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The Effect of Five Disinfection Methods on *Candida albicans*-Infected Chair-Side Soft Liners: A Comparative In Vitro Study

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Abstract

Background: Soft-liners, particularly used for patients with poor hygiene habits, can become a favorable environment for the aggregation of microorganisms. Therefore, disinfection is considered a critical process for preserving the health of oral mucosa. This study aimed to determine the antifungal efficacy of five different disinfectant methods on silicone resilient lining materials.

Material and Method: A total of 60 test samples were prepared and inoculated with 2 ml of *Candida* solution and incubated for 2 hours. The inoculated specimens were then randomly divided into six groups (n=10): Control group, 0.5% sodium hypochlorite (NaOCl) group, Corega® denture cleansing tablet group, microwave water group, diode laser group, and 4% iodine solution group. A swab was taken from the surfaces of the soft-liner discs in each group, planted on aerobic culture media, and the colonies were counted separately for each treatment.

Results: A significant difference ($p < 0.05$) in inhibiting the growth of *Candida albicans* was observed with all five types of disinfection methods compared to the control group.

Conclusion: According to the results, the iodine group and the laser group demonstrated the best disinfection effects, respectively.

Keyword: *Candida albicans; resilient soft liner; denture disinfection; diode laser; denture hygiene.*

1. Introduction

Denture stomatitis may affect 65% of denture wearers [1], and *Candida albicans* is identified as one of the primary etiological agents [2]. *Candida albicans* adheres to dental resin materials, both soft and hard denture liners, as well as acrylic resin used in the construction of any denture prosthesis [3-7]. Soft liner materials are typically used to reline dentures to evenly distribute forces during motion. They are mainly employed in patients with sharp or atrophied alveolar ridges, or those with mucosa that is too thin to tolerate the load applied by the dentures [8, 9].

Many studies have shown that the use of soft liners can promote the development of fungal biofilms over extended periods [10, 11]. The colonization of soft liners by *Candida albicans* increases in the presence of saliva [12-15]. Fungal adhesion to liner surfaces marks the beginning of colonization [16,17], and the fungi can penetrate the soft liner [10, 18]. This is particularly undesirable as it

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decreases the likelihood of effective disinfection using surface-active agents available on the market [19].

Soft liners are prone to infection in the oral cavity, and they are difficult to clean effectively [20,21]. The use of systemic antifungal agents such as nystatin, amphotericin B, or fluconazole may lead to resistance in *Candida albicans* on denture surfaces [22,23]. Topical disinfection methods, including sodium hypochlorite, hydrogen peroxide, and chlorhexidine gluconate, have been shown to cause undesirable side effects, such as changes in the properties of the soft liner [24,25].

In a study by Al-Dwairi et al., exposure of polymethyl methacrylate to nystatin led to an increase in wettability and surface roughness of the denture [26]. Microwave irradiation [27-29] and plasma treatment [30,31] have been proposed as alternatives to decrease *Candida albicans* adhesion. Since the development of the laser by Maiman in 1960 [32], lasers have been widely used in various fields, including dentistry, to reduce denture tissue surface porosity and prevent *Candida* colonization [33-35]. Recent studies have focused on treatment rather than prevention of denture stomatitis [33, 36-39].

Basso et al. suggested that low-level laser therapy (LLLT) had a limited effect on microorganisms [40]. The therapeutic effect of diode lasers on denture stomatitis has recently been investigated [41]. This study aims to evaluate the effectiveness of five different disinfection methods on chair-side silicone resilient lining materials contaminated with *Candida albicans*.

This study seeks to investigate the antifungal efficacy of five different disinfection methods on silicone resilient soft liners contaminated with *Candida albicans*. The specific goals are:

1. To evaluate the antifungal effects of five disinfection methods (Iodine, 940nm diode laser, NaOCl, Corega denture cleansing tablets, and microwave treatment) on the growth of *Candida albicans*.
2. To determine the most effective disinfection method in reducing the colony-forming units of *Candida albicans* on soft liners used in dental prostheses.
3. To analyze the differences in effectiveness of each disinfection method, taking into account their mechanisms of action and exposure times.
4. To explore the potential side effects of disinfection on denture material properties, such as surface integrity, roughness, and durability, ensuring an optimal balance between disinfection efficacy and material preservation.
5. To provide clinical recommendations for dental practitioners on the most effective and practical disinfection protocols for maintaining denture hygiene and preventing infections in prosthesis wearers.

2. Materials and Methods

The resilient liner used in this study was a commercial silicone-based material, Proclinic Expert (DETAX GmbH & Co. KG, Ettlingen, Germany). The material is based on polysiloxanes. Samples were prepared using a custom-made plastic mold of uniform size (10-mm diameter, 2-mm thickness) with smooth surfaces by placing a glass slide over the top, as described by Nikawa et al. [3].

