Peel bond strength and antifungal activity of two soft denture lining materials incorporated with 1% chlorhexidine diacetate

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Two soft denture lining materials (SC-Soft Confort and TS-Trusoft) were investigated with and without the addition of 1.0% of chlorhexidine diacetate (1.0% CHX). To assess peel bond strength, specimens (75×10×6 mm) were submitted to a peel test at 10 mm/min immediately and after 24 h. To evaluate Candida albicans growth inhibition, disc of specimens (10×3 mm) were immersed in a solution with 3×10^6 CFU/mL of C. albicans, and spectral measurements were made following immersion in MTT solution for 2, 4, and 6 days. The agar diffusion test was performed by investigating the diameters of inhibition zones around the disc of specimens (10×3 mm) after 48 h. Data were submitted to statistical analysis (α=0.05) and the failure modes were visually classified. The incorporation of 1.0% CHX significantly decreased the peel bond strength for TS (p=0.001) and SC (p=0.005) for immediate test and for TS after 24 h (p=0.010), but not for SC. C. albicans growth was decreased for both materials over time (p<0.05). SC presented inhibition zones approximately 2.0 times larger than TS. The incorporation of 1.0% CHX inhibited fungal growth without impairment to the peel bond strength for SC after 24 h.

Keywords: Soft denture liners, Candida albicans, Biofilm inhibition, Agar diffusion test, Peel bond strength

INTRODUCTION

Denture stomatitis is the most common oral candidiasis found in wearers of removable dentures9. It generally presents as a reddened area of tissue with an additional area outlined by the overlying denture. In general, denture stomatitis is asymptomatic but it can occasionally result in soreness and burning or tingling of the affected oral mucosa9. Its etiology is multifactorial with the main attribute being an inflammatory hypersensitivity-reaction against C. albicans3,4.

The treatment for denture stomatitis is challenging. This is predominantly mainly due to the roughness of the denture materials, and the adherence of oral microorganisms to the intaglio surfaces and oral mucosa epithelium5. This treatment includes systemic and topical antifungal therapies6,7. In topical antifungal therapy, antifungal agents such as fluconazole and ketoconazole may be applied directly to the affected mucosa or the fitting surface of the denture. This requires adequate good patient compliance, which can prove difficult to obtain when the patient is hospitalized or lacking independence9. Poor patient compliance may also be associated with the unpleasant taste and frequency of dosage of topical agents. Furthermore, topical antifungal therapy does not eradicate the oral microorganisms from the denture surfaces9,11. The achievement of an effective drug concentration on these surfaces is rather difficult due to the fact that the salivary flow, tongue and swallowing movements may dilute and remove the topical agents from the oral cavity9. Therefore, it may be beneficial to use other strategies to decrease or eliminate the fungi from denture surfaces12.

This could be achieved by means of denture lining, specially using acrylic- and/or silicone-based direct soft denture lining materials13,14. Soft denture lining materials are susceptible to deterioration by the hygiene procedures and the use of incompatible denture cleansers15,16, which contributes to an increase in the roughness on the materials surface and encourages the adherence of C. albicans17,18. Concerning these challenges and limitations, the incorporation of antimicrobial19 or antifungal20 agents into the soft denture lining material has been recommended with the purpose of producing a drug delivery system that provides a slow and continuous drug release, and ensures a sustained therapeutic effect against the colonization and penetration on the materials surface by the fungi21.
presents antimicrobial activity against a wide range of oral microorganisms, including the most common opportunist pathogen found on dentures, Candida spp.\textsuperscript{15,22,29}. Denture materials incorporated with CHX have exhibited superior activity against C. albicans biofilm formation in comparison to other antifungal agents, such as fluconazole\textsuperscript{21,24-27}. Bertolini et al.\textsuperscript{19} using an experimental model study, concluded that 1.0% chlorhexidine diacetate (1.0% CHX) incorporated into the soft denture lining materials inhibited the growth of C. albicans. However, it is not clear whether this drug concentration would potentially impair the bonding between the soft denture lining material and the denture base. Moreover, further studies are necessary to ensure the antifungal efficacy of 1.0% CHX incorporated into different soft denture lining materials.

Therefore, the purpose of this study was to evaluate the \textit{in vitro} peel bond strength, the inhibition of \textit{C. albicans} growth and the formation of an inhibition zone in the agar diffusion test of two soft denture lining materials incorporated with 1.0% CHX. The following hypotheses were evaluated: 1) there will be no change in the peel bond strength of the soft denture lining material to the denture base acrylic resin; 2) there will be inhibition of \textit{C. albicans} growth over time; and 3) there will be the formation of an inhibition zone against \textit{C. albicans}, following incorporation of 1.0% CHX.

