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Complete Denture Biofilm after Brushing with Specific Denture Paste, Neutral Soap and Artificial Saliva

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This study compared the levels of biofilm in maxillary and mandibular complete dentures and evaluated the number of colony-forming units (cfu) of yeasts, after using auxiliary brushing agents and artificial saliva. Twenty-three denture wearers with hyposalivation and xerostomia were instructed to brush the dentures 3 times a day during 3 weeks with the following products: Corega Brite denture dentifrice, neutral liquid soap, Corega Brite combined with Oral Balance (artificial saliva) or tap water. For biofilm quantification, the internal surfaces of the dentures were disclosed, photographed and measured using a software. For microbiological analysis, the biofilm was scrapped off, and the harvested material was diluted, sown in CHROMagar™ Candida and incubated at 37°C for 48 h. Data were analyzed statistically by two-way ANOVA and Tukey's test (α=0.05). Mandibular dentures presented a mean biofilm percentage (μ=26.90 $\pm$ 21.10) significantly greater than the maxillary ones (μ=18.0 $\pm$ 15.0) ($p<0.05$). Brushing using Corega Brite combined with Oral Balance (μ=15.87 $\pm$ 18.47) was more effective ($p<0.05$) than using the denture dentifrice (μ=19.47 $\pm$ 17.24), neutral soap (μ=23.90 $\pm$ 18.63) or tap water (control; μ=32.50 $\pm$ 20.68). For the microbiological analysis, the chi-square test did not indicate significant difference between the hygiene products for either type of denture. The more frequently isolated species of yeasts were *C. albicans*, *C. tropicalis* and *C. glabrata*. In conclusion, mandibular dentures had more biofilm formation than maxillary ones. Denture brushing with Corega Brite dentifrice combined with the use of Oral Balance was the most effective method for reduction of biofilm levels, but the use of products did not show difference in yeast cfu counts.

Key Words: complete denture, biofilm, denture cleansers, brushing, yeasts.

Introduction

Complete denture biofilm can be removed by mechanical (brushing and ultrasonic device) and chemical methods (alkaline peroxide and hypochlorite, acids, enzymes and disinfectants). Among these, brushing with conventional and specific dentifrices is the most common method applied for routine denture biofilm control (1) and has been proven effective (2,3). Soap, as an auxiliary hygiene agent, is an accessible abrasive-free product and has been claimed as effective against anaerobic microorganisms and yeasts (4) and stains (5). Studies have indicated that the combination of coconut soap with hypochlorite is an effective cleansing method (6). However, randomized clinical trials regarding its effectiveness as an isolated cleansing method have not been reported (7).

A factor that can adversely affect oral health is xerostomia (dry mouth sensation), which generally is followed by hyposalivation. The reduction of salivary flow affects the lubrication of oral tissues, diminishing the cleaning of residues and the amount of natural antimicrobial salivary agents, favoring the appearance of infections (8). Aiming at restoring the natural antimicrobial capacity of the saliva in xerostomic individuals, an enzymatic system has been investigated, containing lactoferrin, lysozyme and lactoperoxidase with capacity to increase the levels of agents such as tiocianate (SCN^-) and hydrogen peroxide (H_2O_2), thus inhibiting the growth of *Candida albicans* and other microorganisms (9). Reduction of the supragingival biofilm has been observed with the use of this system (10), but there is no available research evaluating the effect of these products on denture surfaces (7,11).

The hypothesis that denture biofilm accumulation can be greater in individuals with xerostomia prompts a need for the evaluation of cleansers and artificial saliva capable of inhibiting biofilm formation with an effective antimicrobial action against yeasts like fungi, which are important etiologic agents of diseases in complete denture wearers.

The objective of this study was to compare the levels of biofilm on the surfaces of maxillary and mandibular complete dentures after the use of two auxiliary brushing agents (specific denture dentifrice and neutral liquid soap)

and artificial saliva (Oral Balance®), as well as to evaluate quantitatively and qualitatively the presence of yeasts on the denture surfaces after the use of each tested cleaning product.

