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Original Article

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The incorporation of chlorhexidine in dental prosthetic materials may interfere on its microbial colonization and surface roughness: a preliminary study

A incorporação da clorexidina em materiais protéticos dentários pode interferir na colonização microbiana e na rugosidade superficial: um estudo preliminar

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ABSTRACT

Objective: the present study aimed to evaluate *in vitro* the antimicrobial activity and roughness interference of relining materials of removable acrylic resin-based prosthesis whenever incorporated with 1% chlorhexidine diacetate. **Material and Methods:** For that purpose, New Truliner™ and Trusoft™ relining resins probes were made with and without the incorporation of the compound. The antimicrobial activity against *Candida albicans* ATCC 10231 of the probes was evaluated by the agar diffusion test, using the inhibition halo measurement and by colony forming units counting. Quantitative analyses of both relining materials roughness were obtained in a non-contact 3D digital profilometer. **Results:** Both materials incorporated with chlorhexidine exhibited reasonable inhibition halos demonstrating the ability to inhibit *C. albicans* growth, in a time-dependent manner. Although an increase in the roughness of both materials was observed over time ($p<0.05$), the incorporation of chlorhexidine did not alter the surface roughness significantly ($p>0.05$). **Conclusion:** Considering the above, this study suggests that the relining materials incorporated with 1% chlorhexidine diacetate have an important antimicrobial activity against *C. albicans*, with no considerable changes in surface roughness and may be considered a suitable tool against prosthetic stomatitis.

KEYWORDS

Antimicrobial activity; *Candida albicans*; Chlorhexidine; Prosthetic stomatitis; Relining material.

RESUMO

Objetivo: o presente estudo teve como objetivo avaliar a atividade antimicrobiana e a interferência na rugosidade de materiais de reembasamento de próteses removíveis à base de resina acrílica, incorporados com diacetato de clorexidina a 1% *in vitro*. **Material e Métodos:** Para isso, foram confeccionados corpos de prova de resinas de reembasamento New Truliner™ e Trusoft™ com e sem a incorporação do composto. A atividade antimicrobiana desses corpos de prova contra *Candida albicans* ATCC 10231 foi avaliada pelo teste de difusão em ágar, utilizando-se a medida do halo de inibição e a contagem de unidades formadoras de colônia. Análises

quantitativas da rugosidade dos materiais de reembasamento foram obtidas em um perfilômetro digital 3D. **Resultados:** Ambos os materiais incorporados com clorexidina demonstraram halos de inibição razoáveis, demonstrando sua capacidade de inibir o crescimento de *C. albicans* de forma tempo-dependente. Embora tenha sido observado aumento da rugosidade de ambos os materiais ao longo do tempo ($p<0.05$), a incorporação de clorexidina não alterou significativamente a rugosidade superficial ($p>0.05$). **Conclusão:** Considerando o exposto, este estudo sugere que os materiais de reembasamento incorporados com diacetato de clorexidina a 1% apresentam importante atividade antimicrobiana contra *C. albicans*, sem alterações consideráveis na rugosidade da superfície, podendo ser considerados uma ferramenta adequada contra estomatite protética.

PALAVRAS-CHAVE

Atividade antimicrobiana; *Candida albicans*; Clorexidina; Estomatite protética; Material de reembasamento.

INTRODUCTION

The unquestionable rising in global life expectancy has led to a growing increase in the world population, making the loss of dental elements a reality [1]. The use of full denture associated with progressive aging and systemic diseases leads to an unfavorable oral health state frequently associated with a poor oral hygiene [2-4]. In fact, an inadequate prosthesis hygiene may promote biofilm accumulation and develop oral infections often called prosthetic stomatitis (PS) [5,6].

It is known that PS has a prevalence of 45-70% among elderly patients with prosthetic dentures [7,8]. Clinically, this condition usually presents itself as a diffuse oral inflammation, reaching preferentially the hard palate region. Although its etiology is considered microbiologically multifactorial, *Candida albicans* has been considered the main etiological factor of this infection [5,9]. The fungal *C. albicans* is a yeast that shows a high ability to colonize and form biofilms on dental prosthesis surfaces, playing an important role on installation and progression of PS [5,10,11].