**MATERIALS AND METHODS**

Two soft denture lining materials (Soft Confort, Dencril, Pirassununga, Brazil; and Trusoft, Trusoft Bosworth Company, Skokie, IL, USA) were used to reline a denture base acrylic resin in two experimental conditions: with the incorporation of 1.0% CHX (Sigma Aldrich, São Paulo, Brazil) powder to the soft denture lining material (experimental group) and with no incorporation (control group). The materials used, as well as their composition, manufacturers and lot numbers are illustrated in Table 1.

**Specimen fabrication**

1. Peel bond strength test

Heat-polymerized acrylic resin specimens (QC 20; Dentsply, Petrópolis, Brazil) \((n=10)\) measuring \(75 \times 10 \times 3\) mm were made according to the manufacturers instructions (Table 1)\textsuperscript{12,28}. The dental flasks were bench cooled for 30 min exposed to running water for 15 min\textsuperscript{29}. The specimens were stored in distilled water at 37°C (Q316M4, Quimis, Diadema, Brazil) for 48 h\textsuperscript{12,29}.

Subsequently, one of the specimen surfaces was polished using #600 silicon carbide paper (3M ESPE, São Paulo, Brazil) in a metallographic polishing (Écomet II, Buehler, Lake Bluff, IL, USA). The specimen was placed in a hollow stainless steel mold with internal measurements of \(75 \times 10 \times 6\) mm. The specimen area (650 mm\(^2\)) not intended for bonding to the soft denture lining material was covered with a polyester strip, exposing it only to the area to be relined (10 mm). Thus, 65 mm remained free to be pulled and 10 mm composed the lining area using the bonding agent according to each soft denture lining material's manufacturer instruction\textsuperscript{12}.

The powder and liquid of the acrylic-based soft denture lining materials were weighed according to the manufacturer's instructions. Prior to the liquid addition, CHX diacetate powder was incorporated of the powder form of soft denture lining materials at a concentration of 1.0% until an homogeneous mixture was achieved. Then, the liquid was added to the powder. The mixed material was then inserted into the hollow mold containing the heat-polymerizing acrylic resin specimen prepared for the relining procedure. This set was covered with a glass slide and kept under finger pressure for the time recommended by the manufacturer. Excess material was eliminated and the relined specimen was removed from the mold. The control group for each material was prepared with no addition of CHX. One experimental group of the specimens \((n=40)\) was immediately submitted to the peel bond strength and other experimental group \((n=40)\) was stored in distilled water at 37°C for 24 h to simulate one day of being in the oral cavity\textsuperscript{12}.

<table>
<thead>
<tr>
<th>Material</th>
<th>Material composition</th>
<th>Manufacturer</th>
<th>Batch number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft Confort</td>
<td>Powder: Polyethyl methacrylate</td>
<td>Dencril, Pirassununga, São Paulo, Brazil</td>
<td>048330-045855</td>
</tr>
<tr>
<td></td>
<td>Liquid: Phthalate ester (plasticizer) and ethyl alcohol</td>
<td></td>
<td>044231-047412</td>
</tr>
<tr>
<td>Trusoft</td>
<td>Powder: Polyethyl methacrylate, and Cadmium pigments</td>
<td>The Bosworth, Skokie, IL, USA</td>
<td>1306-273</td>
</tr>
<tr>
<td></td>
<td>Liquid: Benzyl butyl (plasticizer) and ethyl alcohol</td>
<td></td>
<td>1306-273</td>
</tr>
<tr>
<td></td>
<td>Powder: Methyl methacrylate, N-nitro methacrylate, benzoyl peroxide, colorants and acetate fibers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QC 20</td>
<td>Liquid: Methyl methacrylate, dimethyl methacrylate, hydroquinone, terpinolene, and N-N-dimethacrylate-p-toluidine</td>
<td>Dentsply, Petrópolis, Brazil</td>
<td>386468C</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Powder: Chlorhexidine diacetate salt hydrate &lt;100%</td>
<td>Sigma Aldrich, São Paulo, Brazil</td>
<td>083K0014V</td>
</tr>
</tbody>
</table>

Table 1 Composition, manufacturer and batch number of the materials used in the study
2. Agar diffusion and antifungal activity test

The materials were manipulated as previously described (with and without the addition of 1.0% CHX) and inserted into a disc-shaped mold to produce specimens of 10 mm diameter and 3 mm in thickness (n=3, for the growth inhibition/n=6, for the agar diffusion test). The set was covered with a glass slide and kept under finger pressure for 5 min as specified by the manufacturer. Any irregularities in the disc of specimens were removed with a scalpel blade. All samples were fabricated in a laminar air-flow chamber which created an aseptic environment (410 Pa, Pachane, Piracicaba, Brazil) and after preparation, the specimens were exposed to ultraviolet light for 30 min on each side for sterilization purposes.