Material and Methods

Twenty-three edentulous patients (14 females and 9 males) aged 44 to 84 years (mean age = 65 years), wearers of maxillary and mandibular complete dentures, were recruited from the Complete Denture Clinic of Ribeirão Preto Dental School, University of São Paulo, Brazil. All subjects were required to have good general health but present xerostomia and hyposalivation. The dentures had been worn for about 1 to 5 years and had biofilm scores ≥1 according to the Additive Index (3). Xerostomia and hyposalivation had been diagnosed based on a questionnaire and sialometry test (8), respectively, at the same clinical facility. Hyposalivation was considered when the saliva flow rate was less than 1 mL/min.

Approval for the study was obtained from institutional Ethics Committee (#2003.1.355.58.0) and informed consent was given by all patients.

Initially, the internal surfaces were disclosed with 1% neutral red, cleansed using a denture brush (Medic Denture Brush-Condor S.A., São Bento do Sul, SC, Brazil) with neutral liquid soap (JOB-Química Ltda, São Paulo, SP, Brazil) for total biofilm elimination. Subsequently, each volunteer received a toothbrush (Oral B Indicator 40 soft; Gillette do Brasil Ltda., Manaus, AM, Brazil) and was instructed to brush the dentures after each meal for 2 min with the following auxiliary agents: tap water (control - W); Corega Brite dentifrice for complete dentures (CB; Stafford-Miller Indústria Ltda, Rio de Janeiro, RJ, Brazil); Neutral liquid soap - pH 12 (neutral) (12) (S; Selvática Farmácia Homeopática e de Manipulação, Ribeirão Preto, SP, Brazil) and CB + Oral Balance artificial saliva (OB; Laclede do Brasil Ltda., São Paulo, SP, Brazil). The volunteers were also instructed to rinse the mouth with tap water after brushing and keep the dentures immersed in tap water overnight.

This study was set up using a crossover design. Each volunteer was randomly assigned to wear one of the products for 3 weeks. After this period, the volunteer returned to exchange the product for another. Between products, the volunteer used running tap water (control) for 7 days. Every week, the denture internal surfaces were disclosed with 1% neutral red solution and digital photographs were obtained (Canon EOS Rebel; Canon Inc., Tokyo, Japan) at 45°. The areas (total and disclosed) were measured with Image Tool software, version 3.0 (UTHSCSA, San Antonio, TX, USA) (13). Biofilm percentage was calculated by the relation between the biofilm disclosed area multiplied by 100 and the total analyzed surface. The researcher eliminated the biofilm after taking the photographs as described above.

For the microbiological analysis, at the end of the third week, the biofilm was scrapped off by brushing with saline and collected (14). The dilutions in solution drain plug PBS (10^{-4}) were sown in the CHROMagar™ Candida culture medium (CM) under laminar flow, in duplicate and incubated at 37°C for 48 h. The number of colony-forming units (cfu) of yeast-like fungi was counted and identification was made based on the macroscopic morphology and use of a color code (*C. albicans* - green; *C. dubliniensis* - green; *C. glabrata* - purpura; *C. tropicalis* - blue; *C. parapsilosis* - white). The identity of the isolated yeasts was obtained by the tests of tube formation of germination (GT), chlamydoconidia and tests of fermentation and assimilation.

Data obtained from the efficacy of the denture cleaning products were examined using a two-way ANOVA and Tukey's test. The reduction of cfu counts was analyzed by the chi-square test. The significance level was set at 5% for all analyses.

Results

The mandibular dentures presented a mean biofilm percentage (μ=26.90 ± 21.10) significantly greater than the maxillary ones (μ=18.0 ± 15.0) ($p<0.05$). Brushing using Corega Brite combined with Oral Balance (μ=15.87 ± 18.47) was more effective ($p<0.05$) than using the denture dentifrice (μ=19.47 ± 17.24), neutral soap (μ=23.90 ± 18.63) or tap water (control; μ=32.50 ± 20.68) (Table 1).

The mean percentages of biofilm for each product are graphically illustrated in Figures 1 and 2.

The cfu counts for yeast species were transformed in $\log_{10}$ (Figs. 3 and 4). Table 2 shows that yeast incidence in the maxillary complete dentures was greater than in the mandibular dentures. High counts of *C. albicans* were found in both dentures.

The total frequency values for the maxillary and mandibular dentures (Table 2) were obtained and the chi-squared test did not indicate any statistically significant difference among the brushing products (PHo=76.75%; x^2=1.14).

