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Development of an In Vitro Denture Stomatitis Model Simulating *Candida albicans* Biofilm Formation and Detachment Under Daily Antiseptic Immersion

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Article

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Abstract

Denture stomatitis is frequently attributed to persistent polymicrobial biofilms on prosthetic surfaces, which pose significant therapeutic challenges for conventional antifungal protocols. To address these issues, this study developed a novel in vitro experimental framework to examine the maturation of *Candida albicans* biofilms on denture base substrates under controlled, clinically relevant conditions. Heat-polymerized acrylic resin specimens were fabricated in accordance with ISO 20795-1:2013, with surface roughness polished to below 0.2 µm to ensure standardization. Following inoculation with *C. albicans* and incubation for 24, 48, or 72 h, the efficacy of 0.5% sodium hypochlorite, 0.12% chlorhexidine gluconate, and a deionized water control was evaluated using sonication and MTS assays over a 10-day longitudinal treatment period. Compared to the deionized water control, treatment with the cleansing agents resulted in significant reductions in *C. albicans* loads across both the 24 h and 48 h groups. Furthermore, linear regression analysis demonstrated a significant relationship among treatment duration, cleansing agent type, and *C. albicans* incubation time in both groups (P = 0.05). This study established a reproducible in vitro framework to characterize biofilm dynamics and evaluate the antiseptic efficacy of denture-cleansing agents, facilitating the development of evidence-based strategies for managing denture stomatitis prior to in vivo validation.

Introduction

Removable complete and partial dentures remain one of the most widely used methods for rehabilitating partially or fully edentulous patients worldwide[1]. Despite continuous technological advances in dental biomaterials, most denture bases are still fabricated from poly (methyl methacrylate) due to its favorable handling properties, low cost, and ease of processing[2,3]. However, the acrylic surface that rests against the palatal or alveolar mucosa provides an ideal substrate for microbial colonization, and long-term denture wear is therefore frequently accompanied by opportunistic mucosal infection [3,4]. Among these, denture stomatitis (DS)—a chronic inflammatory condition of the mucosa beneath a removable denture—has been consistently identified as the most common oral mucosal lesion in denture wearers, with a worldwide prevalence ranging from approximately 15% to over 70% depending on the population sampled and the diagnostic criteria applied[5].

The etiology of DS is widely considered multifactorial; however, the predominant oral microbial factor is Candida spp. infection[6]. *Candida albicans*—which exists both as a harmless commensal colonizer of the oral mucosa in 30–70% of healthy individuals and as an invasive pathogen when host defenses fail—is the species most frequently isolated from the fitting surface of affected dentures and from the inflamed mucosa itself[7]. This dual behavior, formally described as the "commensal-to-pathogen" transition, is now regarded as one of the central paradigms of *C. albicans* pathobiology[8].

The pathogenicity of *C. albicans* in DS is driven primarily by its ability to adhere to acrylic surfaces and to form mature, drug-resistant biofilms that consist of yeast cells (blastospores), pseudohyphae, and true

hyphae embedded in an extracellular polymeric matrix[9]. Once the yeast form attaches to the denture surface via hydrophobic and specific protein-mediated interactions, morphological switching to the hyphal form is triggered by local cues such as surface topography, serum, and N-acetylglucosamine, and these hyphae enhance tissue penetration and resistance to mechanical removal[10]. This limitation has shifted the focus of DS prevention toward adjunctive chemical disinfection of the prosthesis, using agents such as sodium hypochlorite, chlorhexidine gluconate, peroxide-based commercial cleansers, and, more recently, polyhexamethylene guanidine hydrochloride.

Among these, 0.5% sodium hypochlorite is currently regarded as one of the most effective and economical agents for controlling *C. albicans* denture biofilm, and clinical studies have confirmed its ability to eradicate viable organisms and physically detach the biofilm from heat-polymerized acrylic[11,12]. In parallel, chlorhexidine gluconate—widely available as a 0.12% mouthrinse—has been shown to reduce the adherence of *C. albicans* to denture acrylic, both by direct fungicidal action and by pre-treating the surface to inhibit subsequent colonization[13,14]. Although both agents are recommended in clinical guidelines, there is wide heterogeneity in the published in vitro methodology used to evaluate their efficacy: sample dimensions, incubation times before intervention, CFU endpoints, and exposure durations vary considerably between studies, making it difficult to compare results or to translate them reliably to clinical practice[14–16]. The 96-well plate-based protocol standardized by Pierce and colleagues has become a reference for susceptibility testing of *C. albicans* biofilms, yet no equivalent protocol has been widely adopted for studies of biofilm development on solid PMMA denture base coupons, as specified by the ISO 20795:2013 standard for denture base polymers[15].

To address these methodological gaps, the present study was designed to develop a reproducible in vitro model of *C. albicans* colonization on heat-polymerised PMMA denture base coupons prepared in accordance with ISO 20795:2013, and to use this model to quantify the residual *C. albicans* load after standardised immersion in two commonly used denture cleansers—0.5% sodium hypochlorite and 0.12% chlorhexidine gluconate—versus deionized water control over a clinically relevant follow-up period. Specifically, the study aimed (i) to establish the time course of *C. albicans* colony formation on PMMA at 24, 48, and 72 h of colonisation, corresponding approximately to the early, intermediate, and mature biofilm phases reported in prior in vitro work; (ii) to compare the antifungal efficacy of the two cleansers using a quantitative MTS cell-viability assay anchored to a log-linear standard curve; and (iii) to examine the relationship between pre-treatment colonisation duration, cleanser type, and post-treatment residual *C. albicans* load using multiple linear regression. The hypothesis tested was that both 0.5% sodium hypochlorite and 0.12% chlorhexidine gluconate would significantly reduce the viable *C. albicans* load relative to a deionized water control, and that the magnitude of this reduction would depend on the duration of pre-treatment biofilm maturation.

Materials and Methods

This study was designed to mimic the pattern of *C. albicans* adherence as clinical denture stomatitis in vitro. The durations of *C. albicans* colony formation were assigned to 3 time points: 24, 48, and 72 hours before treatment stages. Biosafety and Biosecurity permission was approved by NUIBC (MI 64-02-04)

Acrylic resin denture base samples preparation

This experiment used 216 heat-polymerized acrylic resins (Triplex™ hot, Ivoclar Vivadent AG, Schaan, Liechtenstein). A heat-cured PMMA was prepared by mixing 23.4 g of powder with 10 ml of monomer (Methyl methacrylate stabilized, dimethacrylate) for 10 minutes until the mixture reached the dough stage; the working time was approximately 20 minutes. All the above procedures were at 23°C. Next, the dough was pressed into the silicone template, which was created by a metal mold. The sample size was 10X60X3 mm^3, and all details complied with ISO 20795:2013[17]. After that, the polymerization process was performed by placing a closed flask in cold water, heating it to 100°C, allowing it to boil for 45 minutes, and leaving it overnight before the samples were shaped into 10X10X3 mm^3. Finally, all surfaces of the PMMA sample were polished with sandpaper no. 600 to reduce surface roughness, as lower surface roughness levels below 0.2 μm are known to minimize *C. albicans* adherence[18]. Before sonicating in deionized water for 5 min to remove debris, all samples were maintained at 40% humidity to ensure dimensional stability and prevent deformation before sterilization (Motala & Vahed, 2020).