The treatment of PS in patients that use full dentures is toilsome and time-consuming [12]. The maintenance of topical medication at a safe and effective concentration on colonized surfaces and tissues certainly is not possible, since most of the administered drugs able to solve this situation is quickly removed from oral surfaces, due to saliva flow, tongue movement, and swallowing [13-15]. The prevention of the contact between prosthesis and infected tissues, virtually appears to be an effective method for the treatment and prevention of PS, which can be achieved through prosthesis relining, that breaks the cycle of reinfection and reestablishes prosthesis adaptation to the supporting tissues [14].

A promising alternative to prevent PS and eventually treat it is the incorporation of antimicrobial agents into relining materials [16-18], considering its continuous release of antibiotics and low toxicity, reducing the need of pharmacological treatment and prolonging clinical longevity of materials [18]. Among antimicrobials used to treat PS, chlorhexidine has been shown to be the most effective agent, due to the emergence of strains resistant to conventional antimicrobials [19].

Chlorhexidine has a microbiostatic and a microbiocidal activity against Gram-positive, Gram-negative bacteria and fungi, including *C. albicans.* For example, in acrylic resins, the incorporation of chlorhexidine has been shown to be highly effective against biofilm formation of *C. albicans in vitro,* being readily released from the polymer for up to four weeks [20], suggesting that this therapeutic methodology may be useful in patients who need conditioning as a support to treat PS, or who have undergone surgical procedures and need to keep the region decontaminated [14,21,22].

Considering the above, the objective of this study was to evaluate if the incorporation of chlorhexidine diacetate into relining material has an effectiveness antimicrobial activity over time without impairing the biophysical properties of the material, such as roughness *in vitro.*

MATERIAL AND METHODS

Study design

This study was designed as an *in vitro* experimental investigation to evaluate the antimicrobial activity and surface roughness of relining materials for removable prosthesis following the incorporation of 1% chlorhexidine diacetate (CHX). The evaluation was conducted

under standardized laboratory techniques to simulate oral conditions including the use of artificial saliva. Manufactured resin specimens were analyzed at various time periods during the entire experiment to mimic clinical prosthesis usage, as follows: 24 hours after the preparation of the specimens (T0), 7 days after the immersion in proper medium (T1), 14 days after (T2), 21 days after (T3) and 28 days after (T4).

Two commercially available relining materials were used, New Truliner™ and Trusoft™ (Skokie, IL, USA) that were used under two experimental conditions, those with and those without CHX. The materials used, the manufacturers, composition and batch number are shown in Table I.

C. albicans strain ATCC 10231 were obtained from the American Type Culture Collection® and was cultured in sterile Petri dishes 90 mm x 15 mm, Plastbio® (Curitiba, Brazil) containing 20 mL of brain heart infusion agar (BHI) culture medium (Difco®, Rio de Janeiro, Brazil).

Artificial saliva used in this study was composed by Tris-(hydroxymethyl)-aminomethane buffer 1.5 mM calcium, 0.9 mM phosphate, 0.15 M potassium chloride and 0.05 μg fluoride/mL, pH 7.0, at 25° C. Artificial saliva was renewed every couple of days to avoid saturation and allow the release of chlorhexidine throughout experimental time, to simulate oral condition.

Specimens preparation

For the preparation of the specimens, relining materials (New Truliner™ and Trusoft™) were weighed and prepared according to the manufacturer's instructions. In the groups with CHX, they were weighed separately using a precision balance Electronic Balance FA 2104N – Bioprecisa® (Curitiba, Brazil) to achieve a proportion of 1.0% of the total polymer weight for each material. CHX was initially mixed with the polymer powder, followed by the addition of the specific relining material liquid, ensuring complete homogenization. Materials were then inserted into silicone molds shaped as discs with standardized dimensions of 12.0 mm in diameter and 3.0 mm in thickness.

