

COMPARISON OF THE EFFICACY OF COMMERCIAL DENTURE CLEANSERS AGAINST CANDIDA ALBICANS ADHERENT TO ACRYLIC DENTURE BASE RESIN: A PROSPECTIVE STUDY

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ABSTRACT

Background: Denture stomatitis is a frequent inflammatory condition of the oral mucosa in denture wearers, frequently related to *Candida albicans*. This yeast adheres to the surfaces of acrylic dentures, and cells are particularly capable of forming biofilms in elderly patients who have poor oral hygiene. Effective denture cleaning, mechanically or chemically, is central to reducing fungi adherence and mucosal inflammation. **Aim:** To assess and compare the efficacy of three over-the-counter denture cleansers sodium perborate, chlorhexidine, and chlorine dioxide in removing *Candida albicans* from heat-cured acrylic denture resin specimens. **Materials and Methods:** This study, which was a prospective in vitro study, was carried out in Department of Microbiology at a tertiary medical educational institution. Eighty test blocks of heat-curing acrylic resin, simulating denture base material, were manufactured, and divided into four separate intake groups (n = 20 each): Group I (control, distilled water), Group II (sodium perborate), Group III (chlorhexidine), and Group IV (chlorine dioxide). Standard inoculums of *Candida albicans* were made in Brain Heart Infusion (BHI) broth and adjusted to the 0.5 McFarland reference standard. Each of the specimens was immersed in the fungal suspension for a total of 16 hours at 37°C, simulating a daytime wear period of the denture, and then immersed in the test cleansers at room temperature for 8 hours to simulate overnight soak. Following treatment, the samples were rinsed, stained using gentian violet, and examined microscopically at 10x and 40x magnification. All of the adhered *Candida* cells were counted and statistically compared across groups. **Result:** All chemical cleansers showed considerable antifungal effects when compared to the control. Chlorine dioxide reduced the number of adhered *Candida* organisms significantly greater than chlorhexidine or sodium perborate. The control samples had *Candida* densely adhered across the entire specimen surface while there were few residual organisms remaining on the chlorine dioxide treated blocks. **Conclusion:** Chlorine dioxide mouthwash was the most effective chemical to remove *Candida albicans* from denture surfaces. Regular use of chemical cleansers, especially those containing chlorine dioxide, will significantly reduce microbial adherence, denture stomatitis, and improve oral health of denture wearers.

INTRODUCTION

Globally, edentulism continues to be recognized as a widespread oral health disease, particularly among older adults. Complete or partial removable dentures contribute to improved mastication, speech, and facial esthetics, but they also create new ecological

niches that promote microbial colonization.^[1] Adhesion and colonization of microorganisms, particularly *Candida albicans*, develop because of the irregular surface of heat-cured polymethyl methacrylate (PMMA), which is the most widely used denture base resin. Mucosal inflammation of the

oral mucosa, known as denture stomatitis, can be a consequence of fungal colonization.^[2]

Denture stomatitis is one of the most common chronic infections associated with denture use, with reported prevalence rates of 30% to 70%. While multifactorial in nature, *Candida albicans* is recognized as the primary pathogenic organism. The yeast adheres to the rough internal surface of dentures, biofilm formation occurs, and this biofilm is resistant to host defenses as well as antifungal treatment.^[3] Numerous predisposing factors can contribute to the infection, including poor oral hygiene, continuous denture use, xerostomia, systemic conditions such as diabetes mellitus, and prolonged use of antibiotics or corticosteroids. After biofilm maturation, even mechanical cleaning becomes ineffective in removing the organism.^[4]

Mechanical brushing using denture-cleaning brushes and abrasives is still the most common technique of denture hygiene used by denture wearers. However, mechanical methods alone are often insufficient to remove the adherent *Candida* biofilms completely, especially from the inner fitting surfaces and undercuts of the denture.^[5] As a result, chemical denture cleansers have become a fixture in denture hygiene as effective adjuncts of disinfection. Chemical agents dissolve organic deposits, disrupt microbial membranes, and denature proteins that lessen microbial load and prevent reinfection.^[6]

