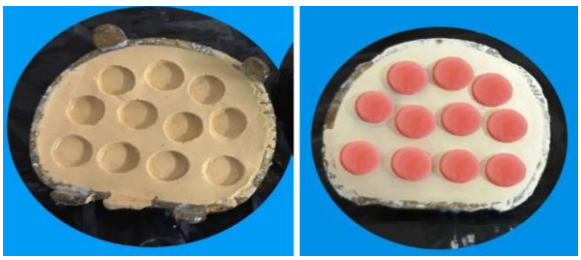

Fig. 2. Samples. Metallic Dental flask filled with acrylic resin.

Alternative methods to reduce the adhesion of microorganisms have been tested by altering the surface charge of denture base resins. The adherence of *Candida albicans* to denture base surfaces in vitro has been associated closely with the hydrophobicity of the microorganism. *C. albicans* adheres more readily to hydrophobic surfaces than to hydrophilic surfaces. In addition to that *C. albicans*, as other living cells, has a net negative surface charge, providing an environment of electrostatic repulsion through the negative-negative charge interactions with the polymer. So, preventing the adhesion of *C. albicans* will reduce the development of denture stomatitis.[6]

Another important limitation is the deposition and formation of biofilm on the surface of PMMA resins, which acts as a reservoir of microorganisms and contributes to oral diseases and tissue damage. The intaglio surface of the denture is not polished before insertion, so the rough uneven imperfect areas in the denture may serve as a breeding ground for opportunistic oral fungi. Poor oral hygiene causes the adhesion of microbial cells and possible dissemination of pathogens from denture biofilm in immunosuppressed patients can cause severe systemic infections.[7]

Synthetic acrylic resins are susceptible to microbial adhesion which offer a reservoir for microorganisms associated with infections. Therefore, attention should be paid to the bacterial population in dentures as a potential source of oral and systemic diseases. In addition to the significant Gram-positive and fungal isolates, the Gram-negative infections that become systemic are of particular concern because they possess lipopolysaccharides (endotoxin), which may initiate cascades of harmful cytokines such as tumor necrosis factor. The already difficult chemotherapy of these microorganisms has been further complicated in recent years by the well-documented overall increase in antimicrobial resistance. Therefore, it is essential for clinicians to be cognizant of the importance of appropriate prosthesis hygiene in order for denture-related diseases to be avoided.[8]

The increasing use of removable dentures has caused an increase in denture related infections like stomatitis or other infections. Management of denture related infections is challenging and infected dentures generally need to be disinfected.[9, 10]

The removal of biofilm deposited on denture surfaces is commonly accomplished by mechanical methods. Due to patient's lack of motor coordination, such methods may be ineffective, and thus demand alternative means such as chemical cleansing. The rate at which deposits accumulate on dentures may vary between individuals and can be affected by factors such as saliva composition, dietary intake, surface texture and porosity of the denture base material, duration for which the dentures are worn, and the denture-cleansing regimen adopted by the wearer. Several disinfectants have been suggested for the disinfection of dentures. The best disinfectant should fulfil most of the requirements of an ideal agent while not causing any alterations in the structure of the dentures.[11]

Sodium hypochlorite is inexpensive, presents a broad spectrum of activity, and requires a short period of time for disinfection. Chau et al.,[12] observed that besides superficial disinfection of acrylic resin, 1% sodium hypochlorite was also effective in the elimination of microorganisms from the inner surface of the material after 10 minutes. Glutaraldehyde based disinfectants are often used in dentistry. Tabs of sodium perborate and alkaline peroxide based denture cleansers are commonly used for denture cleaning and for helping mechanical hygiene.

Gornitsky et al.[13] verified the existence of antimicrobial activity of these solutions on microorganisms adhered to the dentures, but suggested that the use of denture cleaning agents might be controlled. McCabe et al.[14] stated that the denture cleaning agents are complementary to denture hygiene and must be employed in association with mechanical cleaning for more effective biofilm elimination. However, the effect of chemical disinfectants on mechanical properties need to be investigated, the purpose of this article is to investigate the effect of different disinfecting agents on biofilm as well as on surface roughness of denture base material.

2. Materials and Methods

The locally available heat cured acrylic resin was used in this study to prepare the tested samples (PYRAX- Germany). The method of disinfection was sub-immersion for 10 minutes. Three types of disinfectants were used (sodium hypochlorite 5% - chlorhexidine gluconate 0.12% and hydrogen peroxide 6%).