New Truliner™ samples were polymerized for 10 to 15 minutes at room temperature, and their irregularities were removed using 600-grit waterproof sandpaper Norton Saint-Gobain® (Guarulhos, Brazil) under irrigation. The Trusoft™ samples were polymerized for 5 minutes at room temperature, and their irregularities were removed using a 12-scalpel blade Solidor® (Barueri, Brazil). The specimens were examined for uniformity under magnifying glass (20x), and any specimens with visual defects were excluded from the study.

All specimens were sterilized by exposure to ultraviolet radiation (UV) on all sides for 60 min. Specimens were microbiologically tested to ensure their sterility previously each experiment [16].

Sample size calculation

The number of specimens was determined using the Bioestat® software, version 5.0 (Belém, Brazil), and a significance level of 5% was considered significant. Twenty specimens were included for each group, according to the determinate experimental points (T0, T1, T2, T3, and T4), with the presence or absence of CHX.

Inoculum preparation

C. albicans ATCC 10231 were cultured in sterile Petri dishes containing 20 mL of BHI agar and subsequently incubated in aerobiosis at 37 ºC, for 48 hours. Two isolated colonies were transferred to 20 mL of BHI broth supplemented with 2% sucrose and incubated at 37ºC. Culture was evaluated in a spectrophotometer Biospectro, WMED® (São Paulo, Brazil), at 600 nm to adjust the final concentration, according to the McFarland scale 0.5 adapted for yeast.

Table I - Manufacturer, composition, and batch of relining materials and CHX

Material	Manufacturer	Composition	Batch
New Truliner™	The Bosworth Co., Skokie, IL., USA.	Powder: Polyethyl methacrylate (PEMA) Liquid: Isobutyl methacrylate (IBMA) and di-n-butyl phthalate (DBP)	1305-244
Trusoft™	The Bosworth Co., Skokie, IL., USA	Powder: Polyethyl methacrylate (PEMA) and pigments. Liquid: Alkyl phthalate (plasticizer) and ethyl alcohol	1306-273
Chlorhexidine P.A.®	Sigma Aldrich Co., Belgium	Chlorhexidine diacetate (hydrated)	083K0014V

Antimicrobial activity of incorporated chlorhexidine against *C. albicans*

Specimens containing or not CHX were evaluated for the antimicrobial activity using two different methodology approaches, as follows:

By agar-diffusion

CLSI M-44 document with minor modifications was used. Briefly, *C. albicans* ATCC 10231 adjusted inoculum was spread over BHI agar plates to reach a concentration of $1.5x10^7$ cell/mL on the inoculated surface. Resins specimens with or without CHX were positioned on the plates and aerobically incubated at 37ºC for 48 hours. Diameter of inhibition zones were measured with a calibrated digital caliper SC-6 Mitutoyo Digital Caliper® (Tokyo, Japan). Three measurements were taken for each disc and diameter value was calculated as the mean and standard deviation diameter.

By colony forming units (CFU) visual counting

The specimens were placed individually in a 24 wells microtiter plate for each experimental period. Two hundred μL of the adjusted *C. albicans* 10231 inoculum was added to each well and incubated at 37ºC for 48 hours and replaced every 24 hours. The specimens were gently washed twice with PBS pH 7.2 to remove nonadherent planktonic cells and transferred individually to 15 mL Falcon tubes TPP® (Trasadingen, Switzerland) in which 1 mL of sterile 0.85% NaCl solution was added and vortexed for a minute to release attached cells, serial dilutions were made and aliquots of 20 μL were inoculated in Sabouraud Dextrose Agar (SDA) Difco® (Detroit, USA) at 37 ºC for 48 hours. CFU was determined by visual counting, and results were expressed in CFU/mL.

Roughness of specimens incorporated with chlorhexidine diacetate

Quantitative analyses of the roughness (Sa) of the relining materials that were maintained immersed in artificial saliva under study condition that included T0, T1, T2, T3, and T4 that represent 24, 168, 336, 504 and 672 hours, respectively, were determined in a non-contact 3D digital profilometer Nanovea PS50 Optical® (Irvine, USA). The scanned area of each specimen was measured as 1 mm x 1 mm, with a scanning speed of 3 μm/s and a refractive index of 10,000 Hz, totaling a reading time of 12 min. The results were processed by Nanovea Professional 3D software® (Irvine, California, USA) and three-dimensional images represent the topography of the surface of the materials.