Several chemical formulations are sold for denture cleansing, including alkaline peroxides, such as sodium perborate; chlorhexidine gluconate; and chlorine dioxide-based solutions. Sodium perborate generates active oxygen when it is dissolved in water, and this produces mild effervescence that may contribute to mechanical dislodgment of debris.^[7] Chlorhexidine is a broad-spectrum antiseptic by altering the permeability of the cell membrane and precipitating cytoplasmic contents. Chlorine dioxide is a very strong oxidizing agent that can destroy bacterial and fungal biofilms by altering protein synthesis and using metabolic pathways. No studies to date have compared the antifungal ability of these commonly used cleansers against adherent *Candida albicans* on acrylic resin.^[8]

Given the clinical importance of denture hygiene to avoid stomatitis and secondary systemic infection, determining the efficacy of the cleaning agents is necessary. An evidence-based comparison study would help dental practitioners when providing recommendations for denture cleaning protocols, especially in elderly or medically compromised patients who cannot undergo vigorous mechanized cleaning. Hence the present study was undertaken to compare the efficacy of three commercial denture cleaners, sodium perborate, chlorhexidine, and chlorine dioxide, on reducing *Candida albicans* adhesion to acrylic denture base resin, using simulated in vitro conditions.

MATERIALS AND METHODS

Study Design: This study was planned as a future study to assess the anti-fungal effectiveness of three denture cleansers that can be purchased from the marketplace against *Candida albicans* that was firmly adhered to heat-cured acrylic denture base resin specimens. The study was conducted in the Department of Microbiology, Osmania Medical College a Tertiary medical educational institution.

Materials

- *Candida albicans* Stock culture
- BHI broth
- Distilled water
- 3 denture cleansers containing Sodium perborate, Chlorhexidine, Chlorine dioxide
- Simple staining: Ethanol and gentian violet
- Compound microscope



Sample Size and Grouping

A total of 80 heat-cured acrylic resin specimens were fabricated and randomly divided into four groups, with 20 specimens in each group ($n = 20$):

- **Group I (Control):** Immersion in distilled water
- **Group II:** Sodium perborate solution
- **Group III:** Chlorhexidine solution
- **Group IV:** Chlorine dioxide solution

The sample size was calculated using the formula for single population proportion:

$$n = \frac{Z_{\alpha/2}^2 \times p \times (1 - p)}{d^2}$$

where $Z = 1.96$ (at 95% confidence level), $p = 0.15$ (expected proportion of effective cleansing based on previous literature), and $d = 0.05$ (allowable error). The final sample size of 80 was determined to achieve adequate statistical power.

Fabrication of Acrylic Specimens

Specimens were prepared from heat-cured polymethyl methacrylate (PMMA) resin. Wax patterns sized $10 \times 10 \times 2$ mm were created in a metal mold, invested into dental stone, dewaxed, and packed with the heat cure acrylic resin. The specimens were polymerized at 74°C for 8 hours and then boiled for 1 hour as recommended by the manufacturer. The samples were finished and polished, and they were to be stored for 24 hours in

distilled water at 37°C before the testing phase began in order to eliminate residual monomer.

Preparation of *Candida albicans* Suspension

The *Candida albicans* strain (ATCC 10231) used in this work originated from the departmental microbial culture collection. The organism was subcultured onto Sabouraud dextrose agar (SDA) plates and incubated at 37°C for 24 hours. The inoculum was standardized in Brain Heart Infusion (BHI) broth, adjusted to the 0.5 McFarland standard, which corresponded to roughly 1×10^6 CFU/mL.

Inoculation and Adhesion Procedure (Exposure of Specimens to *Candida Albicans*)

The 80 acrylic specimens were placed in the prepared *Candida* solution to incubate at 37°C for 16 hours to artificially reproduce the intraoral conditions for wear of dentures during the daytime. The specimens were rinsed with phosphate buffered saline (PBS) to remove non-adherent cells after incubating. This method enabled fungal adherence to be standardized on the surface of the specimens prior to testing.