Peel bond strength test

A universal testing machine (DL2000, FDMS, São José dos Pinhais, Brazil) was used for the peel bond strength test of the lined test specimens at an angle of 180 degrees. The portion of the incorporated soft denture lining material not bonded to the resin base (65 mm) was folded upwards and fixed onto the top hook of the lining material. The alternative un-lined portion of the specimens was subjected to tension to a speed of 10 mm/min until failure. The failures modes were classified as: 1) peel, when the debonding occurred in the soft denture lining material interface only; 2) snap, when the debonding occurred at the denture base resin– soft denture lining material only; 3) tear, when the debonding occurred in the soft denture lining material fractured.

Antifungal activity tests

To perform the antifungal activity tests, there was a preliminary stage of activation of Candida albicans (C. albicans) strains from a C. albicans commercial strain (#10231) according to the American Type Culture Collection (ATCC). Colonies were incubated in 20 mL of sterile brain heart infusion medium (BHI-Difco, Franklin Lakes, NJ, USA) for 48 h at 37°C. Two isolated colonies were transferred to 20 mL of sterile liquid BHI culture and incubated at 37°C for another 48 h to achieve maximum growth. A 3 mL aliquot was analyzed with a spectrophotometer (SP-220, Biospectro, Curitiba, Brazil) at 625 nm ($A_{625}$) to quantify the colony forming units (CFU), taking as a basis the interval between $A_{625}$ 0.08 and 0.14, which corresponds to 1.5×10^8 CFU/mL.

1. C. albicans growth inhibition test

The initial solution was diluted in liquid BHI supplemented with 2% sucrose. The final inoculum consisted of 1.2 mL to each well containing 3×10^6 CFU/mL of C. albicans suspension. Three wells of a 24 well plate were used for each group (Soft Confort and Trusoft with and without 1.0% CHX) and for each time point (2, 4 and 6 days). A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Aldrich) was prepared as a saturated solution at 5 mg/mL and filter sterilized with a 0.22 µm pore filter.

Prior to the experiment, an aliquot of MTT solution was dissolved in phosphate buffered saline (PBS) (Sigma Aldrich) to obtain a concentration of 0.5 mg/mL. Every 48 h, non adherent cells were removed from the suspension and 1.5 mL of MTT solution was added to each well and the plates were incubated for 4 h at 37°C. The precipitate was diluted with 600 µL of 0.04 N HCl (Hydrochloric acid, BHerzog, Rio de Janeiro, Brazil) in isopropanol (BHerzog) and plates were incubated for an additional 1 h in the dark at 21°C.

Three aliquots were removed with 100 µL of solution from each well and transferred to a 96-well plate. Cell viability was measured by spectrophotometry at wavelength of 550 nm in an Enzyme-linked immunosorbent assay (ELISA) reader (TP-reader basic, Thermoplate, São Paulo, Brazil). Each well was read in triplicate, and the average of 9 values was calculated.

2. Agar diffusion test

Petri plates containing 10 mL of BHI agar were perforated with a sterile punch to hold the reline specimens. The 100 µL inoculum (1×10^6 CFU/mL of C. albicans) suspension was uniformly spread over the surface of the culture medium and disc resins were inserted into the modified petri plates. Each plate held two fixed specimens were fixed and there were six specimens per group. The plates were incubated at 37°C for 48 h and the diameters of the inhibition zones of C. albicans were measured using a digital caliper (SC-6, Mitutoyo Corporation, Tokyo, Japan) and reflected light. Three measurements were made for each diameter, and following subtraction to the disc resin diameter, the average was calculated.

Statistical analysis was performed using Statistical package for the social science (SPSS version 16.0, SPSS, Chicago, IL, USA) using a confidence interval of 95%. The Shapiro-Wilk test was used to evaluate the normality of data. For analysis of the peel bond strength test data, two-way analysis of variance (ANOVA) test was performed. Kruskal-Wallis and Mann-Whitney tests were used for the failure mode, metabolic activity, and agar diffusion test.

