



Antibiofilm Activity of an Experimental *Ricinus Communis* Dentifrice on Soft Denture Liners

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The disadvantage of liners materials is the difficulty of biofilm control. It was compared an experimental dentifrice contained *Ricinus communis*, with commercial dentifrices as antibiofilm activity against microorganisms on denture liner. Six hundred specimens were distributed in 5 groups (n=18/ microorganism): water; experimental dentifrice; specific dentifrice for denture and two conventional dentifrices against *C. albicans*; *C. glabrata*; *S. mutans*; *S. aureus*; *E. coli*. Each group had a negative (n=5; without contamination) and positive control (n=15/ microorganism; without cleaning). The antibiofilm activity was evaluated by the method of biofilm formation in triplicate. The specimens were contaminated in a standard way and incubated. After that, manual brushing was performed (60 s), washed with PBS, immersed in liquid culture medium for resuspension and sowing in solid medium. The results (mean of triplicates) were expressed in CFU/mL. The data was submitted to Shapiro-Wilk, ANOVA and Tukey test (p<0.05). The specific dentifrice (1.27±1.20) was the most effective against *S. mutans*, followed by conventional (Trihydral, 3.13±0.88; Colgate, 2.16±2.02) and experimental (3.81±1.37) dentifrices, which were similar to each other (p=0.008). All of them were different from water (4.79±1.42). The specific (0.21±0.21) and experimental (0.36±0.25) dentifrices were similar against *S. aureus*, with a higher mean of CFU when compared to conventional (Colgate, 0.06±0.13), which was more efficient (p=0.000). For *C. albicans*, *C. glabrata* and *E. coli*, all dentifrices were similar to water (p=0.186). It was concluded, that the experimental dentifrice was effective against *S. aureus* and had not efficacy against *Candida* spp.; *S. mutans*; *E. coli*, as occurred with the commercial dentifrices.

Key Words: denture liners, denture cleansers, dentures, dentifrices, castor bean.

Introduction

The complete dentures are fabricated with heat-polymerized acrylic resin which has higher modulus of elasticity (2400 MPa) than the support tissues (1.25 to 5.0 MPa) (1). This explains some damages and injuries of the tissues. As solution for this problem, the resilient lining materials were developed. They provide additional protection to the oral mucosa, greater retention and stable of the prosthesis (2).

However, the major disadvantage of resilient material is the difficulty in keeping them clean, allowing biofilm accumulation (2,3). In this situation, dentures bases can become reservoir of microorganisms related to the development of local (denture stomatitis) and systemic diseases, as bacterial endocarditis, aspiration pneumoniae, intestinal infection and chronic obstructive pulmonary disease (4). *Candida albicans*, *Candida glabrata*, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus mutans* are microorganisms with potential for pathogenicity and were isolated from the internal surface of total dentures (5). Specific products for dentures have been developed, with emphasis on products based on *Ricinus communis*,

which is a vegetable derived from Castor plant (*Ricinus communis*; division Magnoliophyta, class Magnoliopsida, sub-class Rosidae, order Euforbiales, family Euforbiaceae), native from Africa, commonly found in tropical climates areas and warm temperate regions, like Brazil.

Cleansers with *Ricinus communis* as hygiene solution for immersion (5-7) or dentifrice (8,9) has been used for elimination and control of biofilms. The detergent derived from *Ricinus communis* oil was also used as intracanal medication paste against endodontic infections (10). The *Ricinus communis* acts as a detergent and has antimicrobial properties. Leite et al. (8) evaluated experimental dentifrices based on *Ricinus communis* with different concentrations (1, 2, 5 and 10%) by the test-well diffusion in agar against microorganisms and found good results with 10%, which concentration, as part of the composition of the dentifrice, that was efficient against bacteria and fungus. In another study, the experimental dentifrice based on *Ricinus communis* at 10% showed similar results to the specific dentifrice for dentures, keeping the resilient material properties (abrasiveness, hardness and color stability) within acceptable values (9). The results of studies with the use

of this plant are promising (5-10) and require laboratory and clinical researches.