C. albicans (ATCC 90028) preparation and standard curve creation

The Sabouraud dextrose broth (Difco™ Sabouraud Dextrose Broth, REF: 238230, MD, USA) was prepared by following the datasheet. Briefly, 30 g of powder was dissolved in 1,000 ml of purified water and mixed until the powder clusters disappeared. In addition, Sabouraud dextrose agar was modified by adding 1.5 g/L of food-grade agar (JR F&B Company Limited, Bangkok, Thailand) to 1,000 ml of Sabouraud dextrose broth, and all agents were then autoclaved at 121°C. At 15 psi for 15 min, 2 mL of liquid agar was added to a well of untreated 12-well culture plates, and the broth was kept at 4°C after cooling.

A standard curve was generated by measuring the concentrations of 1–5×10^6/mL *C. albicans* using a spectrophotometer at 530 nm, with a 0.5 McFarland standard. The calibration number ranged from 0.11 to 0.14[19]. Then, that concentration was diluted 10-fold by 10^6-10^3, respectively. Next, the standard curve and formula were generated from the results of the MTS cell viability assay kit (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega, USA). The procedure was performed by sequentially adding 100 µl of the sample and 20 µl of the agent to a non-treated 96-well culture plate (NEST®, NEST Biotechnology Co., Ltd., Wuxi, China). The mixtures were gently blended to ensure homogeneity before being incubated at 37°C for 3 h in each examination to reduce bias. The result was measured by a Microplate reader (xMark™ Microplate Absorbance Spectrophotometer, BIO-RAD, California, USA) with a wavelength of 490 nm. The data were analyzed and presented using Microplate Manager software 6.0 (Bio-Rad Laboratories Inc., USA).

Incubation of heat-polymerized acrylic samples in C. albicans and treatment processes

216 heat-polymerized acrylic samples were placed in 12-well plates containing 2.5 mL of Sabouraud dextrose agar and 100 µL of a 10^2 *C. albicans* suspension in Sabouraud dextrose broth for the experimental groups. On the other hand, the control samples were placed under the same conditions but without *C. albicans*. All of them were incubated at 37°C for 24, 48, and 72 h. The 24-hour group comprised 72 samples and was categorized into 18 groups (4 samples/group; 3:1 for the experiment and control groups, respectively). All 18 groups were divided into 3 subgroups according to the different interventions: 0.5% sodium hypochlorite[20], 0.12% chlorhexidine gluconate combined with 0.5% cetylpyridinium chloride[21], and 8 h of deionized water. Then, in each group, 6 groups received the intervention. The excess *C. albicans* was removed from the samples using deionized water, and each sample was placed in 2 mL of Sabouraud dextrose broth in a 15 mL centrifuge tube. *C. albicans* load was measured at Day 0 (baseline), Day 1, Day 3, Day 5, Day 7, and Day 10 by the sonication technique with an ultrasonic machine (bath type) at 35,000 Hz, 100% intensity for 5 min (Buckingham-Meyer et al., 2022). These processes were done in the same way as the 48 h and 72 h groups.

C. albicans load measurement and Alive *C. albicans* tracing after treatment

After sonication, groups of samples were placed in new 90 mm and 35 mm culture dishes (intervention and control groups, respectively), which contained Sabouraud dextrose agar covered with 500 µL and 100 µL of Sabouraud dextrose broth. Then, each group was incubated at 37 ℃ for 72 h. The proliferation of *C. albicans* was recorded.

The sonicated Sabouraud dextrose broth in each tube was used to measure *C. albicans* loads using an MTS cell viability assay kit (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega, USA). The protocol was described the same as above.

Statistical analysis

All data were analyzed using IBM SPSS Statistics 29.0 (SPSS Inc., Chicago, USA), as *C. albicans* loads were treated as non-parametric due to the small sample sizes. First, the data from the microplate reader were converted to log10 values using the standard curve's formula. Second, these data about intra- and inter-group differences were analyzed by the Kruskal-Wallis and Mann-Whitney U tests. Finally, the relationship between *C. albicans* loads and their factors was examined using linear regression with bootstrapping. Statistical significance was set at $P < 0.05$.

Results

The standard curve and formula creation.

After 10 serial dilutions from 10^6 to 10^0 CFU/ml, the absorbance results were 1.265778, 1.009889, 0.256833, 0.068656, 0.00267, and 0.005, respectively. After that, the log10 values of both results were taken to create a linear graph and a standard curve. The log10 absorbance values were 0.102357,

0.004274, -0.59035, -1.16332, -1.32483, -1.57349, and − 2.30103, corresponding to *C. albicans* loads of 6, 5, 4, 3, 2, 1, and 0, respectively. The linear standard curve, formula, and R^2 are shown in Fig. 4.

The data were summarized, and the formula was:

$y = 2.444x + 5.390$

$r^2 = 0.969$.

The difference in *C. albicans* loads at 24 h, 48 h, and 72 h among variant conditions

The results of *the* 24 h *C. albicans* model showed variants that differed significantly across days. At baseline (Day 0), all groups showed high qualitative loads of *C. albicans*. In the 0.5% sodium hypochlorite group, the amount of *C. albicans* decreased significantly at Day 1. However, the results from the daily-treated and incubated samples showed that, on Day 3, the number of *C. albicans* significantly rebounded*. Then, the results after Day 5th revealed that the amount of *C. albicans* decreased continuously and significantly at Day 10th, compared with Day 5th. As with the 0.12% chlorhexidine gluconate, the results showed a significant decrease when compared with baseline. Even though the *C. albicans* count decreased and rebounded on Days 3rd and 5th. However, the decreasing trend in the number of *C. albicans* remains significantly different, especially when compared with Day 10th. In the last group of the 24 h section, the results reveal that although the statistics differ significantly between the baseline Day 1st and Day 3rd, Day 5th, Day $7^{th,}$ and Day 10th groups, the Day 10th group showed high *C. albicans* loads. Third, although the amount of *C. albicans* was also measured on the final day, significant differences among the groups were observed.

Next, the 48 h results also revealed significant differences among variant timelines. First, the 0.5% sodium hypochlorite group showed significant decreases from baseline at Day 1, Day 3, Day 5, Day 7, and Day 10. Second, the 0.12% chlorhexidine gluconate group showed significant differences between baseline and all groups. Moreover, significant differences were detected between Day 1 and Day 7, and between Day 1 and Day 10. In addition, *C. albicans* loads at Day 5 were significantly lower than at Day 7 and Day 10.

Lastly, the results for the 72 h group showed no significant differences across treatment dates in the 0.5% sodium hypochlorite and 0.12% chlorhexidine gluconate groups (this point was discussed later). However, in the de-ionized water group, a significant decrease was observed relative to the other groups, and the *C. albicans load* was distinctly low.

The relationship between treatment duration, cleansing agent, and differences in *C. albicans* colonized duration

The results of linear regression were presented for the 24 h and 48 h embedded acrylic resin groups, because although the trends among subgroups of the 72 h embedded acrylic resin group decreased, the number of *C. albicans* was very low. The correlation data revealed significant associations between *C.*

albicans loads and treatment duration (b = -0.405 and − 0.818 at 24 h and 48 h, respectively) and cleansing agent (b = 0.907 and 1.125 at 24 h and 48 h, respectively). Interestingly, both 24 h and 48 h were used as models to test the further cleansing agent, but there was some discrepancy in the results for the *C. albicans* load on Day 1 in the 0.5% sodium hypochlorite groups (this result was discussed later).