Statistical analysis

Statistical analysis used SPSS software 17.0® (Chicago, USA). The significance level adopted was set at 5%. The normality of the data was assessed using the Shapiro-Wilk test. The premises were established for antimicrobial activity and the roughness for the New Truliner™ used parametric tests. The assumptions of normality were established for the roughness of Trusoft™, and non-parametric statistical tests were used.

Agar diffusion was evaluated using the Tukey test in a post-hoc way. Comparison between groups in the same experiment were evaluated using the Mann-Whitney test, and comparison of the same group over time were evaluated by Wilcoxon test.

RESULTS

Antimicrobial activity by diffusion in agar

Inhibition halo diameters for both materials are presented in Table II. Results showed the formation of inhibition halos around the specimens against *C. albicans* in both New Truliner™ and Trusoft™ resin incorporated with 1% CHX, while specimens without CHX incorporation showed no visible halos.

Antifungal activity by CFU visual counting

CFU counting for both relining materials across the experimental times are presented in Table III and in Figure 1. The results demonstrated that at T0 the Trusoft™ CHX group exhibited the lowest CFU count with a statistically significant difference compared to the other groups ($p<0.05$). At T1 there was a significant inhibition of *C. albicans* growth in the groups containing CHX compared to their counterparts without CHX, demonstrating that the effect of CHX released may be efficient even after 7 days ($p<0.05$), which was not observed at any other moment of the experiment. Additionally, after 21 and 28 days, the specimens from the Trusoft™ group with and without CHX, showed a higher accumulation of *C. albicans* ATCC 10231 compared to the groups of New Truliner™ material ($p<0.05$).

Table II - Mean ± standard deviation (mm) of the inhibition halos formed by agar diffusion

New Truliner®	New Truliner® + CHX	Trusoft®	Trusoft® + CHX	*p*-value
0.0	3.81 ±1.34	0.0	4.19±1.56	0.008

Table III - Mean ± standard deviation of CFU of both materials, over experimental times

Time (days)	Relining Resin			
	New Truliner®	New Truliner® + CHX	Trusoft®	Trusoft® + CHX
0	6.84 ± 0.52^{a,A*}	3.34 ± 0.05^{b,A}	7.97 ± 0.96^{a,A}	1.95 ± 0.66^{c,A}
7	8.32 ± 0.53^{a,A,B}	7.07 ± 0.17^{b,B}	8.11 ± 0.06^{a,A}	6.74 ± 0.09^{b,B}
14	7.99 ± 0.14^{a,B}	7.71 ± 0.26^{a,C,D}	8.33 ±0.12^{a,A}	7.92 ± 0.11^{a,C,D}
21	7.93 ± 0.10^{a,B}	7.94 ± 0.07^{a,D}	8.17 ± 0.04^{b,A}	8.14 ± 0.14^{b,D}
28	9.44 ± 0.06^{a,C}	9.38 ± 0.09^{a,E}	10.53 ± 0.02^{b,B}	10.44 ± 0.06^{b,E}

(*) different lowercase letters in the same row indicate statistical difference between groups and different uppercase letters in the same column indicate statistical difference between groups.