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The soft-liner material was mixed using supplied automix cartridges and placed directly into the plastic mold at room temperature (20°C ± 5°C and 50% ± 5% relative humidity). To ensure uniformity, all samples were prepared by the same researcher. A total of 60 test samples were prepared (n=10 per disinfection method and 10 control samples) and allowed to auto-polymerize for 30 minutes at room temperature. Samples were stored in distilled water for one week prior to inoculation.

3. Inoculum and Growth

Candida albicans was grown aerobically at 37°C on Sabouraud Dextrose Agar (SAD) and incubated for 24–48 hours. The growth was inoculated with nutrient broth, suspended in PBS, and glucose solution. The concentration was adjusted to 10^7 CFU/ml using distilled water by comparing the optical density to 0.5 at 540 nm with McFarland's standard solution.

The acrylic resilient liner specimens were inoculated with 2 ml of Candida solution, incubated for 2 hours, washed gently with water three times, and air-dried. A smear was taken from the contaminated liner surface, planted on SAD media, incubated at 37°C for 24–48 hours, and colonies were counted as a standard control.

4. Experimental Groups

The inoculated samples were randomly divided into six groups (n=10):

1. **Control group (CG):** Immersed in 37°C distilled water.
2. **NaOCl 0.5% group:** Immersed in 0.5% sodium hypochlorite for 10 minutes per day.
3. **Corega® denture cleansing tablet group (Corega):** Dissolved in 200 ml of warm tap water (40°C ± 2°C), immersed for 3 minutes per day.
4. **Microwave water group (MW):** Exposed to 650W microwave for 3 minutes.
5. **Diode laser group (940 nm EPIC™ BIOLASE, 10W):** Irradiated for 30 seconds at 0.8W.
6. **Iodine 4% group:** Immersed in 4% iodine solution for 10 minutes per day.

All samples were subsequently rinsed for 10 s with normal saline after the disinfection procedure to remove excess disinfectant solution and stored in a plastic container. In each of the six groups, swabs were taken from the surface of the resilient liner and cultured on aerobic media at 37 °C. The samples were incubated for 24-48 hours, after which the results were recorded, and the colonies were counted for each treatment. Data were analyzed using SPSS version 27 (IBM Corp.). A significance level of $p < 0.05$ was set for statistical comparisons.

5. Results

According to the statistical analysis, all five disinfection methods showed a significant reduction ($p < 0.05$) in Candida albicans growth compared to the control group (Table 1). The highest inhibition was observed in the iodine group, followed by the diode laser group. The denture cleansing tablet group showed the least inhibition as displayed in Fig. 1.

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Table 1: The mean and standard deviation (SD) of the Colony Forming Unit/ml (CFU/ml) of all experimental groups.

Treatment	Mean ± SE (CFU/ml)	Probability
1. Control	191.40 ± 3.80 ^{A}	P < 0.001
2. Corega ® denture cleansing tablets	106.20 ± 2.42 ^{B}	
3. Iodine	50.0 ± 1.64 ^{F}	
4. 940nm Diode Laser	70.40 ± 1.78 ^{E}	
5. Microwave	93.80 ± 1.46 ^{C}	
6. NaOCl	86.80 ± 1.83 ^{D}	
Upper case different letters represented statistically different results (P < 0.05)		

When compared with the control group, the highest inhibition of Candida albicans growth was observed in the Iodine experimental group, while the lowest inhibition was observed in the denture cleansing tablet group, as shown in Fig 1.

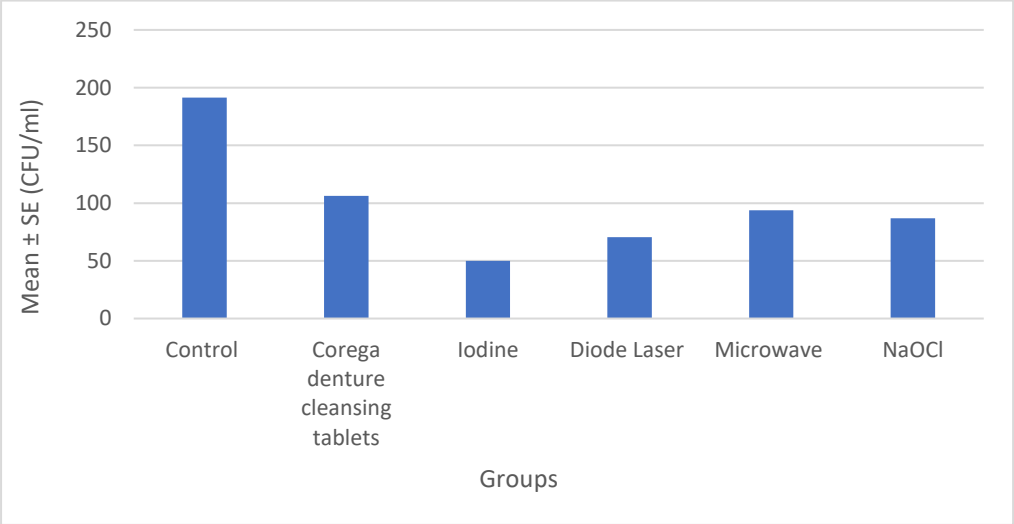

Fig. 1: The mean and standard deviation (SD) of the Colony Forming Unit/ml (CFU/ml) between the control and the experimental groups.