Table 1
The correlation between *C. albicans* loads and treatment duration, and cleansing agent

		24h embedded acrylic resin				**48h embedded acrylic resin**			
	Model	***b***	***SE B***	β	**p-value**	***b***	***SE B***	β	**p-value**
1	Constant	4.569 [3.610, 5.389]	0.460		< 0.001	5.475 [4.486, 6.465]	0.493		< 0.001
	Treated duration	-0.405 [-0.629, -0.143]	0.118	-0.429	0.001	-0.818 [-1.072, -0.564]	0.127	-0.667	< 0.001
2	Constant	2.783 [1.231, 4.231]	0.587		< 0.001	3.266 [2.108. 4.424]	0.577		< 0.001
	Treatment duration	-0.405 [-0.607, -0.186]	0.103	-0.429	< 0.001	-0.836 [-1.042, -0.630]	0.103	-0.682	< 0.001
	Cleansing agent	0.907 [0.463, 1.378]	0.215	0.460	< 0.001	1.125 [0.700, 0.430]	0.212	0.445	< 0.001

24h embedded acrylic resin: Step 1, $R^2 = 0.169$, Step 2, $\Delta R^2 = 0.211$

48 embedded acrylic resin: Step 1, $R^2 = 0.435$; Step 2, $\Delta R^2 = 0.198$

Note

The data of linear regression were revealed with beta [lower, upper bound of 95% confidence interval value]

Discussion

As background information, DS is an infection caused by multiple oral microorganisms and multifactorial causes[22]. However, this study focused mainly on the oral microorganism *C. albicans*, which is considered the causative fungus of DS[4]. We designed an experiment to simulate *C. albicans* colonization and eliminate it after denture cleansing, as a model of DS. In contrast, the study by Gould et al. in 2023 reported a three-dimensional tissue model for the interaction between the host and *C. albicans* (Gould et al., 2023). Although this study clearly demonstrated the host response to pathogens, our study focused on a simple model to test new cleansing agents or modified surface denture bases that affect *C. albicans colonization*. First, PMMA resin bases were prepared in accordance with ISO 20795:2013, the standard for the examination of denture base material quality. Then, the acrylic resin denture base sticks were cut to 10 × 10 × 3 mm^3 to reduce scale in the biological examination.

The process of *C. albicans* colonization of acrylic samples was designed to mimic real conditions. In fact, *C. albicans* colonizes and grows in the mucosal epithelium of immunocompromised hosts [23]. Then, the Sabouraud Dextrose agar acted as an oral mucosal surface; Sabouraud Dextrose agar with *C. albicans*, which coated the agar, represented the infection in the epithelium. DS results from an imbalance between host immunity and factors such as hyposalivary flow, overnight denture wear, and inadequate denture-cleaning habits[24,25]. Whatever the cause, these factors increase *C. albicans* loads. For these reasons, this study gradually designed the process to start with low *C. albicans* loads, simulating conditions in the normal oral cavity, and incubated overnight. However, in real life, it takes a very long time because the host immune response is required to eliminate the infection. Moreover, by detecting DS, the dentist identified the lesion based on its mucosal changes. Then, the primary microorganism in DS, *C. albicans*, was identified. It was a different in vivo study; based on basic knowledge, this study used agar with *C. albicans* in broth to mimic the infected oral mucosal surface. Moreover, a small broth volume limited *C. albicans* growth. High potential for *C. albicans* proliferation was observed in the initial incubation stages. According to the study results, the *C. albicans* loads and linear regression analyses demonstrated that both 24 h and 48 h incubation times were associated with treatment processes involving antiseptic agents such as 0.5% sodium hypochlorite and 0.12% chlorhexidine gluconate. Moreover, residual *C. albicans* was still found after continuous incubation (Supplementary Figs. S1–S3). Compared with the clinical treatment phase, the treatment duration is typically 10 to 14 days, consistent with epithelial cell turnover cycles, suggesting that in vitro models should ideally incorporate extended exposure periods to better approximate the sustained antimicrobial effect required to disrupt biofilm maturation[4]. In addition, the *C. albicans* load was suddenly reduced after day 3 at 24 h and after day 7 at 48 h (Supplementary Figs. S1 and S2). However, the *C. albicans* colony slowly grew and died after 72 h of incubation due to sufficient nutrition. After the initial washing or sonication to remove non-adherent cells, the remaining *C. albicans* attached to the acrylic surface matured into a biofilm [26,27]. Moreover, a frequency of 40kHz is commonly used in ultrasonic cleaning protocols to enhance cavitation, thereby facilitating the disruption of complex polymicrobial biofilms embedded within the microporosities of the denture base [27]. However, for *C. albicans* detachment, we used a 35kHz ultrasonic frequency to facilitate the removal of adherent biofilm structures from the acrylic surface, an approach also shown to be effective in the study of Kaneko et al. [28]. This

standardized mechanical intervention serves to isolate the chemical efficacy of the cleansing agents from physical biofilm disruption, as the retention of *C. albicans* is often exacerbated by hyphal penetration into heavily abraded resin surfaces[9].

In clinical treatment, the dentist suggests that the patient apply antifungal cream to both the lesion and the denture base [29]; the infected dentures are then immersed in appropriate antiseptic or cleansing solutions[25,29], emphasizing that effective biofilm management requires both chemical disruption and mechanical removal to prevent persistent colonization[30,31]. In addition, for an unfit denture, patients were advised to replace it, as chronic irritation from an ill-fitting prosthesis exacerbates mucosal trauma and creates retentive niches that facilitate fungal adherence and biofilm maturation[24]. Moreover, the dentist treats not only the DS lesion but also improves the oral environment to minimize the conditions that favor the proliferation of *C. albicans*. In addition, for patients with systemic diseases that promote the DS condition, the dentist addresses the underlying systemic health by coordinating with the patient's primary care provider to manage conditions such as diabetes or immunosuppression, which are known to exacerbate oral yeast colonization[29]. Therefore, this study aims to present a repeatable methodological framework that mimics the clinical treatment protocol for evaluating the efficacy of various denture materials, providing a baseline for comparing novel antifungal interventions against standardized chemical cleaners.

The limitation of this study was the reliance on a monomicrobial model using *C. albicans* in isolation, which fails to capture the complex, multi-species interactions and inter-kingdom signaling characteristic of natural denture biofilms[6,32]. This protocol could be modified depending on the aim of the study. Moreover, this framework requires greater repeatability and validation due to funding constraints and limited investigator capacity. In future work, this framework could be applied to incorporate dual-species biofilms, including both fungal and bacterial pathogens, to more accurately simulate the commensal and synergistic dynamics observed in clinical DS. In addition, this framework might be expanded to evaluate the efficacy of newer antimicrobial agents, which have demonstrated superior antifungal properties compared to conventional treatments, and to examine the inhibitory capacity of functionalized resins and bioactive additives designed to prevent microbial adhesion.

Conclusion

In summary, this study establishes a robust methodological framework for evaluating antifungal efficacy and surface properties of denture materials and underscores the potential to integrate antimicrobial agents directly into denture base materials to proactively mitigate *C. albicans* colonization and reduce the clinical burden of denture-induced stomatitis. The results demonstrate that 24 h and 48 h were suitable times for embedding *C. albicans* for colonization, and that post-treatment reductions were significant, supporting the efficacy of these protocols in inhibiting microbial growth on acrylic surfaces.

Declarations

Competing interests:

The authors declare no competing interests.