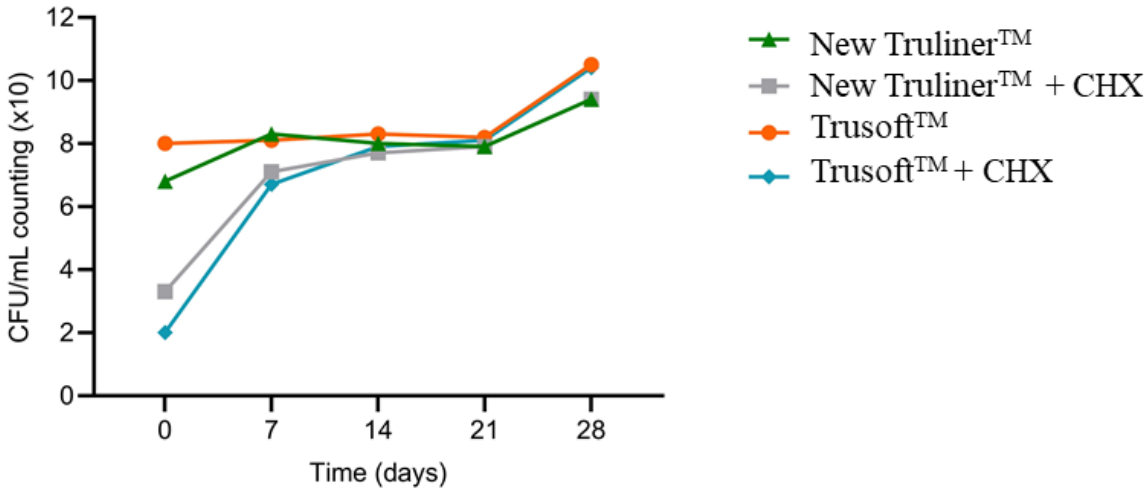

Figure 1 - CFU counting average during experimental times, methodology can be consulted in Material & Methods section above.

Analyzing the CFU counting over time, a greater accumulation of *C. albicans* ATCC 10231 was observed in specimens for longer periods. Both materials incorporated with CHX exhibited similar behavior, with the lowest count recorded at T0 ($p<0.05$), demonstrating the initial action of CHX. CFU progressively increased over time, stabilizing at T2 and T3 ($p>0.05$) showing a significant increase at T4 ($p<0.05$).

New Truliner™ had an evident difference starting at T2 compared to T0 ($p<0.05$), with the highest counting recorded at T4 ($p<0.05$). For the Trusoft™ material, the CFU counting at T0 remained stable through T3 ($p>0.05$), but an increase was observed at T4 ($p<0.05$).

Roughness measurement

New Truliner™

The mean roughness (Sa) values of the rigid recoating material with and without CHX can be seen in Table IV. In fact, no significant differences were observed in Sa between the New Truliner™ relining material with and without CHX ($p>0.05$). However, the groups T1, T2, T3 and T4 were statistically different from T0 ($p<0.05$) but no differences were observed from each other ($p>0.05$).

Although an increase in roughness was observed in T0, the New Truliner™ group with

CHX did not differ from the New Truliner™ without it ($p>0.05$), suggesting that the incorporation of CHX did not significantly alter the surface of the relining material.

Three-dimensional images were also generated to represent the surface topography of the New Truliner™ relining material and are shown in Figure 2.

Trusoft™

The roughness medians of the resilient relining material Trusoft™ with and without CHX can be seen in Table V and revealed a result similar to those found in New Truliner™ with or without CHX.

Three-dimensional images were generated to represent the surface topography of the Trusoft™ relining material and can be seen in Figure 3.