2.1. Sample Preparation

A total of seventy-four discs shape samples were prepared (15 mm-diameter and 4 mm thickness) using a putty former filled with base plate wax (Fig. 1).[15] All the wax patterns were invested with a dental stone in metallic dental flasks (Fig. 2). After the setting of stone, the flask halves were separated, the wax was eliminated, and the stone mold was cleaned with hot water to remove remaining wax. The resin was manipulated, packed and pressed into the mold according to the manufacturer's instructions. The heat polymerization method was carried out in water bath at 73°C for 90 min, followed by 94°C for 30 min. All flasks were allowed to cool at room temperature before opening. Polishing was done only on one surface of the samples, and the other surface was left unpolished to represent the fitting surface of denture base.

Fig. 3. Placing of clinically isolated microorganisms by swab in culture

Fig. 4. Tested samples in petri-dish incubated with microorganisms for colonization.

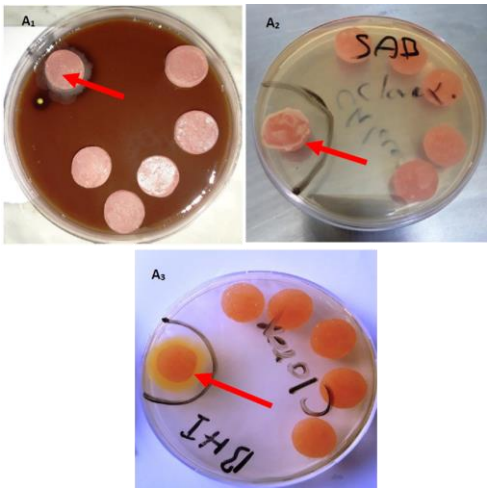

Fig. 5. Group A: Effect of sodium hypochlorite on microorganism growth. (A_1 - Strepto, A_2 - Candida, A_3 - Staph. Arrow – distilled water control sample)

2.2. Microorganisms Preparation and Disinfectant Procedures

A total of fifty-four-disc shape samples were used for this part of the study. The microorganisms have been isolated by a swab from the oral cavity and allowed to grow in their selected culture using an incubator. Specimens for bacteria and other for fungal contamination were prepared and selected from the Microbiology Laboratory at Faculty of Medicine University of Benghazi. Then, the specimens were separately placed in petri-dishes with the respective culture media. The brain heart infusion (BHI) culture media was used in Petri plates to recover/count *Staphylococcus aureus*, chocolate agar for *St. viridans* and Sabourauds dextrose agar (SDA) for *C. Albicans.*

The samples were divided into three main groups according to three different types of disinfectants; each group of samples was placed in petri dishes while the intaglio surface contacted the microorganisms (Fig. 3). Then, all samples were placed again in the incubator at 37°C for 24h to allow the microorganism to grow over the rough side of the samples. All groups were disinfected by immersion in disinfectant for 10 minutes.

Group: A consisted of eighteen samples which were disinfected by 5% Sodium Hypochlorite (SHC).

Group: B consisted of eighteen samples which were disinfected by 6% Hydrogen peroxide (HPO).

Group: C consisted of eighteen samples which were disinfected by 0.12% chlorhexidine (CHX)

Then, each group was again divided into three subgroups as following:

Group A: was divided into three subgroups GA1, GA2, and GA3.

Group1 B: was divided into three subgroups G1B1, G1B2, and G1B3.

Group1 C: was divided into three subgroups G1C1, G1C2, and G1C3.

Each sub group contains six samples. Five of six samples for each sub-group were colonized in the laboratory by *S. aureus, St. viridans*, and *C. albicans* respectively. The remaining single sample was considered as a control sample (Fig. 4). After the colonization of microorganisms, each group was disinfected by the corresponding disinfectant for 10 minutes, and then the samples were placed in their respective culture media and incubated at 37°C for 24 hours. After that the samples were checked for re-colonization of microorganisms in respect to disinfectant agent.

2.3. Surface Roughness Evaluation

A total of twenty samples of heat cure acrylic were used. The surface roughness (Ra-µm) was analyzed with a surface roughness profilometer (Mitutoyo SJ-210, Mitutoyo Corporation, Tokyo, Japan) having a diamond stylus (tip radius 5µm). The surface roughness is the average of the absolute values of the measured profile height of surface irregularities and measured from a mean line within a preset length of the specimen. The profilometer was set to move the diamond stylus across the specimen surface under a constant force of 4 m/N, passing across a length of 4 mm at 0.5 mm/s to the nearest of 0.01 µm. The cut-off length was 0.8 mm. An orientation jig was fabricated to position the stylus of the profilometer in the same location on the specimen for repeated measurements. The mean of the three measurements obtained from each disinfectant agent were compared to control samples (distilled water).[6] Data were analyzed with SPSS statistical software (IBM SPSS statistic; version 22). A one-way analysis of variance (ANOVA) was used to assess the effects of each disinfectant on surface roughness, followed by a Tuckey's post-hoc test among groups. The level of significance was set at p = 0.05 for all statistical analysis.