Ethics declarations

This study did not involve human participants or vertebrate animals. Institutional biosafety and biosecurity approval for the use of *Candida albicans* was obtained from the Naresuan University Institutional Biosafety Committee (NUIBC; approval no. MI 64-02-04).

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Author Contribution

T.K. designed this study with P.J.; P.J. prepared the acrylic resin, and T.K. performed C. albicans colonization, detachment, and quantification. T.K. wrote the major part of the manuscript with the help of P.J.; All authors reviewed and approved the final manuscript.

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Data Availability

The datasets generated and/or analyzed during the current study are available within the paper and its Supplementary Information. In addition, the raw absorbance data for Candida albicans are provided in Supplement 2.

References

1. Jayaraman, S. et al. Final-impression techniques and materials for making complete and removable partial dentures. *Cochrane Database of Systematic Reviews* **2018**, CD012256 (2018).

2. Murat, S., Alp, G., Alatalı, C. & Uzun, M. In vitro evaluation of adhesion of *Candida albicans* on CAD/CAM PMMA-based polymers. *J. Prosthodont.* **28** (2018).
3. Aguayo, S. et al. Early adhesion of *Candida albicans* onto dental acrylic surfaces. *J. Dent. Res.* **96**, 917–923 (2017).
4. Salerno, C. et al. *Candida*-associated denture stomatitis. *Med. Oral Patol. Oral Cir. Bucal* **16** (2011).
5. Gendreau, L. & Loewy, Z.G. Epidemiology and etiology of denture stomatitis. *J. Prosthodont.* **20**, 251–260 (2011).
6. Manzoor, M., Pussinen, P.J., Saarela, R.K.T., Hiltunen, K. & Mäntylä, P. Metagenomic analysis of the denture-associated oral microbiome in patients with denture stomatitis. *Sci. Rep.* **15**, 31913 (2025).
7. Hellstein, J.W. & Marek, C.L. Candidiasis: red and white manifestations in the oral cavity. *Head Neck Pathol.* **13**, 25–32 (2019).
8. Jacobsen, I.D. The role of host and fungal factors in the commensal-to-pathogen transition of *Candida albicans*. *Curr. Clin. Microbiol. Rep.* **10**, 55–65 (2023).
9. Jackson, S., Coulthwaite, L., Loewy, Z.G., Scallan, A. & Verran, J. Biofilm development by blastospores and hyphae of *Candida albicans* on abraded denture acrylic resin surfaces. *J. Prosthet. Dent.* **112**, 988–993 (2014).
10. Gow, N.A.R., Veerdonk, F.L. van de, Brown, A.J.P. & Netea, M.G. *Candida albicans* morphogenesis and host defense: discriminating invasion from colonization. *Nat. Rev. Microbiol.* **10**, 112–122 (2011).
11. Silva, P.M.B. da et al. Microscopical analysis of *Candida albicans* biofilms on heat-polymerised acrylic resin after chlorhexidine gluconate and sodium hypochlorite treatments. *Mycoses* **54** (2011).
12. Salles, M.M. et al. Antimicrobial activity of complete denture cleanser solutions based on sodium hypochlorite and *Ricinus communis* - a randomized clinical study. *J. Appl. Oral Sci.* **23**, 637–642 (2015).
13. Mima, E.G. de O. et al. Effectiveness of chlorhexidine on the disinfection of complete dentures colonised with fluconazole-resistant *Candida albicans*: in vitro study. *Mycoses* **54** (2011).
14. Pellízzaro, D. et al. Effectiveness of mechanical brushing with different denture cleansing agents in reducing in vitro *Candida albicans* biofilm viability. *Braz. Dent. J.* **23**, 547–554 (2012).
15. Pierce, C.G. et al. A simple and reproducible 96-well plate-based method for the formation of fungal biofilms and its application to antifungal susceptibility testing. *Nat. Protoc.* **3**, 1494–1500 (2008).
16. Dahlan, A.A., Haveman, C.W., Ramage, G., López-Ribot, J.L. & Redding, S.W. Sodium hypochlorite, chlorhexidine gluconate, and commercial denture cleansers as disinfecting agents against *Candida albicans*: an in vitro comparison study. *Gen. Dent.* **59**, e224–e229 (2011).
17. International Organization for Standardization. ISO 20795-1:2013 Dentistry — Base polymers — Part 1: Denture base polymers (ISO, Geneva, 2013).
18. Abuzar, M. et al. Evaluating surface roughness of a polyamide denture base material in comparison with poly (methyl methacrylate). *J. Oral Sci.* **52**, 577–581 (2010).

19. Muñoz-Escobar, A. & Reyes-López, S.Y. Antifungal susceptibility of *Candida* species to copper oxide nanoparticles on polycaprolactone fibers (PCL-CuONPs). *PLoS One* **15**, e0228864 (2020).
20. Fernandes, F.S. de F. et al. Can 0.5% sodium hypochlorite treat *Candida*-associated denture stomatitis? *Pesqui. Bras. Odontopediatria Clin. Integr.* **24**, e056 (2024).
21. Ghazzar, F.Z., Kaoun, K., Rochd, T. & Bellemkhannte, S. Comparative in vitro study of the effect of chlorhexidine and sodium hypochlorite on the colonization of delayed-setting resin by *Candida albicans*. *European Journal of Dental and Oral Health* **6**, 1–6 (2024).
22. Gleiznys, A., Zdanavičienė, E. & Žilinskas, J. *Candida albicans* importance to denture wearers: a literature review. *Stomatologija* **17**, 54–66 (2015).
23. Kirchner, F. et al. Persistence of *Candida albicans* in the oral mucosa induces a curbed inflammatory host response that is independent of immunosuppression. *Front. Immunol.* **10**, 330 (2019).
24. Han, Y., Liu, X. & Cai, Y. Effects of two peroxide enzymatic denture cleaners on *Candida albicans* biofilms and denture surface. *BMC Oral Health* **20**, 193 (2020).
25. Almeida, L. de F.D. de et al. Efficacy of citronella and cinnamon essential oils on *Candida albicans* biofilms. *Acta Odontol. Scand.* **74**, 393–398 (2016).
26. Fortes, C.V. et al. Evaluation of biofilm formation, adhesive strength and effectiveness of cleaning protocols on adhesive-containing acrylic resin specimens: an in vitro study. *Dent. Med. Probl.* **62**, 843–851 (2025).
27. Moraes, G.S. et al. Is there an optimal method to detach *Candida albicans* biofilm from dental materials? *J. Med. Microbiol.* **70** (2021).
28. Kaneko, C. et al. Effectiveness of ozone treatment, ultrasonic treatment, and ultraviolet irradiation in removing *Candida albicans* adhered to acrylic resins fabricated by different manufacturing methods. *Materials* **19** (2026).
29. Altarawneh, S. et al. Clinical and histological findings of denture stomatitis as related to intraoral colonization patterns of *Candida albicans*, salivary flow, and dry mouth. *J. Prosthodont.* **22**, 13–22 (2013).
30. Jose, A. et al. Reducing the incidence of denture stomatitis: are denture cleansers sufficient? *J. Prosthodont.* **19**, 252–257 (2010).
31. Duyck, J. et al. Impact of denture cleaning method and overnight storage condition on denture biofilm mass and composition: a cross-over randomized clinical trial. *PLoS One* **11**, e0145837 (2016).
32. Lamfon, H. Denture biofilm and denture-associated stomatitis: a literature review. *Egyptian Dental Journal* **67**, 775–787 (2021).