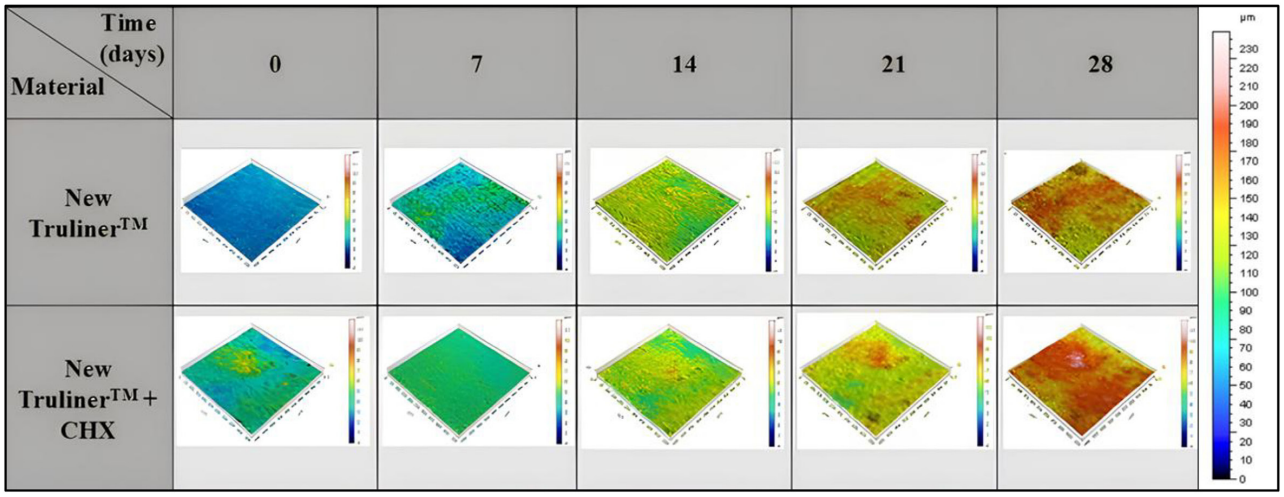

Figure 2 - Three-dimensional images of the New Truliner™ relining material over the experimental periods. Scale on both axes in each figure separates specimens in intervals of 0.1mm. The color gradient scale on the right identifies the volumetric changes in roughness on the overall surface of the specimen (µm).

Table IV - Mean ± standard deviation (µm) of the roughness (Sa) values for the New Truliner™ relining material with and without the incorporation of 1% chlorhexidine diacetate

Time (days)	Relining material (µm)	
	New Truliner™ + CHX	New Truliner™
0	1.00±0.83	0.73±0.21
7	1.27±1.04	1.31±1.01
14	0.93±0.13	0.82±0.26
21	1.03±0.57	0.86±0.38
28	0.72±0.09	0.84±0.27

Table V - Median (µm) of the roughness values (Sa) for the Trusoft™ relining material, with and without the incorporation of 1% chlorhexidine diacetate, over the experimental times

Time (days)	Relining material	
	Trusoft™	Trusoft™ + CHX
0	28.63 (6.12;74.85)	13.25 (3.07;73.05)
7	39.27 (6.02;62.39)	6.78 (2.98;61.5)
14	9.02 (4.09;77.5)	12.57 (3.88;48.54)
21	8.94 (4.06;58.94)	7.39 (2.64;65.93)
28	24.69 (3.89;71.62)	12.19 (2.63;47.29)

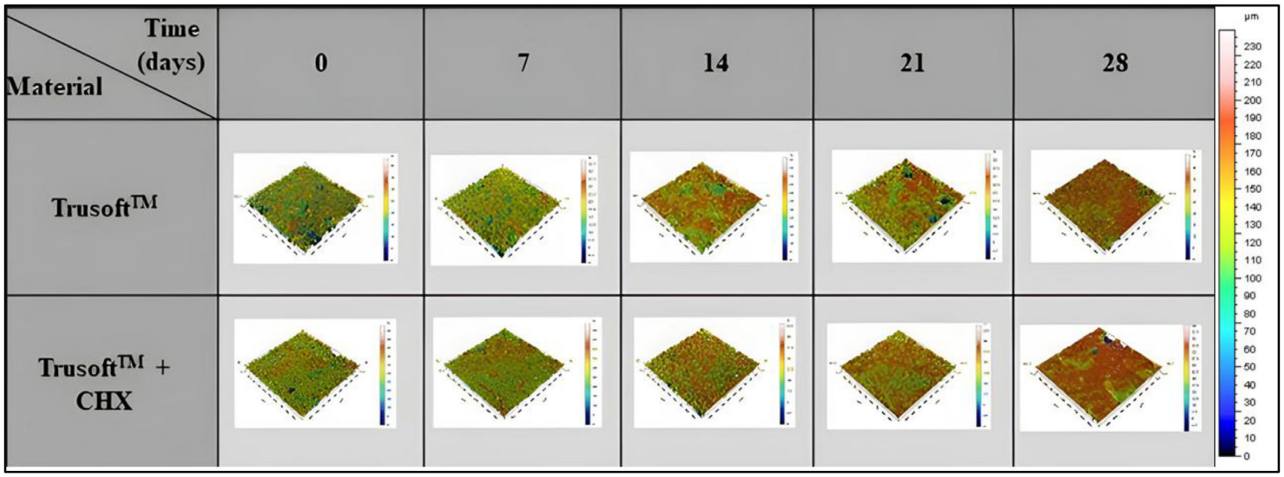

Figure 3 - Three-dimensional images of the Trusoft™ relining material over the experimental periods. Scale on both axes in each figure separates specimens in intervals of 0.1mm. The color gradient scale on the right identifies the volumetric changes in roughness on the overall surface of the specimen (µm).