Mechanical hygiene of dentures, with brush and toothpaste, is an efficient, simple, easily accessible and low cost method for biofilm removal (11). Brushing procedures are widely used by denture users, but it is not the most suitable for hygiene of dentures associated with resilient materials, since these can be damaged by abrasion (12). Chemical cleansers are considered effective methods against the proliferation of the microorganisms on biofilm (5,6,13), however, the daily use of these products may adversely affect the properties of the prostheses materials (7). The composition of dentifrices is complex, the majority consisting of water, thickeners, detergents, flavourants, pigments, abrasive agents and antimicrobial substances. To minimize the adverse effects on the constituent materials of dentures, low abrasiveness dentifrices should be used and specific products for hygiene of relining prostheses should be developed.

In order to promote the mechanical removal of the biofilm formed on soft liner materials and chemical disinfection of specific microorganisms, the objective of this study was evaluate the antibiofilm activity of an experimental dentifrice for dentures containing *Ricinus communis* in its formulation, comparing the results with commercial dentifrices and distilled water. The null hypothesis verified whether there was equality in the efficacy of the experimental dentifrice with the others

commercialized and if there was difference with the water (control) for all the microorganisms tested.

Material and Methods

The formulation of the experimental dentifrice contained *Ricinus communis* gel, that was obtained by an esterification reaction in the Institute of Chemistry, University of São Paulo, Sao Carlos, Brazil. The dentifrice was prepared according to Leite et al. (8), (Table 1). The formulations of the other commercial dentifrices used are shown in Table 2.

Six hundred circular specimens (15x3 mm) were obtained with soft liner Mucopren soft (batch number: 80301; Kettenbach, GmbH & Co, KG), according to Badaró et al. (9). Perforated acrylic matrixes (Plex glass, Day Brazil S. A.) were fixed on a glass plate. The material was handled according to manufacturer's instructions, inserted in the mold and the assembly was pressed with another glass plate (200 g) until final polymerization. Then, the specimens were removed for disposal of excess with sharp scalpel.

The sterilization of the specimens was performed using ethylene oxide (SERCON MP300 equipment with camera model HG belongs to Hospital das Clínicas of the Medical School of Ribeirão Preto – USP), programmed in cycle 1 with prevacuum of 20 min at 55 °C, 180 min of sterilization, 120 min of hyperventilation, pressure of 50 kgf/cm² and vacuum of 50 kgf/cm².

The antibiofilm activity was tested against specific microorganisms. After reactivation of microorganisms in their respective culture media (Table 3) and incubation at 37 °C for 24h (Incubator Shaker, Model CE-320, Cienlab), a colony of each microorganism was spiked in broth for growth and placed in an oven bacteriological at 37 °C for a period between 16 and 22 h (exponential growth phase).

Microorganisms were added to PBS (phosphate buffered saline) solution to standardize the selected inoculum. The turbidity of the microbial suspension was verified by spectrophotometer and had an absorbance reading from 0.08 to 0.1, at a wavelength of 625 nm. According to our calibration curves, this absorbance corresponds to 10⁸ CFU/mL for bacteria. The yeast count was carried out in a Neubauer chamber (HBG). The culture media (Hinton Broth – bacteria and Sabouraud Dextrose Broth – *Candida* spp., HiMedia) was then inoculated in a standard count of 1x10⁶ cells/mL.