6. Discussion

The current study evaluated the efficacy of five disinfection methods—0.5% sodium hypochlorite (NaOCl), Corega® denture cleansing tablets, microwave irradiation, diode laser, and 4% iodine solution on Candida albicans-infected chair-side soft liners. The results demonstrated statistically significant reductions in fungal colony counts across all experimental groups as shown in Fig. 2. The most substantial reduction in Candida albicans growth was observed with 4% iodine solution and diode laser treatment, followed by NaOCl, while microwave and Corega® denture cleansing tablets displayed the least inhibition. These findings have notable implications for clinical practice, particularly in the treatment and prevention of denture stomatitis caused by Candida albicans colonization.

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Fig. 2 Images for the biofilm inhibition on the surface of the soft liner between the experimental groups (A: Corega denture cleaning tablet, B: 920nm Diode laser, C: Microwave, D: Iodine, E: NaOCL).

Effectiveness of Iodine as a Disinfectant: Iodine has long been recognized for its antimicrobial properties, especially in its ability to inactivate various microorganisms, including fungi, by disrupting cellular function through oxidative stress mechanisms. Iodine's efficacy in this study (50.0 ± 1.64 CFU/ml) aligns with previous research findings that emphasize its role in denaturing proteins and nucleotides, which leads to irreversible cellular damage in pathogens [42]. The high antifungal effectiveness of iodine observed in this study could be attributed to the increased oxidative stress and reduced antioxidant activity caused by iodine, as reported by Rufino et al. [43], which makes it an ideal disinfectant for treating biofilm-forming pathogens like *Candida albicans*.

In a clinical context, iodine's high disinfection efficacy is essential, particularly for patients with a history of denture stomatitis or those at risk of fungal infections. However, concerns about the material compatibility of iodine with soft liners remain, especially regarding the potential degradation of the soft liner's physical properties over time. The study did not assess the long-term effects of iodine on the liner material, which warrants future research focusing on the impact of prolonged iodine exposure on the physical and mechanical properties of dental prostheses.

Diode Laser as a Disinfection Method: The use of diode lasers as a disinfection tool has gained increasing attention due to their ability to reduce microbial load through photothermal effects. In this study, diode laser treatment at 940 nm demonstrated a significant reduction in Candida albicans colonies (70.40 ± 1.78 CFU/ml), second only to iodine [45]. The photochemical effects produced by the laser, particularly the inhibition of mitochondrial ATP production, have been implicated in the destruction of fungal cells [44].

Previous studies corroborate the findings of the present research, highlighting diode lasers' efficacy in reducing biofilms on denture surfaces. Maver-Biscanin et al. [46] and Seyedmousavi et al. [47] also demonstrated similar outcomes with laser treatments, albeit with different wavelengths and power settings. However, the present study reveals that although the diode laser significantly reduces the

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colony count, it does not completely eliminate fungal growth. This partial reduction could be due to factors such as insufficient irradiation time, suboptimal power settings, or mode of irradiation. Further optimization of laser parameters could potentially increase its fungicidal efficacy, making it an even more valuable tool in clinical disinfection protocols.

NaOCl as a Commonly Used Disinfectant: NaOCl is widely employed in clinical settings due to its proven antimicrobial efficacy against a broad spectrum of microorganisms, including bacteria, fungi, and viruses. The present study confirmed that 0.5% NaOCl effectively reduced Candida albicans colonies (86.80 ± 1.83 CFU/ml), consistent with previous reports of NaOCl's antifungal activity [48]. Webb et al. [49] highlighted the capacity of NaOCl to coagulate fungal cells and disrupt fungal adhesion to denture surfaces.

However, concerns remain regarding NaOCl's impact on the mechanical properties of soft liners. Several studies, including those by Yilmaz et al. [50], have indicated that extended exposure to NaOCl can lead to surface roughness and degradation of denture liner materials. The findings of this study suggest that while NaOCl is an effective disinfectant, clinicians must balance its use with the potential for material damage, particularly in patients requiring long-term use of soft liners.