DISCUSSION

Chlorhexidine diacetate has been widely used in dentistry and is recognized for its antimicrobial activity against a wide range of bacteria and fungi, including *C. albicans* [14,21,22]. Its use as an antifungal agent may be a useful alternative to fluconazole, ketoconazole, amphotericin B, nystatin, or miconazole, since the development of resistance for these compounds has been vastly observed. The antimicrobial effectiveness of chlorhexidine within acrylic resins also confirms that the polymerization of the material does not affect the efficacy of the drug [12,23]. In fact, CHX was selected for this study because it is widely used both for the treatment of oral infections, but also as antiseptic and disinfectant of full dentures, demonstrating greater stability compared to CHX gel or solution formulation [17,19,23-25]. This study revealed that both the New Truliner™ and the Trusoft™ resins incorporated with 1% chlorhexidine diacetate (CHX) showed an antifungal activity that was evidenced by the formation of inhibition halos, resulting from the diffusion of the incorporated substance through solid medium. On the other hand, the probes without CHX did not present these halos indicating that these resin materials, in their original formulations, do not demonstrate antifungal action against *C. albicans* [15,26,27].

The antifungal activity presented here agrees with earlier investigations that used CHX in rigid resins of Polyethyl Methacrylate and Methyl Polymethacrylate [12,14,19,25]. Those studies showed a double sized inhibition halo of *C. albicans* compared to the inhibition halo formed by the same resin incorporated with 10% fluconazole [10]. However, differently from our study, such concentration makes the clinical use unfeasible, since it was classified as severely cytotoxic [16].

Previous studies have already proposed that the incorporation of chlorhexidine and miconazole in a gel formulation to relining materials based on acrylic resin may be useful, however the physical consistency of the materials after the incorporation of these substances did not present the same inhibitory activity on *C. albicans* [19,25].

Bertolini et al. found that concentrations of 1% and 2% of chlorhexidine diacetate incorporated into the resilient relining material based on acrylic resin exhibit significant inhibition zones without significant cytotoxic impairments. Furthermore, no inhibition halos were observed around the specimens with 0.5% chlorhexidine diacetate suggesting that 1% concentration of CHX must be the effective concentration for antifungal activity, with no cytotoxic effect [16]. Their results were related to the inhibition of *C. albicans* found in studies that used resilient resins for the relining of prosthesis, but with the incorporation of other substances that were not so common, such as silver nanoparticles and essential oils such as *Melaleuca alternifolia* [12,13,16].

In fact, the release rate of diacetate chlorhexidine is known to be high in the first four days [26]. The process is initiated when the storage solution diffuses through the polymeric matrix of the material, dissolving and releasing CHX. As this

release raises, the pores created by this release promote the acceleration of the antimicrobial agent liberation [27]. In the present study, materials with the incorporation of CHX showed the ability to inhibit the growth of *C. albicans,* which was not observed in specimens without it.

Differently, Patel et al. used a rigid resin based on polyethyl methacrylate with the incorporation of CHX and verified the inhibition of *C. albicans*, in different concentrations, ranging from 4.5% to 12%. This inhibition was demonstrated by plating the cell suspension in contact with the specimens and CFU counting after 24 hours [28,29]. However, no long-term analysis was performed on their study, which could have led to an overestimating result, considering the high concentration of CHX incorporated.