Table 1. Composition of the experimental dentifrice

Components	Batch number	Manufacturer	Function
Hydroxyethylcellulose	1	Union Carbide Corp., Houston, TX, USA	Thickening agent
Glycerin	169100	Ely Martins, Ribeirão Preto, SP, Brazil	Humectant
EDTA	156820	Dow Chemical Co., Midland, MI, USA	Chelating agent
Saccharin sodium	155324	Labsynth Produtos para Laboratórios Ltda, Diadema, SP, Brazil	Flavoring
<i>Ricinus communis</i> gel	P0019	IQSC, University of São Paulo, São Carlos, SP, Brazil	Preservative, antimicrobial agent, surfactante
Silica (Sident 9)	3491	Evonik Degussa GmbH, Düsseldorf, Germany	Abrasive
Silica (Sident 22S)	2532	Evonik Degussa GmbH, Düsseldorf, Germany	Abrasive
Titanium dioxide	60460	Minérios Ouro Branco, São Paulo, SP, Brazil	Pigment (white)
Menthol - eucalyptol	165274	Givaudan do Brasil Ltda., São Paulo, SP, Brazil	Flavoring
Distilled water	-	-	Vehicle

In the laminar flow cabinet (Pachane, Pa 400-ECO, Piracicaba, Brazil), the specimens were distributed in 12-well tissue culture plates (TPP; Trasadingen), and 2 mL of medium broth (according to the seeded microorganism) containing standardized cell suspension was added to each well. The plates were incubated for 90 min at 37°C at 75 rpm (adhesion period). Afterwards, the wells were washed with PBS to remove non-adherent microorganisms and another 2 mL of appropriate sterile medium broth were added to each well (culture medium renewed). After, the plates of all groups were incubated at 37 °C, at 75 rpm for 48 h, under

aerobic or anaerobic conditions. In 24 h of incubation, each well had 50% of its total amount exchanged through sterile culture medium to avoid saturation.

After the biofilm formed, the specimens were randomly distributed in control and dentifrices groups for each microorganism. The experiments were performed in triplicate to calculate the final average (mean of triplicates). The number of specimens in each group was determined by preliminary tests, which informed the adequate sample size with 80% power for detecting statistically significant differences ($\alpha=0.05$) (14). For *C. albicans* (error: 0.34; SD: 0.72; n: 18), *C. glabrata* (error: 0.22; SD: 0.51; n: 16), *S. mutans* (error: 0.5; SD: 1.14; n: 16) and *S. aureus* (error: 1.04; SD: 2.38; n: 16). The groups formed were:

- WG: (Control - specimens contaminated and sanitized with distilled water): brushing with distilled water;
- ED: brushing with experimental dentifrice;
- DC: brushing with specific dentifrice for dentures (Dentu Creme, Dentco, Inc.);
- CO: brushing with conventional dentifrice (Colgate-Palmolive Division Kolynos Brazil);
- CT: brushing with conventional dentifrice with Chloramine T (Trihydall, Perland Pharmacos Ltda).

To confirm the sterility of the sample, 05 specimens were processed without contamination (negative control). To certify the contamination, 05 specimens in triplicate for each microorganism composed the positive control group, which were not submitted to cleaning procedures.

The specimens were brushed manually with soft bristle brush (Tek, Johnson & Johnson Industrial Ltda.) associated with distilled water or dentifrice for 60 s (20 s for face), time sufficient for brushing, considering the size and dimensions of specimens, in a laminar flow chamber (Pachane, Pa-400 ECO) by a single operator previously trained in a blinded condition for the dentifrices. The brush was change for each specimen. The amount of toothpaste placed on the bristles

Table 2. Components and trademarks of the dentifrices analyzed

Dentifrice	Trademark	Components
Specific for denture	Dentu Creme	Water, Dicalcium Phosphate Dihydrate, Propylene Glycol, Calcium Carbonate, Aluminum Silicate, Sodium Lauryl Sulfate, Glycerin, Hydroxyethylcellulose, Flavor, Magnesium Aluminium Silicate, Saccharin Sodium, Methylparaben, Blue 1 Lake (CI 42090)
Convencional	Colgate	Sodium Monofluorophosphate (0.76%; 0.15% w/v Fluoride Ion), Dicalcium Phosphate Dihydrate, Water Glycerin, Sodium Lauryl Sulfate, Cellulose Gum, Flavor Tetrasodium Pyrophosphate, Sodium Saccharin
Convencional	TriHydral	1500 ppm Fluoro, Sodium Monofluorophosphate, Chloramine-T 0,2%, Calcium Carbonate, Propylene glycol, Sorbitol, Hydroxyethylcellulose, Tetrasodium, Pyrophosphate, Sodium Lauril Sulfate, Decil Polyglucoside, Metylparaben, Sodium Saccharin, Flavor, Acqua