RESULTS

Peel bond strength test

Mean peel bond strength values (N/mm²) are shown in Table 2. The addition of 1.0% CHX significantly decreased the peel bond strength between Trusoft and the denture base acrylic resin immediately and following 24 h of immersion in distilled water ($p=0.001$ and $p=0.010$, respectively), and for Soft Confort when it was
immediately submitted to peel bond strength ($p=0.005$). However, this antifungal agent did not affect the peel bond strength between Soft Confort and the denture base acrylic resin after 24 h of immersion in distilled water ($p=0.921$). Results indicated that Soft Confort demonstrated statistically higher peel bond strength values when compared to Trusoft at 24 h of immersion in distilled water ($p<0.001$), regardless of the presence or absence of 1.0% CHX. When the peel bond strength was assessed immediately, it was not found difference between these soft denture lining materials ($p>0.05$).

The failure modes obtained after performing the tests are illustrated in Table 3. The immediate peel bond strength demonstrated 100% of snap debonding for both denture soft lining materials with or without 1.0% CHX. After 24 h of immersion in distilled water, in regards to Soft Confort, the predominant mode of debonding was snap. Peeling away from the denture base was only observed for Soft Confort, regardless of the incorporation of 1.0% CHX. However, a decreased percentage of the snap mode of debonding was found when this material was incorporated with the antifungal agent (70%) when compared with the control group (90%). Results demonstrated that in the Trusoft samples, the main debonding mode was snap (100%), regardless of the presence of 1.0% CHX.

### C. albicans growth inhibition test

Mean optical density values (nm) are graphically represented in Fig. 1. Data showed that the incorporation of 1.0% CHX significantly decreased *C. albicans* growth for both materials for all time intervals evaluated ($p<0.01$). However, the comparison of the same material for each experimental condition at intervals of 2, 4 and 6 days revealed a statistically significant difference over time for Trusoft ($p<0.02$). In terms of Soft Confort, this difference was significant only after 2 and 4 days in the experimental group, and after 4 and 6 days in the control group ($p<0.01$). There was no statistical difference in the *C. albicans* growth inhibition with the incorporation of 1.0% CHX for Soft Confort over time ($p>0.05$).

### Agar diffusion test

The mean values of the inhibition zones (mm) for each tested material are shown in Table 4. The agar diffusion test showed clear inhibition zones around the disc of specimens with 1.0% CHX for both materials. Additionally, no inhibition zones were observed around the discs in control groups (Fig. 2). Comparing both tested soft denture lining materials, Soft Confort presented inhibition zones almost 2.0 times larger than Trusoft ($p<0.01$).
Table 4  Means and standard deviation of inhibition zones obtained by agar diffusion test (mm) of Soft denture lining materials without and with incorporation of CHX

<table>
<thead>
<tr>
<th>Soft denture lining materials</th>
<th>1.0% CHX Without</th>
<th>With</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soft Confort</td>
<td>0.00±0.00 aA</td>
<td>7.82±0.78 bA</td>
</tr>
<tr>
<td>Trusoft</td>
<td>0.00±0.00 aA</td>
<td>4.01±1.40 bB</td>
</tr>
</tbody>
</table>

Note: Data in the same line with the same small letters indicate no statistically significant difference ($p>0.05$). Data in the same column with the same big letters indicate no statistically significant difference ($p>0.05$).

DISCUSSION

The conservation of satisfactory softness is one of the most problematical factors when soft denture lining materials are used because they are not stable in aqueous environment. These materials are exposed to saliva, foods, water and denture cleansers, which cause leaching of their components or water absorption.
The equilibrium between the loss of components and fluid absorption interfere with the performance of soft denture lining materials, resulting in alterations as expansion, distortion, microbial colonization, and increased hardness. Therefore, the hardness changes occurring during clinical use of these materials determine their ability to absorb impacts and are related to their modulus of elasticity. Although soft denture lining materials are initially very soft, the rapid loss of ethanol and plasticizers results in a significant increase in their hardness and solubility, which leads to a gradual loss of cushioning effect. Consequently, the material life cycle is limited to a relatively short period.