3. Results

3.1. Microbiology Evaluation

3.1.1. Effect of sodium hypochlorite (GA)

Fig. 5 shows the effect of using sodium hypochlorite as disinfectant agent on growth of microorganisms. It can be seen that there is no signs of microorganism growth (*St. viridans - S. aureus - C. albicans*), compared to the sample being immersed in distilled water, which showed a growth of microorganisms.

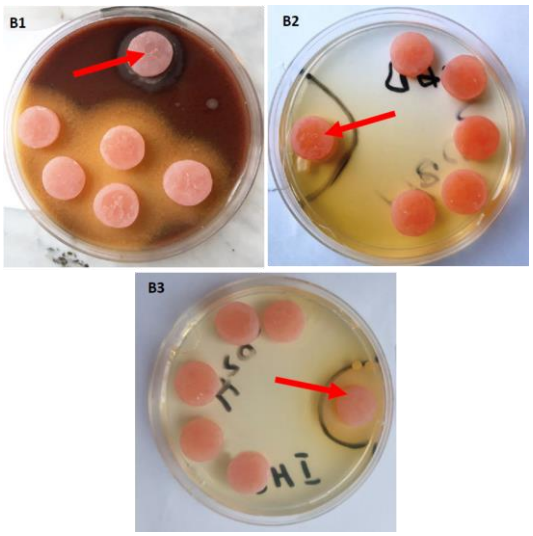

Fig. 6. Group B: Effect of hydrogen peroxide on microorganism growth. (B_1- Strepto, B_2- Candida, B_3- Staph. Arrow – distilled water control sample)

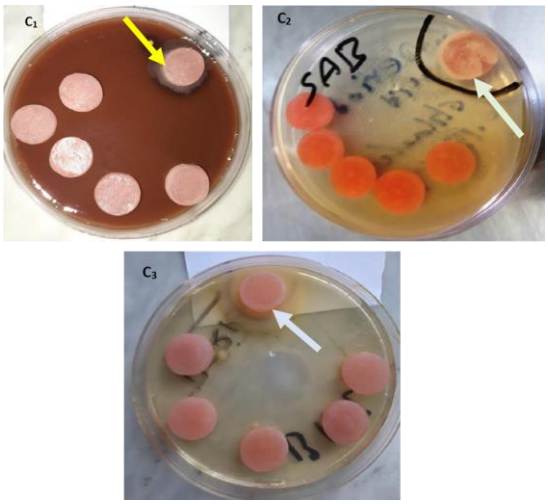

Fig. 7. Group C: Effect of chlorhexidine on microorganism growth. (C_1 - Strepto, C_2 - Candida, C_3 - Staph. C. Arrow – distilled water control sample)

3.1.2. Effect of Hydrogen peroxide (GB)

Fig. 6 shows effect of using hydrogen peroxide as disinfectant agent. It can be seen that there is signs of *St. viridans* growth, furthermore, there is no signs of *C. albicans* and *S. aureus* growth, compared to the sample being immersed in distilled water, which showed a growth of microorganisms.

3.1.3. Effect of chlorhexidine (GC)

Fig. 7 shows effect of using chlorhexidine as disinfectant agent. It can be seen that there is no signs of microorganism growth (*St. viridans* - *S. aureus* - *C. albicans*), compared to the sample being immersed in distilled water, which showed a growth of microorganisms.

Table 1. Shows effect of disinfectant agents on surface roughness (Ra-µm) of denture base material

Samples	Mean (µm)	Std. Deviation	Std. Error
SHC	349.000	32.5115	18.7705
HPO	456.000	20.5183	11.8462
CHX	536.000	32.1403	18.5562
DW	299.333	11.5902	6.6916
Dry	299.000	21.1660	12.2202

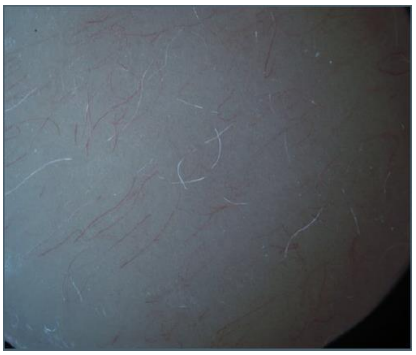

Fig. 8. Microscopic photo 100 magnification of control sample.