Table 1. Mean percentage of biofilm on the internal surface of the complete dentures after use of the products

Product	Means and S.D.
Tap water (control)	32.50 ± 20.68[a]
Liquid neutral soap	23.90 ± 18.63[ab]
Corega Brite denture dentifrice	19.47 ± 17.24[bc]
Corega Brite + Oral Balance (artificial saliva)	15.87 ± 18.47[c]

Same letters indicate statistically similar means (Tukey's value = 0.46).

Discussion

The lactoperoxidase enzyme system found in artificial saliva is indicated to replace or improve the antimicrobial capacity (9), presenting antibacterial activity *in vitro* and clinical efficacy when incorporated in dentifrices (3). The artificial saliva used in this study does not present any systemic or local side effects, alleviates xerostomia symptoms (15) and does not cause discoloration or pigmentation of the acrylic resin (16).

In this study, using an auxiliary agent was proven necessary, as it promoted greater biofilm reduction than seen in the control group (Table 1). In the search for an alternative material, soap was chosen due to the absence of abrasiveness (17), easy access and low cost for patients. No medicaments or antiseptic agents were added to its composition, enabling to evaluate its cleaning capacity (12). The neutral pH aimed at diminishing the possibilities of allergic reactions or unpleasant taste, and the liquid form to facilitate application to the brush (3,12). Sodium sulfate lauryl was associated as a prevailing agent, as well as sulfate lauryl triethylamine and betaine cocamidopropyl, also found in the formulation of the toothpaste Corega Brite. According to Landa et al. (18), the association of tensoactive products facilitates penetration of the product into the biofilm. The incorporation of sulfate lauryl in hygienic cleansers by immersion (Kleenite and Mersene) resulted in greater efficacy, acting as a soluble agent of proteins (4).

In the present study, brushing with Corega Brite was more effective than brushing with soap, a fact explained by the presence of solid components, such as titanium dioxide, silicon and silica dioxide in the dentifrice, which increases the mechanical clinical ability (3,19). Efficacy of the detergents depends mainly on their penetration into

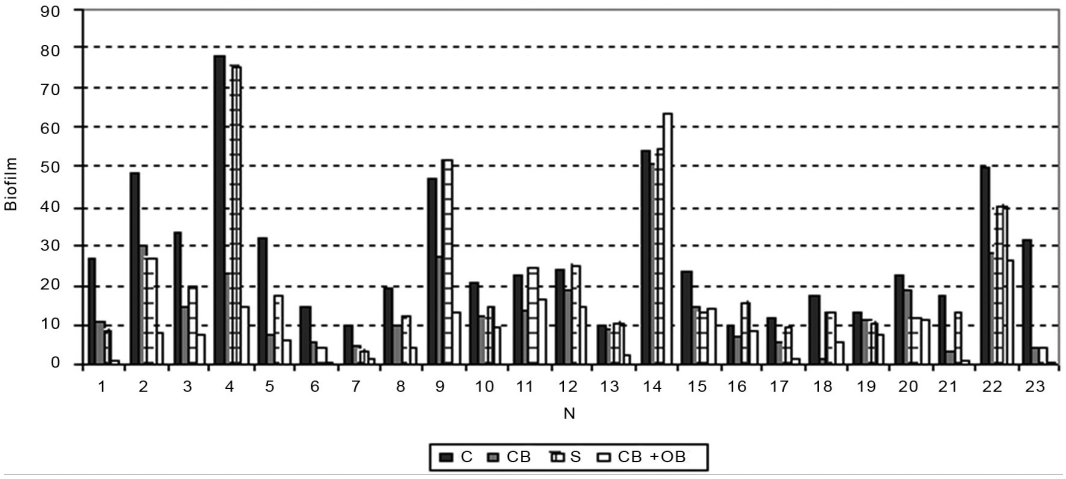

Figure 1. Average percentage of biofilm in the internal surface of the maxillary complete denture after the use of tap water (control - C), Corega Brite (CB), Neutral Soap (S) and Corega Brite associated with daily use of artificial saliva (CB + OB).