Figures

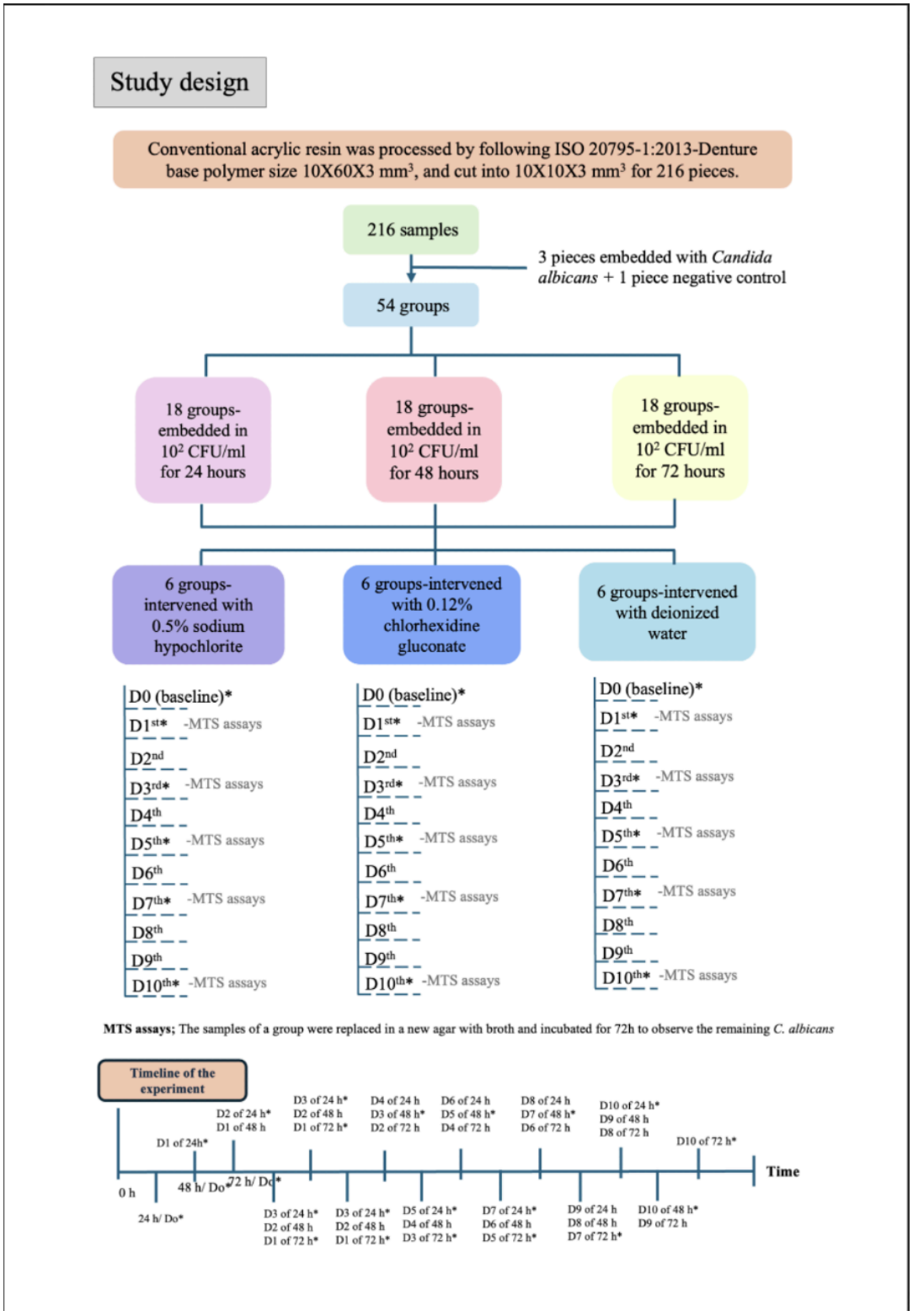


Figure 1

This diagram demonstrated the study design to evaluate C. albicans loads.

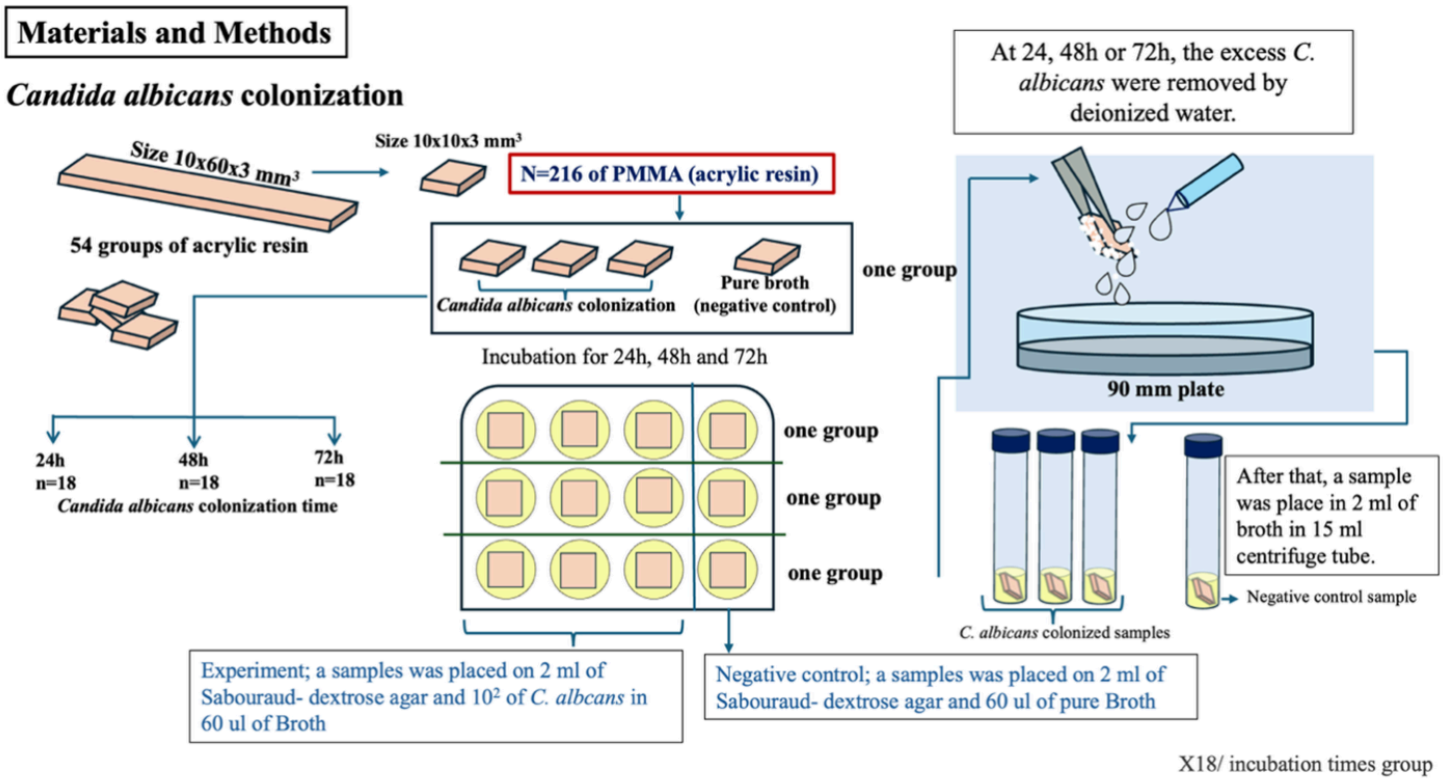


Figure 2

This picture demonstrated the preparation of acrylic resin denture base samples. After the bars were cut into small pieces (10×10×3), the debris was removed by ultrasonic bath (35kHz) for 15 min, then rinsed with deionized water and kept in 40% moisture. Then, all samples were contained in clear plastic and sealed before sterilization with UVC for 20 mins. Next, the samples were categorized into 3 groups by *C. albicans* embedding time; within each group, they were divided into 6 subgroups by treatment duration, each comprising 3 experimental and 1 control sample. The samples were placed into a 12-well plate with Sabouraud-dextrose agar and 10^2 CFU in 60 µL of Sabouraud-dextrose broth, except that the control groups contained no *C. albicans colonies.* According to the timeline, a group of resin samples that exceeded the *C. albicans* threshold was removed and placed in a 15 mL centrifuge tube containing 2 mL of broth.