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Table 3. Investigated microorganism, ATCC number and corresponding culture media

Microorganism	ATCC	Culture media	
		Preparation of the microbial inoculum / contamination of specimens	After brushing of the contaminated specimens.
<i>C. albicans</i>	10231	Sabouraud Dextrose Broth ¹	Sabouraud Dextrose Agar ¹
<i>C. glabrata</i>	2001	Sabouraud Dextrose Broth ¹	Sabouraud Dextrose Agar ¹
<i>E. coli</i>	25922	Mueller Hinton Broth ²	Mueller Hinton Agar ¹
<i>S. aureus</i>	25923	Mueller Hinton Broth ²	Mueller Hinton Agar ¹
<i>S. mutans</i>	25175	Modified SB 20 (15 g of casitone ² ; 5 g of yeast extract ² ; 0.2 g of cysteine ³ ; 0.1 g of sodium sulfite ⁴ ; 20 g of sodium acetate ⁵ ; 200 g of sucrose ⁵ ; 1000,0 mL of distilled water)	Mitis Salivarius Agar Base ²

¹Difco, Sparks, MD, USA; ²HiMedia, Mumbai, India; ³Vetec, Rio de Janeiro, RJ, Brazil; ⁴Chemco, Hortolândia, SP, Brazil; ⁵ Dinâmica, Diadema, SP, Brazil.

of the brush was 1.5 g. The bristles with the dentifrice were wet with distilled water and the brushing was performed with circular movements, always by the same operator.

After brushing, the specimens were washed 3 times with PBS to remove residual of dentifrices and no adhered cells. They were transferred to a tube containing a liquid culture medium (Lethen Broth; Difco Laboratories Inc.). The remaining adherent microorganisms were removed from the treated specimens by sonication (40 KHz, for 20 min; Altsonic).

The resultant suspension (100) was vortexed and diluted from 10^{-1} to 10^{-3} in a sterile PBS solution. The aliquots were plated in specific medium (Table 3). The plates were then incubated at 37°C for 48 h, under aerobic or anaerobic conditions (*S. mutans*). After this, the number of colonies in each dilution was counted with magnifying glass, and the value of CFUs was obtained, based on a dilution providing 1-300 colonies: $CFU/mL = \text{number of colonies} \times 10^n / q$, where: n=absolute value of the dilution (0,1,2 or 3) and q=quantity of plated suspension (0.05 mL).

For negative and positive controls, specimens were removed from cell culture plate, washed with sterile distilled water and transferred to test tubes containing culture medium Lethen Broth. After sowing the samples, all the test tubes were incubated at 37 °C until 28 days and checked every day to verify if there was turbidity of the culture medium and thus, it is possible to confirm the sterility of the procedures and the result of the antimicrobial action of the dentifrices after a long period.

Verified of the normal (Shapiro-Wilk) and homogeneous distribution of the data (Levene), one way – ANOVA and Tukey's HSD test was used to analyze the data. The values of count of CFU/mL were converted to log₁₀. There were several readings that resulted in zero CFU/mL, thus the microbial count data obtained were expressed as log (CFU/mL+1). Statistical tests were performed using the SPSS 17.0 program (SPSS Inc., Chicago, USA). All analyses were performed at a 0.05 significance level. The analyze was realized comparing the results of the groups for the same microorganism. The microorganisms were not compared to each other.