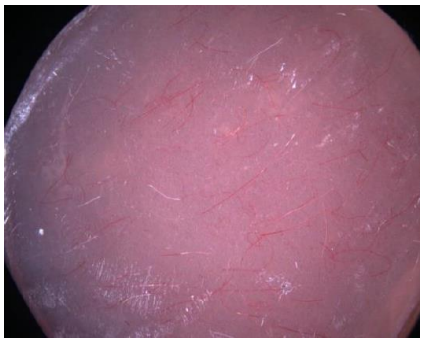

Fig. 9. Microscopic graph at 100 magnifications of sample immersed in SHC.

3.2. Surface Roughness Evaluation

Table 1 shows effect of disinfectant agents on surface roughness of denture base material. In a comparison between sodium hypochlorite (SHC), distilled water (DW) and dry samples, there was no significant differences between SHC and two control groups (DW and Dry), (P<0.180 and P<0.176 respectively). While hydrogen peroxide groups displayed higher surface roughness with significant differences compared to all tested groups (DW and Dry samples, P<0.000, SHC P<0.003, and CHX P<0.018). The CHX groups showed also higher surface roughness compared to DW, Dry and SHC samples (P<0.000), as well as with HPO P<0.018).

Furthermore, in a comparison between tested groups under microscope (Fig. 8), which shows the effect of SHC sample under microscope at 100 magnifications in comparison to the control sample (Fig. 9). It can be seen that there was no clear difference between the two groups. Furthermore, Fig. 10 shows the effect of HPO on the surface roughness of the sample under microscope at 100 magnifications, it can be seen that there was an effect on surface roughness as compared to control samples. While, Fig. 11 shows the effect of CHX on the surface of the denture base under microscope at 100 magnifications, it is a clear that the CHX was caused surface roughness to denture base samples.

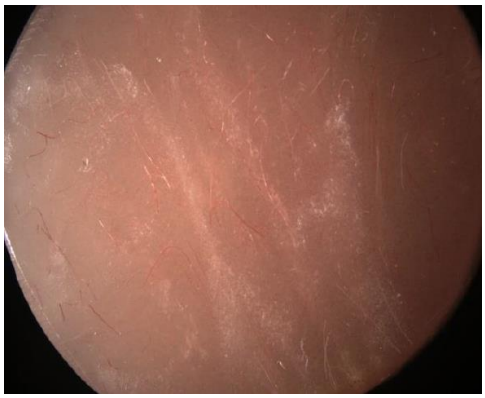

Fig. 10. Microscopic graph at 100 magnification of sample immersed in HPO.

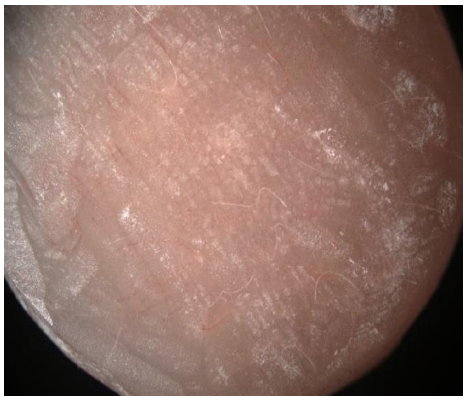

Fig. 11. Microscopic graph at 100 magnification of sample immersed in CHX.

4. Discussion

The Poly methyl methacrylate (PMMA) is the preferred material for making denture bases, due to the fact of their ability to overcome many of the deficiencies of the materials used before. Instead, removable dentures are used in critical conditions of the oral cavity.[16]

Microbial biofilm on oral tissues and surface of acrylic resin denture base is a significant part in the development of denture stomatitis. Appropriate cleaning of dentures is crucial for keeping a healthy mucosa of the oral cavity. Denture cleansing is an essential part in preventing cross contamination and improve oral health of the patients, longevity of the dentures and quality of life. Several products are designated for removal of denture biofilm and categorized into chemical and mechanical products. Cleaning using chemical products consists of placing the denture in liquids with solvent, antifungal, detergent, and antibacterial activities with or without use of brushing or ultrasonic devices. The efficacy of denture cleansers is well known; nevertheless, it is critical that continuing use for long time should not cause any negative effect on the acrylic resin denture base and their mechanical and physical properties should remain unchanged.[17]