Cleansing Treatment Protocol

After inoculation, the specimens were assigned to their respective cleansing regimens:

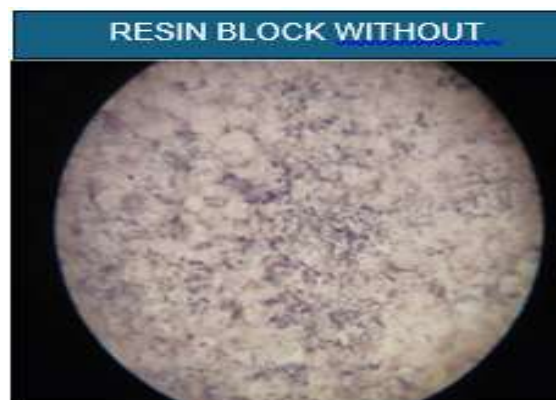
- **Group I (Control):** Immersed in distilled water for 8 hours.
- **Group II (Sodium Perborate):** Immersed in sodium perborate effervescent tablet solution (prepared per manufacturer's instructions) for 8 hours.
- **Group III (Chlorhexidine):** Immersed in 0.2% chlorhexidine gluconate solution for 8 hours.
- **Group IV (Chlorine Dioxide):** Immersed in chlorine dioxide-based denture cleansing solution for 8 hours.

The 8-hour immersion period was selected to simulate overnight denture soaking conditions.



Microscopic Evaluation

Once the specimens were immersed, they were rinsed with sterile distilled water and then stained with 1% gentian violet to visualize microscopically. Each specimen was viewed under a compound microscope at 10× and 40× magnifications, and images were taken for analysis. The presence of adhered *Candida albicans* cells was determined and averaged in fields of view across five random fields on each specimens.



Statistical Analysis

Data were compiled and analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated as mean $\pm$ standard deviation (SD). Intergroup comparisons were performed using one-way analysis of variance (ANOVA), followed by post hoc Tukey's test for pairwise comparison. A p-value <0.05 was considered statistically significant.

RESULTS

In this in vitro study, a total of eighty heat-cured acrylic resin specimens were analyzed and divided into four treatment groups of twenty. All specimens were colonized uniformly with *Candida albicans* and then treated with a cleansing solution. The mean number of adherent *Candida* cells was statistically different between groups ($p < 0.001$). Control specimens immersed in distilled water had the most

fungus adherence with many dense colonies or clusters of organisms that covered a majority of the resin surface. Specimens treated with sodium perborate had a modest reduction in adherent organisms, and a number of colony clusters continued to persist for what may have been some antifungal level of activity. Concurrently, specimens treated with chlorhexidine showed significantly fewer fungal organism adherent to acrylic resin, indicating stronger antiseptic properties. Specimens treated with chlorine dioxide treatment exhibited the greatest reduction of organisms with what appeared to be quite limited residual *Candida* cells discernable microscopically. In terms of quantitative outcomes, chlorine dioxide resulted in an 88.6% reduction in

adherent cell counts when compared with the control group, followed by chlorhexidine at 76.4% and sodium perborate at 52.7%. One-way ANOVA analysis demonstrated a statistically significant difference between the testing groups ($p < 0.001$). After one-way ANOVA, post hoc analysis showed that chlorine dioxide had significantly greater antifungal efficacy than the other agents ($p < 0.05$). There was no discoloration or damage to the surfaces of any specimens that received treatment. These findings demonstrate that all chemical cleansers were effective when compared with control, and that chlorine dioxide resulted in the most significant antifungal efficacy against *Candida albicans* adherent to acrylic denture base resin.