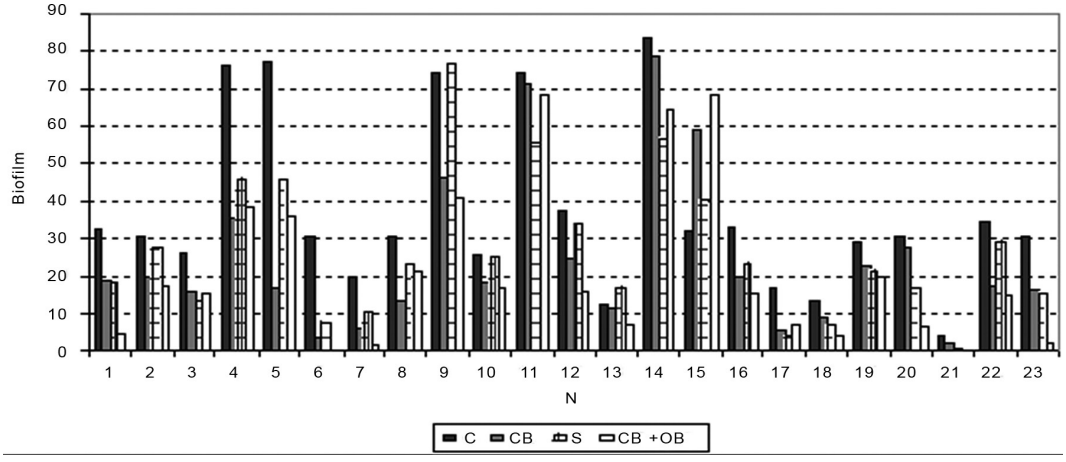

Figure 2. Average percentage of biofilm in the internal surface of the lower complete denture after use of tap water (control - C), Corega Brite (CB), Neutral Soap (S) and Corega Brite associated with daily use of artificial saliva (CB + OB).

the biofilms (18) and the maturation biofilm is a barrier that makes this process slow and partial most of the time. According to Goddson (20), the components of dentifrices and mouthrinses take 2 min on average for satisfactory absorption. Considering the average brushing time and the product absorption into the biofilm, it can be deduced that time was a determinant factor for the efficacy of soap, thus interfering in its cleaning capacity. Longer exposure time to the product before brushing, in addition to incorporating antiseptic agents or tensoactive products with greater penetration capacity, should be assessed. These results disagree from the study where there was no difference between Colgate Anti-tartar dentifrice and soap (5). This disagreement can be explained by the differences in methodologies, as McCabe et al. (5) employed brushing performed by the professional and score attribution methodology for biofilm quantification. In the

Table 2. Frequency of colony-forming units (cfu) in maxillary and mandibular complete dentures after use of the products

Yeast	Maxillary					Mandibular				
	W	CB	S	CB+OB	Total	W	CB	S	CB+OB	Total
C. albicans	12	11	15	9	47	6	6	9	7	28
C. glabrata	7	8	7	9	31	5	5	4	6	20
C. tropicalis	7	4	7	6	24	6	3	7	6	22
C. dubliniensis	6	5	4	2	17	2	4	2	2	10
C. parapsilosis	2	5	1	1	9	3	2	0	3	8
Total	34	33	34	27	128	22	20	22	24	88

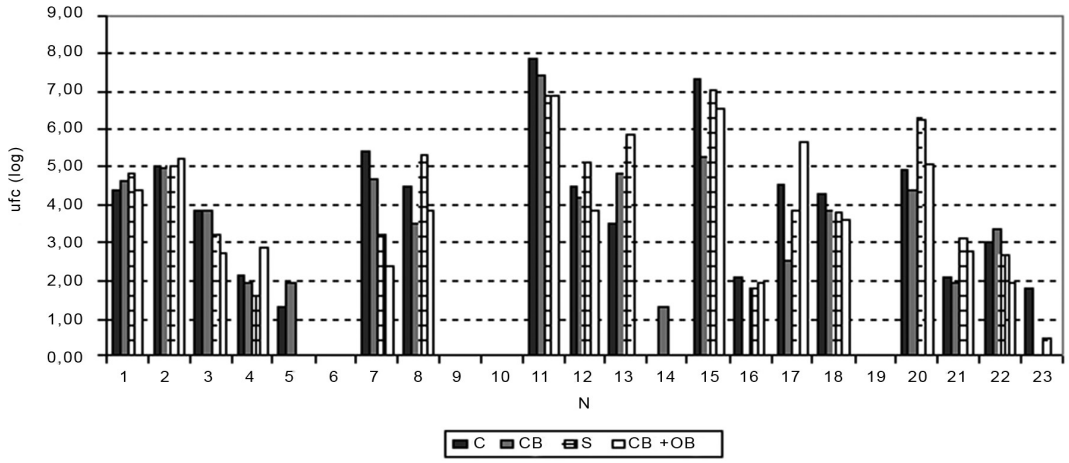

Figure 3. Total of ufcs (Log10) of the yeast found in the internal surface of upper complete denture after use of tap water (control - C), Corega Brite (CB), Neutral Soap (S) and Corega Brite associated with daily use of artificial saliva (CB + OB).