The success of a denture lining relies on the bonding between the lining material and the denture base acrylic resin that should remain intact for the liner to adequately recover the injured tissues. Unfortunately, peeling of the soft denture lining material from the denture base is a common clinical failure found in dentures that are relined. This problem may be worsened with the addition of antimicrobials. Urban et al. observed that CHX exhibited bigger and more irregular particles distributed dispersed within the acrylic soft lining material. The pattern of CHX incorporation in the polymer results in fragility of the matrix and increases the porosity of the modified materials. This porosity contribute to the CHX releasing, which is also favored by the higher solubility of its molecules in water. The addition of a substance such as a drug in a soft material might impair the plasticizers to penetrate in the polymeric chains and form a softened gel. This alteration in gel formation may affect the material properties. Moreover, the lower content of plasticizer because of the incorporation of drugs can reduce the disentanglement of polymer beads, yielding a weak cohesion among the polymer chains. Thus, all these mechanisms may have resulted in hardness increase of the soft lining materials with the 1.0% CHX addition, which leads to a decrease in their peel bond strength to the denture base resin. These findings corroborate with those of a recent study that demonstrated lower peel bond strength values for the Trusoft material after both the addition of CHX and itraconazole, regardless of the immersion time in distilled water. Bertolini et al. suggest that the CHX incorporation did not lead to a clinically relevant change on the material. This statement can be applied for the present study since incorporating the CHX into the soft lining materials immediate or after 24 h of evaluation did not result in values below those recommended for peel bond strength as stated by Craig and Gibbons and Kawano et al.

Differently from that observed with Trusoft, addition of the drug seemed to have no effect on the peel bond strength for Soft Confort after 24 h of immersion in distilled water (p=0.921). This difference may be accounted to the composition of the materials, especially regarding quantity of plasticizers and ethanol concentrations. No information about Soft Confort was found on the Material Safety Data Sheet (MSDS). According to the manufacturer, the liquid of Soft Confort is composed of a phthalate ester plasticizer (not specified) and ethyl alcohol. The MSDS describes that the liquid of Trusoft contains ethanol (10–15%) and benzyl butyl phthalate (50–85%). The leaching of soluble components and fluid absorption of temporary soft lining materials are influenced not only by drug diffusion through channels and pores created in the polymer matrix, but also to the plasticizer and ethanol concentrations. The higher Shore A hardness demonstrated by Soft Confort compared to Trusoft in up to 24 h suggests that the former has a greater initial amounts of plasticizer and ethanol. Another indication that the Soft Confort is softer than Trusoft and is also softer when samples are immediately fabricated can be seen by the lower immediate peel bond strength values demonstrated by the material compared after 24 h of immersion in distilled water with 1% CHX (p<0.001) or without (p=0.004). The softness of the materials is responsible for the stretch. Soft Confort is submitted to tension forces over a longer period and disrupts at a higher force than a less soft material, like Trusoft. Being softer, even with the addition of 1.0% CHX, the peel bond strength of Soft Confort did not significantly change after 24 h. Moreover, because of the higher amount of soluble components, the dynamic properties of Soft Confort was not affected in up to 24 h, despite the great ethanol loss in up to 24 h as well the leaching of plasticizer in this period. It is important to emphasize that differences of softness between different materials is also affected by type, particle size and molecular weight of power, and powder/liquid ratio.

In the present study, the peel bond strength test was conducted immediately and 24 h following immersion in water. In fact, the influence of immersion time on the peel bond strength is a very important factor. Sánchez-Aliaga et al. evaluated the peel bond strength after immersion in distilled water for 24 h, 7 and 14 days. The results indicated that the values of peel bond strength significantly increased over time for the materials evaluated, which could be considered an advantage for the lining because it prevents biofilm formation at the interface with the denture base acrylic resin, allowing better prosthesis hygiene and patient comfort. On the other hand, as previously stated, the increase in peel bond strength may be associated with less softness and viscoelasticity, which reduce the material capacity to absorb impact, impairing its ability to promote the needed comfort to the patient. Previous studies...
demonstrated an increase in hardness of temporary soft lining materials after CHX addition; however these changes were observed after longer periods (14 days\(^{40}\) and 3 months\(^{46}\) in water than those tested in this study. Therefore, a limitation of the present study is the absence of information over the time.