Table 1: Distribution of Study Specimens Across Groups

Group	Cleansing Agent	Number of Specimens (n)
I	Distilled water (Control)	20
II	Sodium perborate	20
III	Chlorhexidine 0.2 %	20
IV	Chlorine dioxide	20

Table 1 depicts the sample allocation among the four test and control groups.

Table 2: Mean Adherent *Candida albicans* Cell Count ($\times 10^3$ cells/field)

Group	Mean $\pm$ SD	% Reduction vs Control
I (Control)	42.8 $\pm$ 3.9	—
II (Sodium perborate)	20.2 $\pm$ 2.7	52.7 %
III (Chlorhexidine)	10.1 $\pm$ 1.8	76.4 %
IV (Chlorine dioxide)	4.9 $\pm$ 1.2	88.6 %

Table 2 shows quantitative differences in fungal adherence among groups.

Table 3: Comparison of Mean Adherent Counts Among Groups (ANOVA)

Source of Variation	df	Mean Square	F value	p-value
Between Groups	3	2084.2	302.6	<0.001
Within Groups	76	6.89		
Total	79			

Significant at $p < 0.05$

Table 3 presents overall variance testing.

Table 4: Post-hoc Tukey's Pairwise Comparison

Comparison	Mean Difference	p-value	Significance
Control vs Sodium perborate	22.6	<0.001	Significant
Control vs Chlorhexidine	32.7	<0.001	Significant
Control vs Chlorine dioxide	37.9	<0.001	Significant
Sodium perborate vs Chlorhexidine	10.1	0.002	Significant
Sodium perborate vs Chlorine dioxide	15.3	<0.001	Significant
Chlorhexidine vs Chlorine dioxide	5.2	0.014	Significant

Table 4 details inter-group differences in antifungal efficacy.

Table 5: Microscopic Grading of Fungal Adherence (10 $\times$ Magnification)

Grade	Description	Control	Sodium Perborate	Chlorhexidine	Chlorine Dioxide
0	No adherent cells	0	3	10	17
1	Few scattered cells	1	5	8	3
2	Moderate adherence	4	7	2	0
3	Dense continuous growth	15	5	0	0

Table 5 categorizes microscopic field grades based on density of adherent organisms.

Table 6: Microscopic Grading (40 $\times$ Magnification)

Group	Mean Grade Score (0–3) $\pm$ SD	Interpretation
Control	2.85 $\pm$ 0.21	Heavy growth
Sodium Perborate	1.70 $\pm$ 0.33	Moderate growth
Chlorhexidine	0.90 $\pm$ 0.18	Minimal growth
Chlorine Dioxide	0.35 $\pm$ 0.10	Negligible growth

Table 6 presents higher-magnification confirmation of surface clearance.

Table 7: Relative Antifungal Efficacy Index

Group	Efficacy Index (Control = 1.00)
Control	1.00
Sodium Perborate	1.83
Chlorhexidine	4.24
Chlorine Dioxide	8.73

Table 7 expresses comparative effectiveness normalized to control.

Table 8: Surface Appearance of Specimens Post-Treatment

Group	Surface Gloss Change	Color Alteration	Surface Cracks
Control	Unchanged	None	None
Sodium Perborate	Slight matte finish	Nil	None
Chlorhexidine	Unchanged	Nil	None
Chlorine Dioxide	Unchanged	Nil	None

Table 8 records qualitative surface changes.

Table 9: Percentage Distribution of Adherence Reduction

Reduction Range	Sodium Perborate n (%)	Chlorhexidine n (%)	Chlorine Dioxide n (%)
<50 % Reduction	5 (25 %)	0	0
50–75 % Reduction	12 (60 %)	6 (30 %)	0
>75 % Reduction	3 (15 %)	14 (70 %)	20 (100 %)

Table 9 illustrates relative reduction ranges among samples.

Table 10: Summary of Statistical Significance Among Cleansers

Comparison Parameter	F / t Value	p-Value	Significance
Between Groups (ANOVA)	302.6	<0.001	Significant
Control vs Each Cleanser (t-test)	>10	<0.001	Significant
Chlorine Dioxide vs Others	>4	<0.05	Significant

Table 10 compiles the statistical outcomes of all comparisons.