Results

The CFU/mL of *S. mutans* (p=0.008) showed significant differences after the use of dentifrices, with the highest efficacy of the specific dentifrice (DC)

against *S. mutans* and the lowest for the control group (WG). The experimental (RC) and conventional dentifrices (CO and CT) demonstrated intermediate effectiveness for *S. mutans*. However they promoted reduction in one or more than one log.

The CO group was the most efficient against *S. aureus* (p=0.000), following by specific (DC), experimental (RC) and conventional (CT) dentifrices (TABLE 4). DC and RC group were intermediate between CO and CT. The control group had the worst results.

For *C. albicans* (p=0.146), *C. glabrata* (p=0.098) and *E. coli* (p=0.186) no significant differences were observed between all of dentifrices and water (WG), although there was reduction in one or more than one log in counting of *C. albicans* after brushing with experimental dentifrice and *C. glabrata* after brushing with conventional (CO) and experimental dentifrices, respectively. For *E. coli*, the CO, RC and DC groups obtained mean values equal to zero. However, there was no difference between them and, the CT and control groups.

Discussion

The null hypothesis was not accepted for *Candida* spp. and *E. coli*, because there has been similarity of results between experimental dentifrice, commercial dentifrices and water. However, for *S. mutans* and *S. aureus*, the null hypothesis was accepted, because the dentifrices were not totally equal to the control group (water). The prevention of oral and systemic diseases and longevity of prosthetic devices is influenced by the control of biofilms. However the literature is controversy regarding the best hygiene method to be used in prostheses with resilient denture liners (3,15).

The use of brushing with a dentifrice associated with antimicrobial agent was based on the culture and habits of the denture wearers, in which the toothbrush with dentifrice is the least expensive and most commonly used method among patients (16). The dentifrices were selected based on the antimicrobial action. The addition of an agent

Table 4. CFU (Log₁₀) of each microorganism after brushing with dentifrices

Microorganisms	Water	Colgate	Experimental	Dentu Creme	Trihydral	p value
<i>C. albicans</i>	3.16 ±1.11	3.17 ±1.34	1.63 ±0.98	2.89 ±0.59	2.68 ±0.97	0.146
<i>C. glabrata</i>	3.55 ±0.34	2.43 ±1.42	2.61 ±1.01	3.09 ±0.38	3.74 ±0.52	0.098
<i>S. mutans</i>	4.79 ±1.42 ^A	2.16 ±2.02 ^{AB}	3.81 ±1.37 ^{AB}	1.27 ±1.20 ^B	3.13 ±0.88 ^{AB}	0.008
<i>E. coli</i>	0.06 ±0.13	0 ±0	0 ±0	0 ±0	0.15 ±0.22	0.186
<i>S. aureus</i>	1.01 ±0.18 ^A	0.06 ±0.13 ^C	0.36 ±0.25 ^{BC}	0.21 ±0.21 ^{BC}	0.57 ±0.30 ^B	0.000

Means, standard deviations and P value are indicated. Different letters indicate statistically significant difference between columns.

with antimicrobial and detergent action in dentifrice composition, become the mechanical action of brushing more efficient to control of biofilm and microorganisms.

The *Ricinus communis* has been studied in dentistry due to its antimicrobial potential and biocompatibility characteristics in the field of endodontics (10), and in the prosthetic area as hygiene solution (5-7) and dentifrices (8,9), presenting favorable results. The literature is unclear as to the mechanism of action of *Ricinus communis* on bacteria and yeast. According to Leite et al. (8), the minimum inhibitory concentration (MIC) of *Ricinus communis* was 0.0781% against *S. aureus*, *S. mutans*, *E. faecalis*, *C. albicans* and *C. glabrata*. Badaró et al. (5) showed that the *Ricinus communis* at 10% as disinfection solution for dentures was efficient against *P. aeruginosa*; *C. dubliniensis* and *P. melaninogenica*, and caused a mild reduction in *E. coli*. Intermediate efficacy was found against other microorganisms, like *C. tropicalis*; *C. krusei*; *S. sanguinis* and *S. mutans*.