Microwave Disinfection: Microwave irradiation has been proposed as a physical disinfection method capable of eliminating microorganisms through thermal and non-thermal effects. While the microwave group in this study showed a moderate reduction of Candida albicans colonies (93.80 ± 1.46 CFU/ml), it was less effective than iodine and diode laser treatments. The effectiveness of microwave irradiation in reducing microbial load has been documented in previous studies by Dixon et al. [51] and Silva et al. [53], with microwave energy shown to accelerate cellular wall changes and inactivate microorganisms.

One possible explanation for the moderate efficacy observed in this study could be the exposure time (3 minutes) and microwave power settings (650W). Studies have demonstrated that higher microwave power and prolonged exposure can enhance disinfection outcomes [54,55]. Therefore, further investigation into the optimal microwave settings for complete fungal inactivation is warranted, especially given the method's advantages in preserving the structural integrity of soft liners compared to chemical disinfectants.

Corega® Denture Cleansing Tablets: The use of denture cleansing tablets, such as Corega®, showed the lowest reduction in fungal colonies (106.20 ± 2.42 CFU/ml). These tablets primarily function through the effervescent action of alkaline peroxide, which mechanically dislodges biofilm. While they offer convenience and ease of use, their efficacy may be limited compared to more aggressive chemical disinfectants [57].

Previous studies have also highlighted the limitations of denture cleansers like Corega® in eliminating fungal pathogens such as Candida albicans. While denture cleansers are useful for routine hygiene maintenance, they may not suffice as a standalone treatment for severe infections like denture stomatitis [58]. Consequently, their use should be complemented by more potent disinfection methods, especially in clinical scenarios involving immunocompromised patients or those with persistent fungal infections.

Clinical Relevance and Future Directions: The findings of this study have significant implications for clinical practice. Iodine and diode laser treatments emerged as the most effective methods for reducing Candida albicans colonies on chair-side soft liners. These methods should be considered in

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clinical protocols for patients at high risk of fungal infections or those with recurring cases of denture stomatitis. However, the long-term effects of these disinfection methods on denture materials, including surface roughness, hardness, and color stability, must be studied further to ensure they do not compromise prosthesis durability.

Future research should focus on optimizing laser and microwave treatment parameters to enhance their disinfection efficacy while preserving material properties. Additionally, more studies are needed to evaluate the combined use of mechanical and chemical disinfection methods to achieve comprehensive denture hygiene in clinical settings.

Iodine's high inhibitory effect on Candida albicans may be attributed to its concentration or the duration of treatment [42]. Studies have shown that iodine increases oxidative stress and reduces antioxidant activity [43]. It can also interact with cellular membrane structures, causing damage and loss of intracellular substances [44].

Diode lasers are known to reduce Candida colonies due to photochemical effects that decrease ATP production and degrade fungal cells [45]. These findings are consistent with previous studies [46, 47]. Although diode lasers reduced colony count, they did not completely eliminate the colonies, which may be due to laser power, irradiation duration, or mode [45].

NaOCl is widely used in hospitals due to its efficacy in eliminating microorganisms [48]. It has been shown to reduce the adhesion of Candida albicans by coagulating fungal strains and converting blastopores to hyphae [49]. Previous studies have also found significant reductions in microbial adhesion after NaOCl treatment [50].

Microwave irradiation is effective at inactivating Candida by inducing structural changes in cellular walls via thermal and non-thermal effects [51-53]. Studies have demonstrated that microwave energy accelerates these changes, disrupting microbial activity [54, 55]. Immersion of denture specimens in water during microwave treatment enhances microbial inactivation [56].

Corega® tablets showed a significant but lower reduction in Candida albicans colonies. The alkaline peroxide in these tablets reacts with water to produce hydrogen peroxide and oxygen, which exerts a mechanical cleansing effect [58]. However, the relatively short immersion time may have contributed to its lower efficacy compared to other methods.

7. Conclusion

Prosthesis hygiene plays a critical role in maintaining both the health of the soft tissue under the prosthesis and the longevity of the prosthesis itself. Based on the findings of this study, the following conclusions can be drawn:

1. All five disinfection methods significantly reduced the viability of Candida albicans on resilient liners.
2. Immersion in 4% iodine solution for 10 minutes per day was the most effective method, followed by the 940nm diode laser and NaOCl solutions.
3. Disinfection methods such as Corega denture cleansing tablets and microwave treatment, while effective, showed relatively lower antifungal activity compared to iodine and laser methods.
4. Dentists should be aware of the chemical components of the disinfection products used, particularly their effects on both microbial growth and denture material properties.

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5. Future research should focus on the long-term effects of these disinfection methods on denture materials, such as changes in surface roughness, hardness, and color stability, to ensure that disinfection does not compromise material durability.
6. Educating patients about proper denture hygiene and recommending effective disinfection protocols can help in preventing denture-related infections, especially denture stomatitis caused by Candida albicans.

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