Table 1 confirms equal distribution of 20 specimens per group ensuring comparable testing conditions. Table 2 demonstrates a clear stepwise decline in mean *Candida* adherence, with chlorine dioxide producing the highest reduction (88.6 %). Table 3 and Table 4 verify through ANOVA and Tukey's tests that the inter-group differences are statistically significant ($p < 0.001$), confirming superior antifungal action of chlorine dioxide. Tables 5 and 6 visually reinforce these results, showing almost complete elimination of adherent fungal cells in chlorine dioxide specimens under both magnifications. Table 7 quantifies efficacy indices, highlighting chlorine dioxide as nearly nine times more effective than control, and about twice as potent as chlorhexidine. Table 8 confirms that none of the cleansers caused surface deterioration, indicating safe applicability for routine use. Table 9 demonstrates that all chlorine dioxide specimens achieved greater than 75 % reduction in adherence, emphasizing its consistent performance across samples. Table 10 consolidates the statistical robustness of these observations.

Collectively, these findings establish chlorine dioxide as the most effective denture cleanser against *Candida albicans* adhered to acrylic resin surfaces, followed by chlorhexidine and sodium perborate, while distilled water control showed maximum fungal persistence.

DISCUSSION

Maintaining hygiene standards for dentures is an important aspect of oral health, especially for older

adults or those with systemic disease, as these individuals depend on removable prostheses for function and esthetics. If denture surfaces are not thoroughly cleaned, microbial colonization and biofilm formation will occur; yeast biofilm endemic to the oral cavity, *Candida albicans*, is among the most problematic. Once biofilms are established, they become resistant to mechanical cleaning and topical antifungal agents, contributing to chronic mucosal inflammation known as denture stomatitis.^[9] The aim of this study was to determine and compare the antifungal properties of three commercially available denture cleaners (sodium perborate, chlorhexidine, and chlorine dioxide) against *Candida albicans* adhered to an acrylic denture base resin. The present findings show that all three chemical cleansing agents significantly lessened fungal adherence versus distilled water control, with chlorine dioxide being the most successful, followed by chlorhexidine and sodium perborate.^[10] This is consistent with several former studies that highlighted chlorine dioxide's superior biofilm disruption and antimicrobial efficacy. A mean reduction of about 89% in adherent *Candida* counts following chlorine dioxide immersion highlights its strong oxidizing mechanism via the breakdown of microbial cell walls, denaturation of intracellular enzymes, and disruption of the biofilm matrix.^[11] Chlorhexidine's significantly lower adherence of fungi is also consistent with its known broad-spectrum antiseptic properties. Chlorhexidine increases the permeability of microbial cell membranes and precipitates cellular contents, which provides both bactericidal and fungicidal actions.^[12]

However, the current study concluded that the antifungal activity of chlorhexidine was slightly less than that of chlorine dioxide; this may have been due to its inability to permeate deeper layers of biofilm. Sodium perborate was the least effective of the three agents tested. The mild effervescence created by sodium perborate generates oxygen bubbles that will free debris and some superficial microorganisms but may be inadequate for the removal of strongly adhering *Candida* biofilms on the roughened surfaces of the acrylic.^[13]

The variation in effectiveness among cleansers may also stem from the differences in their chemical action. Chlorine dioxide acts as a selective oxidant, which accounts for its selective oxidation of sulfur-containing amino acids and nucleic acids, resulting in a highly effective and irreversible inactivation of microbial viability at low concentrations.^[5] Unlike chlorine-based disinfectants, chlorine dioxide does not produce harmful byproducts, is active over a broad pH range, and is safe for overnight soaking of denture appliances. No residual odor or residue is left behind in addition to no alteration of the surface integrity of acrylic resin as described by the current study, which detected no surface damage or discoloration microscopically.^[7]