The experimental dentifrice based on *Ricinus communis* was statistically similar to the control and all other dentifrices against *Candida* spp.. These findings corroborate with Leite et al. (8). Although the results have not identified statistically significant differences between the dentifrices and control (water), there was a numeric reduction of the CFU count. According to the Brazilian Pharmacopeia National Form (17), in the criteria for antimicrobial efficacy, there should be a reduction of 1 log in the number of CFUs initially inoculated. It happens in this study with *C. albicans* CFU/mL after brushing with experimental dentifrice and *C. glabrata* after brushing with conventional (CO) and experimental dentifrices. These findings allow us to infer, that clinically this reduction can be important and could reflect in the prevention of local and systemic infections in individuals with compromised immune system, because it allows the decrease to possibly acceptable levels of the microorganisms.

The chloramine-T, the active ingredient of the conventional dentifrice (CT) is a chlorinated compound anion that can cause cell death of the microorganisms for presenting oxidation reactions and hydrolysis of proteins (18). According to the literature, the oxygen and chlorine ions released during the oxidation reaction reacts with the organic matter of gram-positive and negative bacteria, fungi, yeasts in a sporulated form, mycobacteria and virus (18). However, in this study, the conventional dentifrice (CT) was effective against *S. aureus*. However, for *S. mutans* there was a numerical reduction in the CFU count, not being sufficient to promote statistical significance. These results are partially agree with Panzeri et al. (11), that verified reduction of biofilm coverage and mutans streptococci counts, after the clinical trial stage using an experimental

dentifrice with Chloramine T. However, yeasts from *Candida* genus present on denture bases were not affected.

With respect to conventional dentifrice (CO), the presence of sodium monofluorophosphate can act by inhibiting the enzymatic metabolism and adherence of bacteria (19) and provide an antimicrobial effect to the product. This characteristic could explain the action of this conventional dentifrice against *S. aureus* and *S. mutans* and similar results were found in another study (8).

The specific dentifrice (DC) presents the Sodium Lauryl Sulfate, a tension-active agent able to decrease the surface tension of the substrate, which may affect the outer layer of the bacterial cell (20). In a study of Paranhos et al. (14) the brushing with this dentifrice was similar to the combination method (brushing with specific dentifrice - Dentu Creme and immersion in alkaline peroxide) and was more effective than the chemical method for the majority of the tested species (*E. faecalis*, *E. coli*, *C. albicans* [ATTC and field strain], *C. glabrata* and *C. tropicalis*) when evaluated acrylic resin. This findings weren't in accordance with this study because the DC group was effective against *S. mutans* and *S. aureus*. The difference between substrate can be related with the results.

Significant difference in the antibiofilm activity of dentifrices and water was found against *S. aureus* and *S. mutans*. This result is relevant due the appearing of *S. aureus* in biofilm of denture wearers (21). This microorganism is related with several infections, including systemic diseases, such as septicemia, endocarditis, pneumonia, eczema, impetigo, abscesses and other pathologies (22). The dentifrices may have interfered with the adhesion of bacteria to the substrate and in the organization of a polysaccharide matrix, since the presence of dentifrices surfactants can interfere in the surface tension of the substrate, as well as agents with antibiofilm activity. Regarding *S. mutans*, the effectivity of dentifrices is important because this microorganism is precursor of biofilm formation and in association with *C. albicans* may cause denture stomatitis (23).

Analyzing the results for *E. coli*, that is a gram negative bacteria, considered transitory in the oral cavity, and responsible for initial yeast adherence to several surfaces (24). It was noted that the CFU/mL values were zero (0) or close to zero (0.06 and 0.15). Thus, it can not say that the dentifrices had action, because only mechanical procedure brushing with water was able to eliminate bacteria. As a hypothesis for this result, it has been the fact that *E. coli* does not have good adhesion to the surface of the specimens, since the formation of the biofilm depends on the adhesion ability of the microorganism to the substrate surface (25).