As expected, the second hypothesis investigated in this study was accepted because the addition of the antimicrobial agent significantly decreased C. albicans growth for both soft denture lining materials for all the time intervals analyzed. The inhibition of C. albicans growth was evaluated for six days in this study. This period of time was chosen because CHX release from polymers has been shown to have a high initial release in distilled water for the first four days, followed by a decreased and a constant release from the sixth day thought the twentieth day, and a final decreased release after the twentieth day\(^{25,56} \). The realize begins when water diffuses into the polymer matrix of the material, dissolving and releasing the drug as it comes in contact with the water. Moreover, pores are created on the materials surface, leading to an increase in the contact area and a faster drug release\(^{57}\). In the present study, the CHX release from Trusoft exhibited an increasing flow up to the sixth day, since the C. albicans growth significantly decreased over time. Controversially, Soft Confort may have shown a constant and uniform pattern of CHX release, since no significant difference on C. albicans growth was found. The progressive antifungal activity observed for Trusoft may potentially be caused by less porosity on the surface of the material, which may facilitate CHX release over time and not initially. In these situations, the material initially releases the drug closer to outside surface (burst phase) and later, releases the drug internally (diffusion phase —Fickian process\(^{58}\)). This second phase is a diffusion-controlled complex process in which the drug is released slowly. It involves water cluster formation around CHX diacetate particles and the interaction of these clusters with the water uptake process of the polymer\(^{59} \). Since Soft Confort is a softer and more porous material, it may have released more CHX in the beginning of the test and did not demonstrate any difference over time.

The third hypothesis of this study was also accepted since both soft denture lining materials demonstrated clear inhibition zones around the discs with 1.0% CHX on agar culture. Greater antifungal activity from Soft Confort may be attributed to a higher CHX release due to the presence of a larger amount of plasticizer in this material. This release may have led to increase surface area and subsequently, the release of CHX in the fluid environment. To our knowledge, there are limited studies evaluating the antifungal activity of CHX incorporated into soft denture lining material. In a previous study, the addition of CHX to a tissue conditioner in different concentrations was both effective and dose-related in the inhibition of the growth of C. albicans\(^{19} \). Likewise, Bertolini et al.\(^{18} \) concluded that CHX diacetate incorporated into two soft denture lining materials at concentrations of 1.0 and 2.0% produced inhibition zones in the agar diffusion test, with no significant cytotoxic effect. This is in agreement with the present study, which also demonstrated the antifungal activity of 1.0% CHX with the growth inhibition of C. albicans. In contrast, Radnai et al.\(^{29} \) found that CHX digluconate added to a soft denture lining material, in the form of a gel, had no inhibitory effect on the growth of C. albicans.

Two microbiological investigations were conducted in this study. The first test was used to verify the efficacy of the antifungal agent to inhibit the C. albicans biofilm formation on the surface of the soft denture lining material over time. The second, the agar culture, was a relatively simple and reliable method based on Gould\(^{20} \) and adapted by Wilson\(^{21} \). These microbiological tests revealed coherent results, since the CHX released from the soft denture lining materials was able to induce an antifungal activity against C. albicans on both growth inhibition and agar culture tests. The released CHX from Soft Confort showed lower optical density values and lighter color than that released from Trusoft in the growth inhibition test, indicating lower antifungal activity on C. albicans. This was confirmed in the agar culture, since this soft denture lining material showed higher inhibition zones surrounding the disc of specimens with CHX when compared to Trusoft. The mechanism of action of CHX in the fungal cells is not completely elucidated. It has been stated that CHX may disrupt the cell wall of the fungi by binding to glucan moieties, inhibit cell replication or prevent its adherence to the epithelium of the oral mucosa or acrylic resin of the denture base\(^{46} \).

There are limitations with the current study. Specifically, in vitro tests potentially do not represent the same load to which soft denture lining materials are clinically submitted, since these tests are performed by the application of only one type of force. The interpretation of the peel bond strength results is impaired by the complexity of the bonding phenomenon and by the fact that the shape specimen is different from the prosthesis configuration\(^{14,40} \). However, this test is useful for comparisons between unmodified or modified materials. The microbiological tests results indicate that the CHX-supplemented drug-release had a powerful antifungal effect, demonstrated by its capacity to inhibit the growth of C. albicans for both soft denture lining materials. However, it must be taken into account that only one type of Candida spp. and one concentration of CHX diacetate were evaluated in this present study. Future studies should monitor the performance of chorhexidine over a longer duration in order to simulate the clinical period of up to 14 days\(^{30} \).

CONCLUSIONS

Within the limitations of this in vitro study, it was concluded that the incorporation of 1.0% CHX into the soft denture lining materials demonstrated antifungal activity against C. albicans, without the impairment of peel bond strength for Soft Confort after 24 h.
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