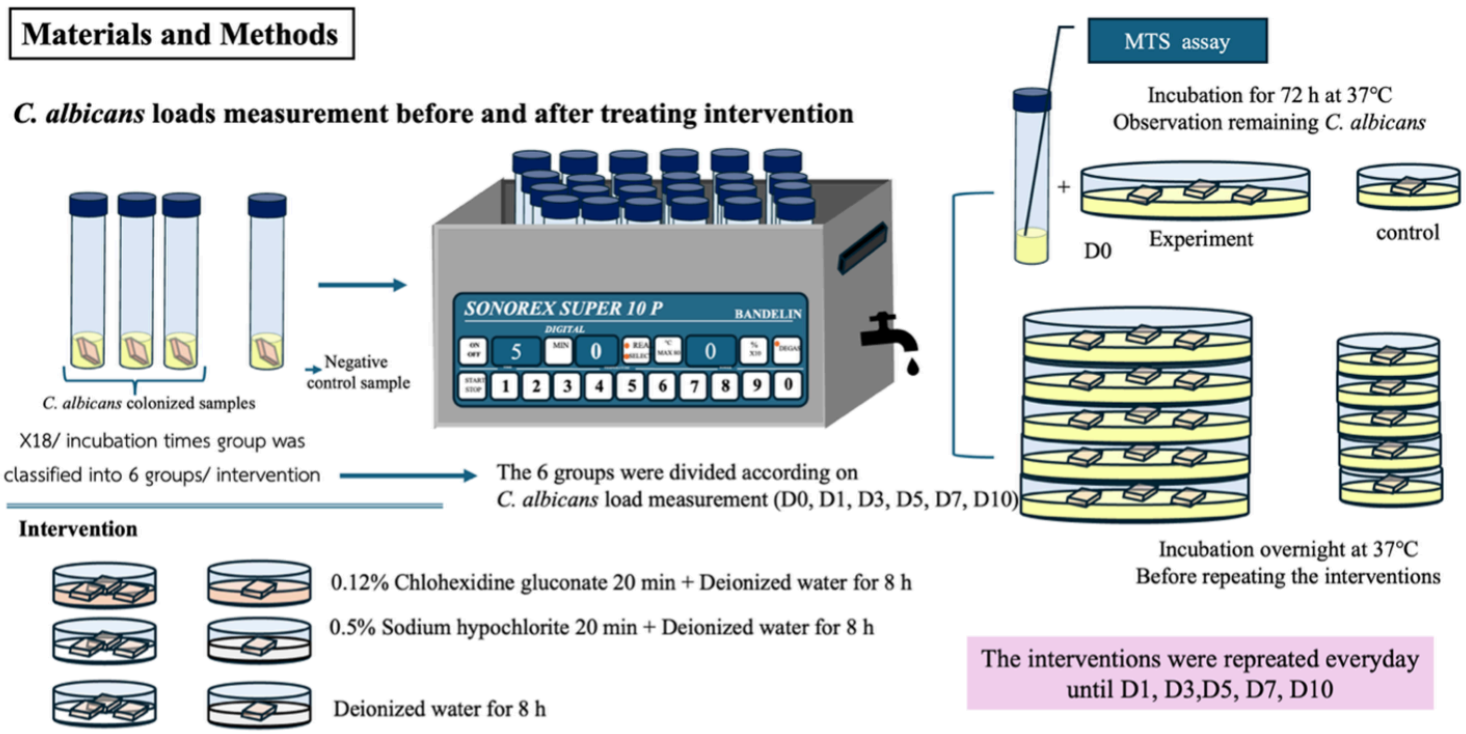


Figure 3

This picture revealed a method for detaching *C. albicans* from resins using sonication (35kHz). After that, all samples were kept in 90 mm tubes with 2.5 mL of agar and 400 µL of broth/group and incubated at 37 °C for 72 h to observe residual *C. albicans*. The C. albicans loads were measured by sonicated broth with MTS assays. For other groups, they were also kept under the same conditions, but the next day all remaining groups were treated with 2 disinfectant agents and deionized water daily until the next examination date.