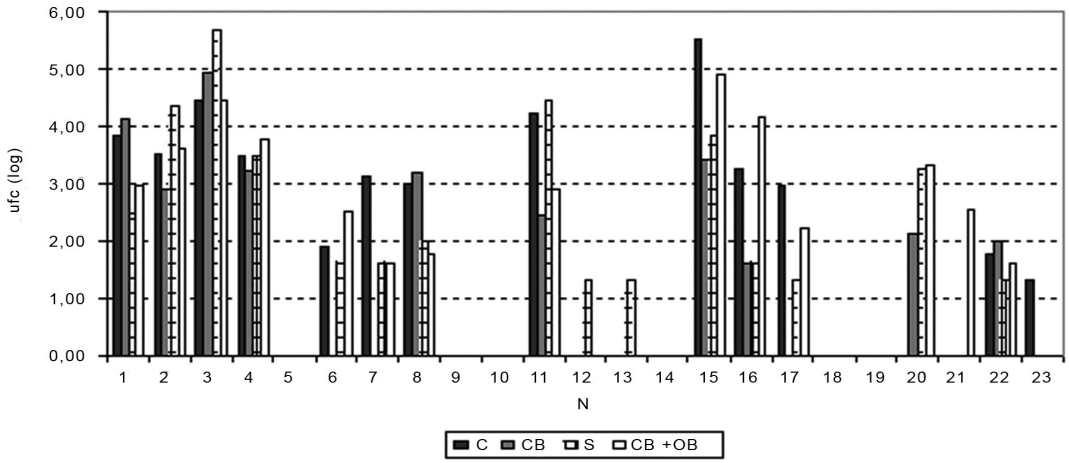

Figure 4. Total of ufcs (Log10) of the yeast found in the internal surface of lower complete denture after use of tap water (control - C), Corega Brite (CB), Neutral Soap (S) and Corega Brite associated with daily use of artificial saliva (CB + OB).

present study, brushing was performed by the volunteers and photography combined with quantitative analysis was employed to obtain an objective evaluation of the biofilm levels (13). The mandibular dentures present a mean biofilm percentage significantly greater than the maxillary ones. This fact could be explained by the retaining capacity of the maxillary dentures as well as its shape. The mandibular dentures form a considerable reservoir of microorganisms, emphasizing the need for good oral hygiene.

Despite observing similarity in the effectiveness of the auxiliary agents employed in this study in terms of reduction of yeasts, it is unknown whether higher alkalinity of soap could generate the same results. Gibbson et al. (21) verified *in vitro* that the acid and alkaline detergents were more efficacious than the neutral detergent in the removal of some types of bacteria (*Staphylococcus aureus* and *Pseudomonas aeruginosa*) initially adhered to the metallic specimens. However, Barnabé et al. (6), using the same microbiological harvesting technique, found that cleaning with coconut soap produced no significant reduction of *Streptococcus mutans* and *C. albicans* on the biofilm of complete dentures.

Regarding Corega Brite denture dentifrice, its denture biofilm removal capacity has been demonstrated, but its antimicrobial activity has not been evaluated. Panzeri et al. (3) found that two experimental dentifrices containing 1% chloramine T and 0.01% fluorosurfactant were able to reduce biofilm coverage and mutans streptococci counts after the clinical trial stage; however, yeasts from Candida genus on denture bases were not affected by the tested dentifrices. Perhaps a significant reduction for Candida sp. counts would only be expected with dentifrices with high concentrations of active substances like cetylpyridinium chloride (19).