The results obtained in our study corroborate the bench experimental studies that evaluated the behavior of CHX release from acrylic resins based on methyl polymethacrylate and ethyl polymethacrylate, which reported a high release rate during the first days, which is the period in which the diffusion gradient of the tested drug is usually significantly higher [30,31]. In addition to the fact that each relining material present different release kinetics, the process of releasing chlorhexidine into the storage solution, as well as its absorption by the material is related to the osmolarity of the solution in contact with the material that received chlorhexidine incorporation [30]. In our study, medium was constantly changed, preventing its saturation that may change release kinetics of CHX resembling the oral environment dynamics where a constant exchange of saliva dilutes any present drug [28,32].

In fact, the higher concentration of CHX incorporated into resin material can lead to modifications in its biomechanical properties which makes mandatory to avoid unnecessary cytotoxicity and changes in resin roughness properties [16,27,30-34]. In our study, although an increase in surface roughness of all evaluated relining materials used was observed over time, the incorporation of 1% chlorhexidine diacetate was not able to significantly alter roughness values compared to the group without CHX ($p>0.05$). Indeed, roughness is an important property to evaluate the surface integrity of relining materials and influences friction surface, such as fatigue, resistance decreasing, optical properties, fluid flow, roughness changes, and any modification on its basis may lead to an increase in surface and mechanical retention of pigments and biofilm [12,17,30]. Nevertheless, in the intraoral environment, these materials undergo physicochemical pressure of chewing and dieting, which can impair the integrity of the surface [11,17].

In our study, a change in roughness was observed after the contact with saliva, but the group with CHX did not differ from the group without it, showing that chlorhexidine did not alter the surface roughness significantly. So, here we demonstrated that both groups showed changes in surface roughness over the days, but the addition of chlorhexidine cannot be held responsible for it. Furthermore, although not investigated in this study, we consider that, in addition to roughness, other factors also need to be evaluated for a better response of the material to stress, such as the evaluation of the response to compression, tension, shear, and bending, which may also interfere with the results. In addition, an evaluation of the different responses to the morphological states of *C. albicans* (hyphae or yeast) would also be an interesting subject for study, since they directly interfere with the response of this microorganism to antifungal agents [35]. Therefore, future studies using new methodological approaches may address the limitations present in this study.

New Truliner™ roughness associated with the results of antifungal activity demonstrate an advantage of the incorporation of CHX. This situation seems to be due to the significant capacity to inhibit the growth of *C. albicans*, especially up to the 7th day, without prejudice to its roughness. On the other hand, Trusoft™ altered roughness (Sa) over time, but the addition of CHX did not make this change greater than the one that would occur naturally, considering the natural gradual increase in roughness over 28 days [28]. According to Urban et al. [36] this behavior is due to the leaching of the plasticizer present in resilient materials, which promotes a decrease in resilience and an increase in hardness and roughness, which ultimately leads to a decrease in the longevity of the material [21,31,33].

In fact, Bertolini et al. [16] have already pointed that biofilm formation decreases roughness in resins incorporated with CHX compared to the resin without it. However, no specific roughness tests were performed, and the porosity left after the release of chlorhexidine could interfere in the roughness of the resin.

CONCLUSION

The results pointed in this study demonstrate a favorable antimicrobial activity with no significant impairment on material roughness. Considering this, the commercial development of prosthesis relining materials with antimicrobial action with low tissue toxicity seems to be very favorable, without prejudice to their intrinsic structural properties. In this sense, the present study provides some perspective for further research to be carried out to verify the clinical and therapeutic efficacy of these relining materials, incorporated with chlorhexidine diacetate.

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None.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author's Contributions

AKA, MGC, JARC, MJSA: Conceptualization. AKA, CSSF, JARC, MJSA: Data Curation. JARC, CSSF, AKA: Investigation. JARC, CSSF, AKA: Methodology. MGC, LCM, MJSA, DCG: Project Administration. LCM, MGC, MJSA: Supervision. CSSF, BNMS: Software. JARC, CSSF, AKA: Writing - Original Draft Preparation. JARC, BNMS: Writing - Review & Editing.

Conflict of Interest

The authors declare that there is no conflict of interest.

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Regulatory Statement

This study did not involve human participants, animal studies, or the use of human-derived samples. Therefore, Ethical approval was not required.

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