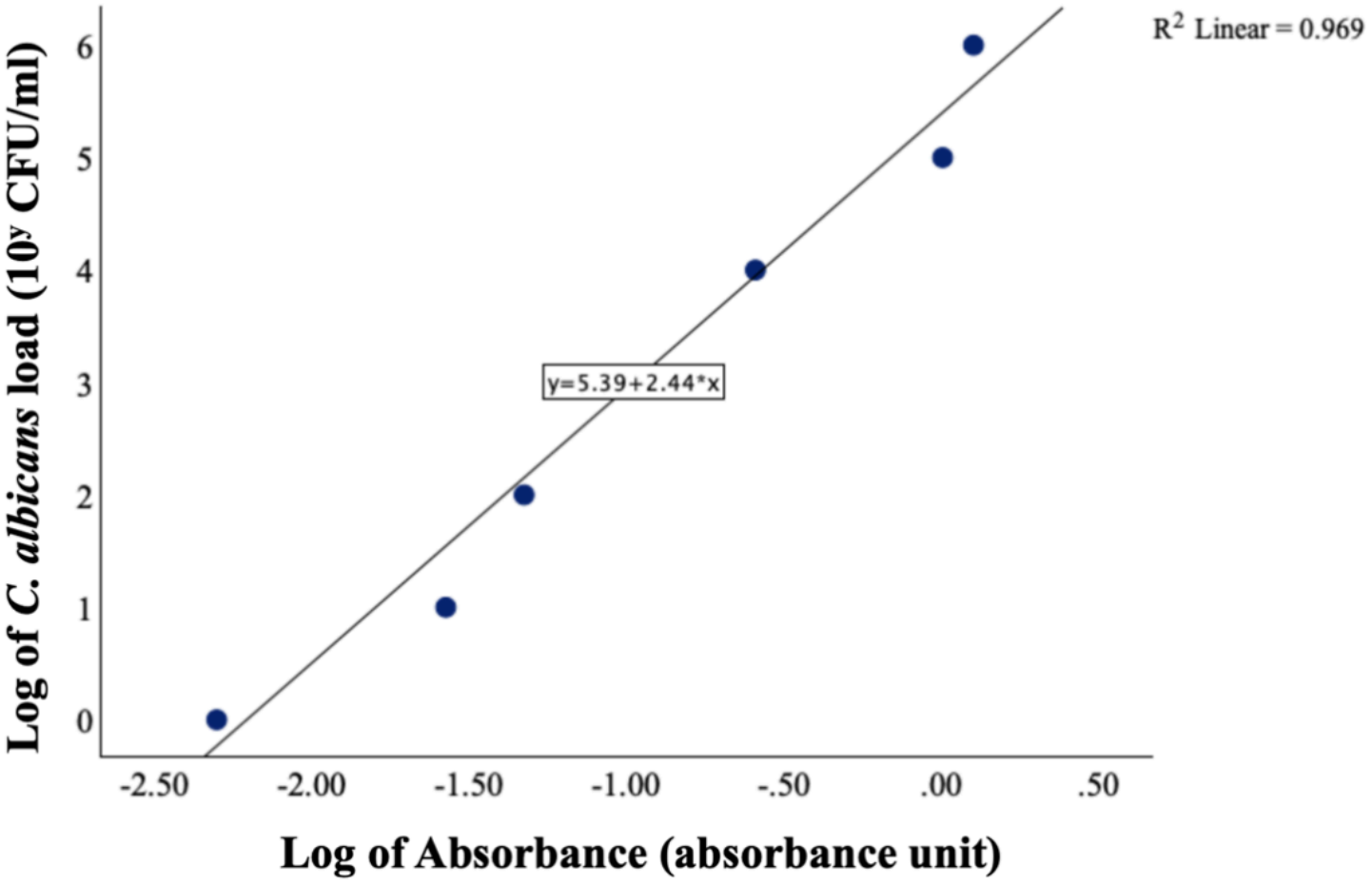


Figure 4

The line graph demonstrated a standard curve with x = log of absorbance units and y = log10 of *C. albicans loads.* The formula was revealed to be y = 2.444x + 5.390, with $r^2 = 0.969$.

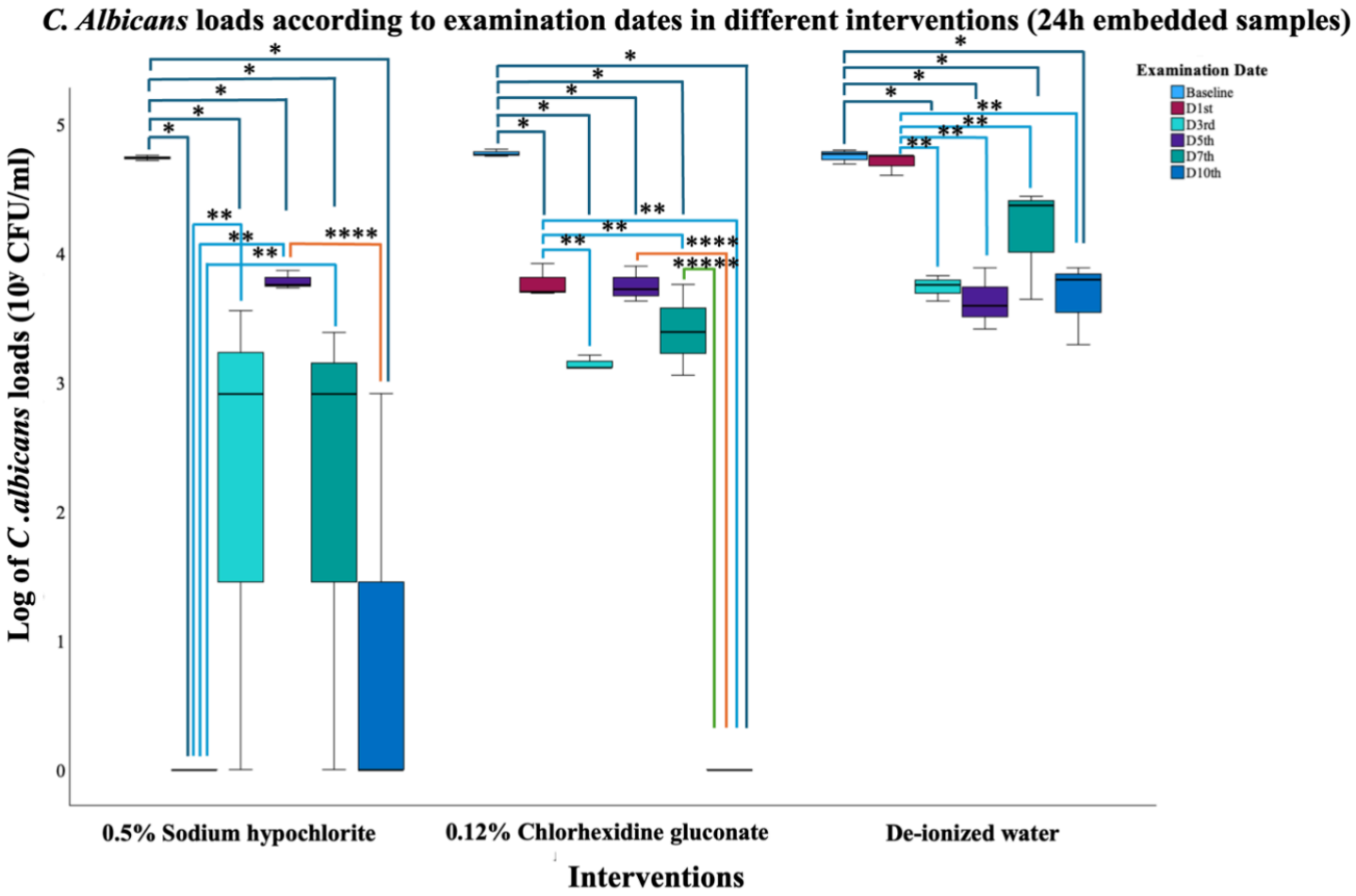


Figure 5

The box plot showed the results for the 24 h group, demonstrating significant differences in variant timelines after daily treatments. First, significant differences were observed in the 0.5% Sodium hypochlorite group between baseline and the other groups; however, Day 1 showed the lowest number of *C. albicans*, as discussed in the next section. Second, the result for the 0.12% chlorhexidine gluconate group showed that the trend in *C. abicans* loads was similar to that of the first group, but the effects were more pronounced towards treatment days. Finally, although the results of the de-ionized water groups reveal significant differences, *C. albicans* loads were high.