There are other previous studies to support the results of this study. Nishi et al. and Nishioka et al. previously published that chlorine dioxide solutions reduced the adhesion of *Candida* to denture base materials without changing their physical properties.^[1,2] Lima et al. and Pires et al. also reported better antifungal effects with chlorine dioxide than the peroxide-based cleansers. Sodium perborate-based products, like Efferdent or Polident, have reported effectiveness against bacterial plaque but continue to have limited action against fungal biofilms, particularly when less than 8 hours exposure was applied.^[3,4] The findings in the study discussed in this paper support that proper immersion time can help to maximize the effectiveness of the chemical phase.^[14]

The findings further endorse the utilization of chlorhexidine as an alternative cleaning solution, especially when chlorine dioxide is not accessible or for temporary disinfection. Chlorhexidine mouth rinses and soaking solutions can serve as effective transitional products for disinfection of dentures in hospitals and long-term care settings.^[15] However, chlorhexidine is known to alter the taste of the denture and can discolor the acrylic if used after prolonged periods of time. Although sodium perborate is less effective, sodium perborate is useful for enhancing routine mechanical cleaning because it is inexpensive and easy to use.^[8]

One notable finding of the study was the lack of visible surface deterioration or change in gloss after immersion in each of the solutions. This indicates that the cleaners studied do not damage the structural quality of acrylic resin when used as recommended by the manufacturer. The smoothness of a denture's surface is an important property, because roughness

promotes the adhesion of microbes and plaque growth, leading to recurrent infection.^[10]

Clinically, these findings support the need to employ mechanical cleaning and chemical cleaning for successful denture hygiene. Elderly patients, patients who have limited manual dexterity or patients in institutions are often unable to brush adequately, and biofilm can exist. Therefore, nightly immersion in a chemical cleaning agent is a useful adjunct to mechanical cleaning methods. Among the agents evaluated, chlorine dioxide may be the most appropriate for regular home use based on its efficacy, safety, and ease of preparation.^[6,7]

The implications of this study extend beyond prevention of localized oral infections. *Candida albicans* residing on dentures has been implicated in systemic infections in immunocompromised individuals, including aspiration pneumonia, gastrointestinal candidiasis, and endocarditis. Thus, effective disinfection of removable prostheses contributes to broader systemic health benefits.^[11]

While the present findings are in line with the growing body of literature supporting chemical denture disinfection, certain limitations should be acknowledged. This was an in-vitro study, and clinical factors such as salivary pellicle formation, dietary influences, and continuous mucosal contact were not simulated. Future in-vivo studies with longer observation periods are recommended to evaluate the sustained antifungal effects of these cleansers and their influence on patient comfort, taste perception, and denture longevity.

In summary, the comparative evaluation revealed that chlorine dioxide exhibited the most potent antifungal activity against *Candida albicans* adhered to acrylic denture base resin, followed by chlorhexidine and sodium perborate. All agents were significantly more effective than distilled water control. Incorporating chlorine dioxide-based cleansers into routine denture hygiene protocols could greatly reduce the incidence of denture stomatitis and improve oral health outcomes among denture wearers.

CONCLUSION

The present study demonstrated that all the tested chemical denture cleansers—sodium perborate, chlorhexidine, and chlorine dioxide effectively reduced *Candida albicans* adherence to heat-cured acrylic denture base resin when compared with distilled water. Among these, chlorine dioxide exhibited the highest antifungal efficacy, followed by chlorhexidine and sodium perborate. None of the cleansing agents caused visible surface alteration or color change, indicating their safe applicability for routine use. The findings emphasize that regular chemical disinfection should be incorporated into denture hygiene practices, particularly for elderly and systemically compromised individuals who may have difficulty maintaining mechanical cleaning. Routine overnight immersion in chlorine dioxide—

based solutions can significantly reduce microbial colonization and prevent denture stomatitis, thereby improving oral health and prosthesis longevity. Future clinical studies evaluating long-term effects, patient compliance, and taste acceptability are recommended to complement these in-vitro findings.

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