The antimicrobial system in the artificial saliva presented clinical efficacy in biofilm removal (Table 1). Studies give emphasis to its antimicrobial effect (bactericidal or bacteriostatic) on planktonic cells, which are more susceptible to the lactoperoxidase system, reducing the cfu counts on the surface of the dentures (22). *In vitro* tests show efficacy of the lactoperoxidase system against fungi (9). However, the microbiological analysis (Table 2) did not evidence any significant effectiveness of this system, agreeing with the previous findings where the use of artificial saliva against the microbiota in geriatric patients with xerostomia produced no significant reduction of yeasts (23). In addition to the barrier promoted by the biofilm mass against the antimicrobials, the efficacy observed *in vitro* is relative, since the cultivated microorganisms may offer less resistance to certain agents (22). The results showed that mechanical biofilm removal alone was not sufficient to promote reduction of yeasts, strains that normally colonize acrylic resin surface. These observations corroborate with the findings of Barnabé et al. (6), reinforcing the need to use of chemical agents to reduce the number of viable microorganisms. The literature has emphasized the ability of decontamination promoted by various chemical agents (4,24) and favors the use of this hygiene, especially in patients with impaired manual dexterity (11). Future studies should assess the effectiveness of these auxiliary agents associated to chemical methods, by a randomized clinical trial design.

Similar to other studies (19,24), *C. albicans* was the most frequently isolated species in the present study (Table 2), followed by *C. glabrata* and *C. tropicalis*. The highest frequency of *C. albicans* and *C. glabrata* found in this study agree with the findings of Grimoud et al. (23), who observed similar prevalence of these species in the saliva of patients with reduced salivary flow. The *C. dubliniensis* is a recently described Candida species combined with oral candidosis and frequently found in immunocompromised patients; it exhibits a high similarity to *C. albicans*. The use of the CHROMagar™ medium is advantageous to facilitate the detection of mixtures of yeast species from different samples on a single isolation plate. This medium is particularly useful as *C. dubliniensis* is often co-isolated with *C. albicans* (25).

From the obtained data, it may be concluded that the mandibular dentures presented a significantly greater mean biofilm percentage than the maxillary dentures. Brushing with a specific denture cleansing paste results in better denture cleansing than brushing with neutral liquid soap or tap water alone. The artificial saliva showed a adjuvant and preventive efficacy to reduce biofilm levels from complete dentures. None of the trial methods resulted in a significant reduction of yeast cfu counts. The most frequently isolated yeast species were *C. albicans*, *C. tropicalis* and *C. glabrata*.

Resumo

Este estudo comparou os níveis de biofilme em próteses totais maxilares e mandibulares, e analisou o número de unidades formadoras de colônias de leveduras, após o uso de agentes auxiliares da escovação e saliva artificial. Vinte e três usuários de próteses totais com hipossalivação e xerostomia foram orientados a escovar as dentaduras 3 vezes ao dia durante 3 semanas com os seguintes produtos: Corega Brite (dentifrício para prótese), sabonete líquido neutro, Corega Brite associado com o uso do Oral Balance (saliva artificial) ou água de torneira. Para a quantificação do biofilme, as superfícies internas das próteses totais foram evidenciadas, fotografadas e o biofilme quantificado com o auxílio de um software. Para a análise microbiológica, o biofilme foi removido por escovação, coletado, diluído, semeado em meio seletivo CHROMagar™ Candida e incubado a 37ºC por 48 h. A análise de variância para dois fatores ($p<0,05\%$) mostrou que as próteses mandibulares apresentaram uma média de porcentagem de biofilme ($\mu=26,90\pm21,10$) maior que as maxilares ($\mu=18\pm15$). O teste complementar de Tukey (0,46; $p<5\%$) mostrou que a escovação com Corega Brite e Oral Balance ($\mu=15,87 \pm 18,47$) foi mais efetiva que o dentifrício ($\mu=19,47 \pm 17,24$), sabonete neutro ($\mu=23,90 \pm 18,63$), ou água de torneira (controle; $\mu=32,50 \pm 20,68$). Em relação à análise

microbiológica, o teste de Qui-Quadrado não indicou diferença entre os produtos de higiene, para ambas as próteses. As espécies de leveduras mais comumente isoladas foram *C. albicans*, *C. tropicalis* e *C. glabrata*. Em conclusão, as próteses mandibulares apresentaram mais biofilme do que as maxilares. Além disso, a escovação das próteses com o Corega Brite associado ao uso do Oralbalance foi o método mais efetivo na redução dos níveis de biofilme, entretanto o uso dos produtos não demonstrou diferença no número de ufc de leveduras.

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