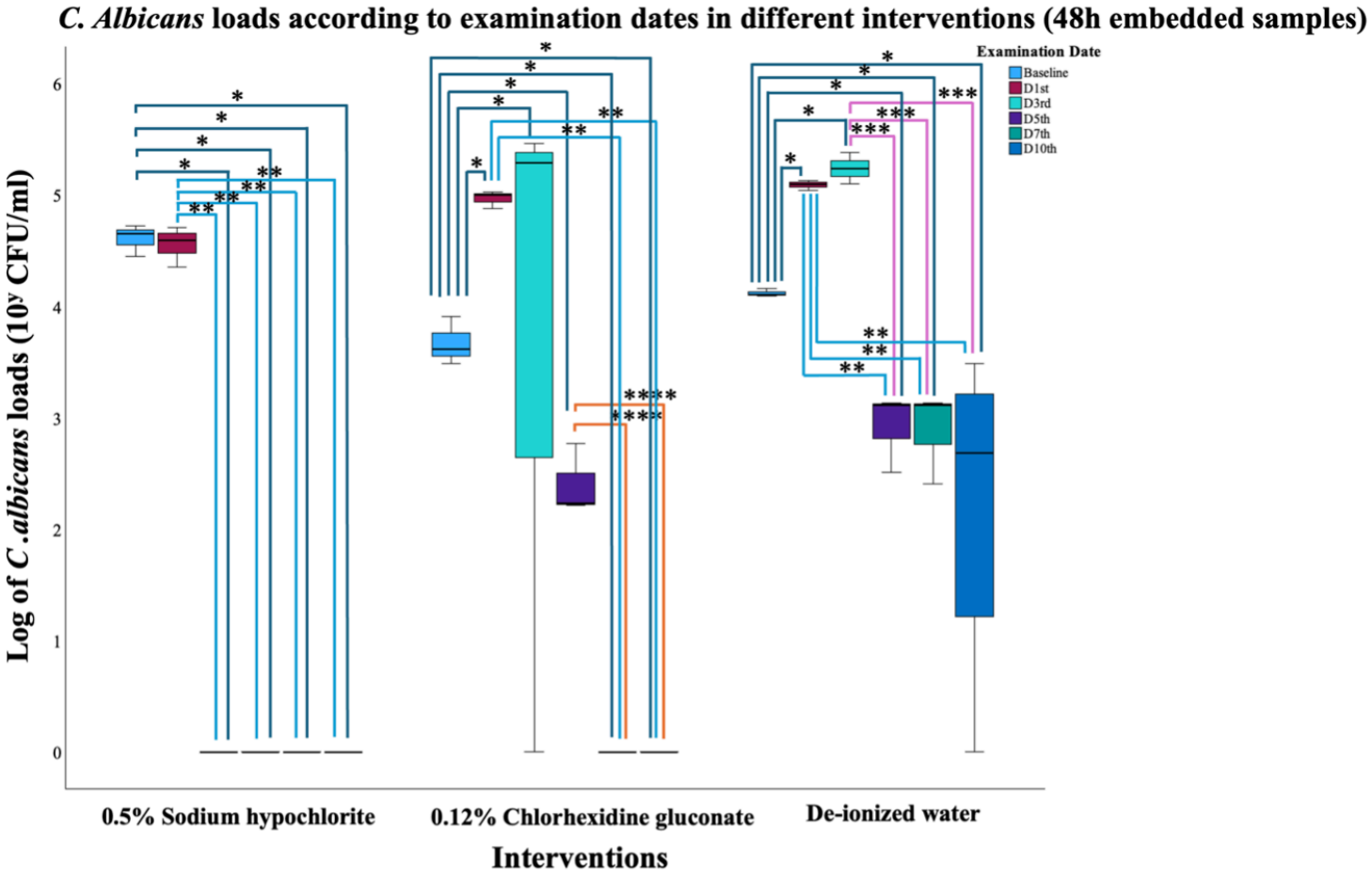


Figure 6

The box plots showed the results of the 48h group, revealing significant differences in variant timelines after daily treatments. First, significant differences were observed in the 0.5% Sodium hypochlorite group between baseline and the other groups, as well as in the Day 1 results. Second, although the result for the 0.12% chlorhexidine gluconate group showed a reduction in *C. albicans* loads, it was similar to that of the first group; the effects were more pronounced towards the end of the treatment period. Finally, although the results of the de-ionized water groups revealed significant differences, *C. albicans* loads were evidently high.

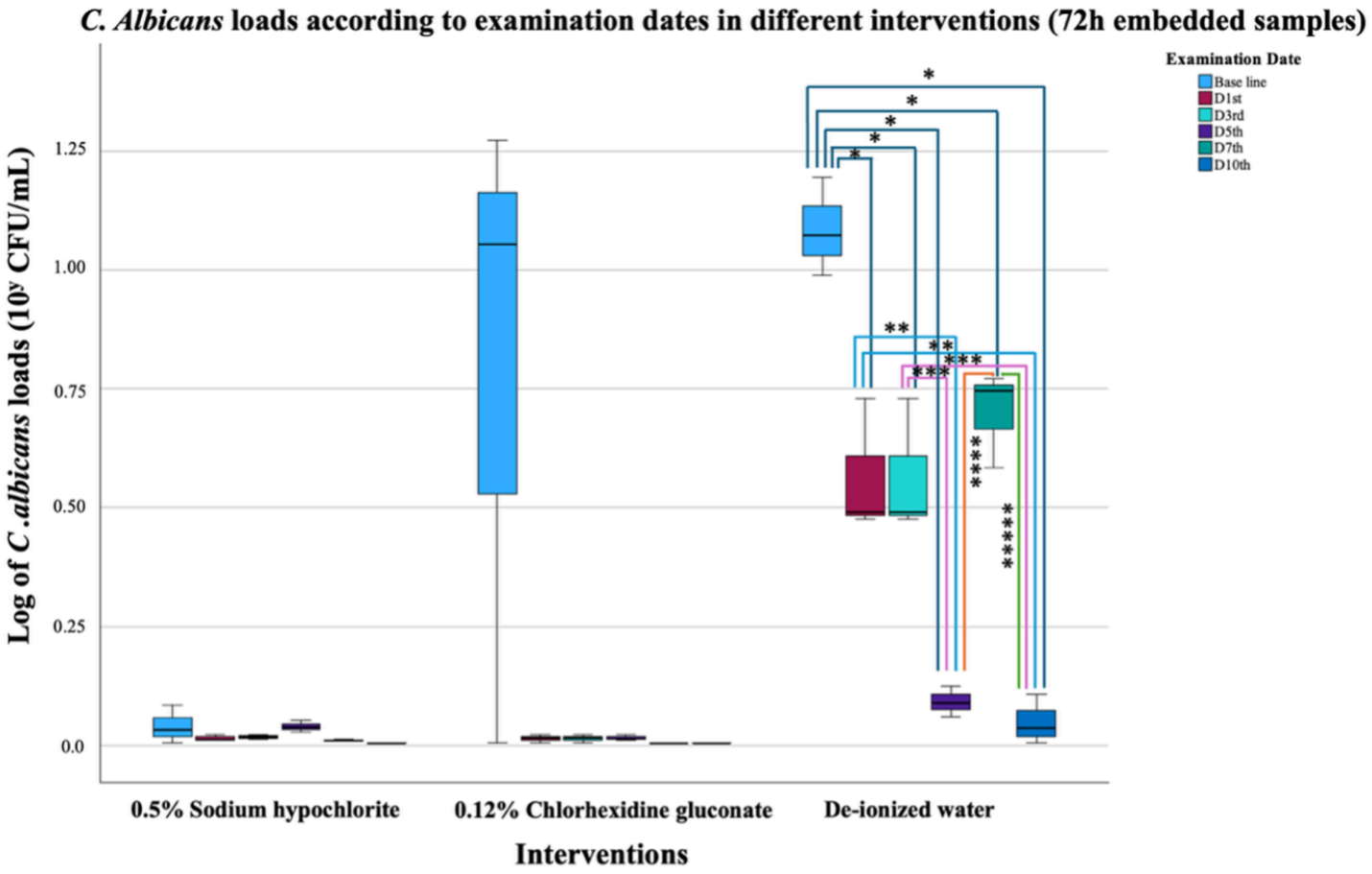


Figure 7

The box plots show *C. albicans loads* across different treatment conditions. The results revealed that the *C. albicans loads* in the 0.5% sodium hypochlorite and 0.12% chlorhexidine gluconate groups did not differ significantly. Although the de-ionized water group showed significant differences, the overall *C. albicans load* was relatively low (as discussed later).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- denturestomatitissupplementaryinformation.docx
- Supplement2rawdataC.xlsx