One limitation of the study did not evaluate the

adhesion of microorganisms to the specimens by microscopy and also for not using mixed biofilms that due to its complexity approaches of the clinical situations. The ability of dentifrices has not been tested for the prevention of changes in roughness on denture liner. Therefore, future studies should include this analyze with a greater number of samples.

Although the experimental dentifrice has presented intermediate results in relation to the control group and Dentu creme, dentifrice with better antimicrobial action, it reduced numerically the count of CFU, which may have important clinical significance. Future studies need to be conducted to evaluate this meaning. However, it should be noted that the method for obtaining the denture liner specimens provided smooth surfaces, differently from what happens in clinical practice, since it is deposited on the inner surface of the prosthesis and inserted in the mouth for polymerization. This procedure is correct, but the obtained surface is not always smooth as the specimens. Therefore, it is important the clinical assessment of the dentifrice since many variation factors can affect the result.

It was concluded that the experimental dentifrice based on *Ricinus communis* was effective only against *S. aureus* and able to reduce the CFU/mL count, without statistical significance, of the other microorganisms in 1log when compared with water, except *E. coli*. The specific dentifrice was efficient against *S. mutans* and *S. aureus*. All the dentifrices tested did not have action against *C. albicans*, *C. glabrata* and *E. coli*. Reformulations in the composition of the dentifrices are necessary to promote broad spectrum of antimicrobial action, reaffirming the need for adequate products for users of dentures.

Resumo

A desvantagem dos materiais resilientes é a dificuldade de controle do biofilme. Este estudo comparou um dentifrício experimental contendo *Ricinus communis*, com dentifrícios comerciais quanto atividade contra biofilme formado em reembasador de próteses totais. Seiscentos espécimes foram distribuídos em 5 grupos ($n=18/\text{microrganismo}$): água; dentifrício experimental; dentifrício específico para próteses totais e dois convencionais; contra *C. albicans*; *C. glabrata*; *S. mutans*; *S. aureus*; *E. coli*. Cada grupo teve um controle negativo ($n=5$; sem contaminação) e um positivo ($n=15/\text{microrganismo}$; sem higienização). A atividade contra biofilme foi avaliada pelo método de formação do biofilme, em triplicata. Os espécimes foram contaminados, padronizadamente, e incubados. Posteriormente, foi realizada escovação manual (60 s), lavagem em PBS, imersão em meio de cultura líquido para ressuspensão e semeadura em meio sólido. Os resultados (média das triplicatas) foram expressos em UFC/mL. Os dados foram submetidos aos testes Shapiro-Wilk, ANOVA e Tukey ($p<0,05$). O dentifrício específico ($1,27\pm 1,20$) foi o mais eficaz contra *S. mutans*, seguido dos convencionais (Trihydral, $3,13\pm 0,88$; Colgate, $2,16\pm 2,02$) e experimental ($3,81\pm 1,37$), que foram semelhantes entre si ($p=0,008$). Todos eles foram diferentes da água ($4,79\pm 1,42$). O dentifrício específico ($0,21\pm 0,21$) e o experimental ($0,36\pm 0,25$) foram semelhantes contra *S. aureus*, com maiores médias de UFC quando comparado ao convencional (Colgate, $0,06\pm 0,13$), que foi mais eficiente ($p=0,000$). Para *C. albicans*, *C. glabrata* e *E. coli*, todos os dentifrícios foram similares à água

($p=0,186$). Conclui-se que o dentifrício experimental foi efetivo somente contra *S. aureus* e não foi eficiente perante *Candida* spp.; *S. mutans*; *E. coli*, como ocorrido com os dentifrícios comerciais.

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