Reports in the literature using experimental testing protocols that would allow a comparison with this current study. The purpose of immersing dental prostheses in a disinfectant solution is to inactivate infectious viruses and bacteria without damaging the dental prostheses. Srinivasan and Gulabani [18] reported that the use of chemical-based denture cleansers reduced the microbial numbers as compared to plain manual cleansing methods in complete dentures. An immersion type or chemical-based cleanser was found to be the most suitable cleanser because of its low abrasion and effective removal of organic debris. The main cleansing agents in this category are effervescent peroxide or sodium hypochlorite. The oxygen released effectively dislodges debris and creates a surface free of plaque.[19-22]

Duyck et al.[23] did a crossover randomized clinical trial and concluded that the use of cleansing tablets during overnight denture storage reduced the total bacterial count on acrylic removable dentures as compared to overnight storage in water.

The present study evaluated the effect of various denture cleansers on microorganism growth and surface roughness of heat cure denture base material. The most common disinfectant materials used in this field were sodium hypochlorite, hydrogen peroxide and chlorhexidine solution. There are controversial opinions in literature related to the effects of denture cleansers on surface roughness and hardness of denture materials. Differing compositions of cleansing solutions and materials, and different testing methods may be responsible for the controversy. The surface roughness of dental materials has been shown to be of particular importance for adhesion of oral bacteria; hence, smoother surfaces will result in denture longevity.[24]

Profilometry and its numerical data have been shown to be useful in the evaluation of the roughness of dental materials. Bollen et al. found a threshold value of 0.2 µm, suggesting that low roughness levels do not influence adhesion.[25] This study compared the efficacy of denture cleaners on contaminated specimens. Among all the agents evaluated against selected microorganisms, 5% sodium hypochlorite solution demonstrated the best cleaning effect on denture base material. No colonization was found in any of the specimens. While, the 6% hydrogen peroxide had no effect on *St. viridans*. Moreover the 0.12% chlorhexidine eliminated all selected microorganisms but it had a strong effect on surface roughness and it was the highest among the other disinfectants.

Pavarina et al,[26] also noted the effectiveness of chlorhexidine as a denture cleanser, though they used the chlorhexidine in a different concentration. In their study, the effectiveness of chemical agents (4.0% chlorhexidine gluconate, 1.0% sodium hypochlorite, and iodophors) for cleansing and disinfecting removable dental prostheses was evaluated, and it was concluded that the 4.0% chlorhexidine gluconate and 1.0% sodium hypochlorite solutions were effective in reducing the growth of the microorganisms in the 10-minute immersion period.[27]

Hydrogen peroxide is widely used to treat cuts and scrapes, but some sources warn that it doesn't reliably kill all bacteria and can even harm healing tissue. The hydrogen peroxide molecule has one more oxygen atom than a water molecule, so it acts as an oxidizer. Some bacteria can defend themselves against this, and some cannot. Hydrogen peroxide is an oxidizing agent, but it does not damage the cell as much as the superoxide anion and tends to diffuse out of the cell. Hydrogen peroxide would not be an effective choice for disinfecting linens, rooms, carpets, etc. because it is not effective enough on streptococci.[28,29]

Peracini et al.[30] demonstrated that commercially available alkaline peroxide denture cleansers altered the color, increased the surface roughness, and reduced the flexural strength of heat-polymerized acrylic denture base resin.

5. Conclusions

Despite the limitations of this study, it can be concluded that the 5% sodium hypochlorite and 0.12% chlorhexidine have great ability to eliminate all selected microorganisms from the samples after 10 minutes of immersion, while hydrogen peroxide had no ability to eliminate the *St. viridans* from all samples. In addition, 5% sodium hypochlorite had no significant effect on surface roughness in comparison to other disinfectants (0.12% chlorhexidine and 6% hydrogen peroxide). Although, 12% chlorhexidine had eliminated all microorganisms from sample surfaces but it had a significant effect on roughness in comparison to other disinfectants.

From a financial point of view the 5% sodium hypochlorite is cheaper than the other disinfectants and it is available in domestic markets. Whereas, the other disinfectants (0.12% chlorhexidine and 6% hydrogen peroxide) are more expensive and they are only found in certain places. Therefore, from the results of the current study it can be recommended that the 5% sodium hypochlorite is the most appropriate disinfectant agent in prosthodontics to clean dentures; this finding was supported and approved by many researchers.

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Conflicts of Interest

The authors declare no conflict of interest.

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