Are Desensitizing Toothpastes Equally Biocompatible and Effective Against Microorganisms?

Samira Esteves Afonso Camargo¹, Noala Vicensoto Moreira Milhan¹, Fernanda de Oliveira Saraiva¹, Jonatas Rafael de Oliveira¹, Luciane Dias de Oliveira¹, Carlos Henrique Ribeiro Camargo²

The aims of this study were evaluate cytotoxicity, genotoxicity, antimicrobial activity of desensitizing toothpastes compared to a common one and the surface roughness of tooth enamel submitted to brushing with these toothpastes. Samples of three desensitizing toothpastes (Colgate Sensitive, Sensodyne and Oral B Sensitive) and common toothpaste (Colgate) were placed in contact with gingival human fibroblasts. Cytotoxicity and genotoxocity were measured by MTT assay and micronucleus test. Antimicrobial activity of the toothpastes extracts against C. albicans, S. mutans and S. aureus were assessed. For surface roughness evaluation, bovine teeth were submitted to 10.000 brushing cycles. The results were analyzed statically using Mann-Whitney U, ANOVA and Z tests (p<0.05). All toothpastes caused cytotoxic effect to the cells (p<0.05), except Colgate Sensitive. The toothpastes did not increase the number of micronuclei compared to the untreated control group. Colgate eliminated all the evaluated microorganisms at lower concentrations compared to Colgate Sensitive and Oral B Sensitive, which were not able to eliminate S. aureus. Sensodyne did not reach the minimum microbicidal concentration. The surface roughness of tooth enamel increased after brushing with Colgate Sensitive and Oral B Sensitive, however the comparison between groups showed no difference on the enamel surface roughness presented by desensitizing toothpastes when compared with the common one (p>0.05). Based on these results, we can conclude that although none toothpaste has induced genotoxicity, Colgate Sensitive was also not cytotoxic. Colgate was the most effective against the microorganisms, and there were no differences on the enamel surface roughness between the groups.

¹Department of Biosciences and Oral Diagnosis, Institute of Science and Technology, UNESP - Univ Estadual Paulista, São José dos Campos, SP, Brazil ²Department of Restorative Dentistry, Institute of Science and Technology, UNESP - Univ Estadual Paulista, São José dos Campos, SP, Brazil

Correspondence: Samira Esteves Afonso Camargo, Av. Eng. Francisco José Longo, 777, 12245-000 São José dos Campos, SP, Brazil. Tel: +55-12-3947-9384. e-mail: samira@ict.unesp.br

Keywords: toothpastes, desensitizing, biocompatibility, antimicrobial, roughness.

Introduction

Dentin hypersensitivity (DH) is a frequent oral health problem in the adult population. This condition is characterized by an acute and transient pain that affects the exposed dentin in response to some mechanical, thermal and chemical stimuli. This condition may be localized or generalized, and may affect one or multiple tooth surfaces simultaneously. Some theories have been suggested in order to explain the biological mechanism of DH, and the hydrodynamic theory is one of the most widely accepted (1). This theory is based on the idea that perturbation of dentinal fluid within the dentinal tubules activates pulpal nociceptors causing pain (1,2).

Numerous desensitizing products for the treatment of dentine hypersensitivity are currently available. These products are divided into two categories: products that occlude open dentine tubules and ones that block/reduce neural transmission (2,3). In this way, desensitizing toothpastes that act occluding tubules can reduce dentine sensitivity (3). These toothpastes can occlude superficially the dentine tubules, process that is dependent on the active ingredients of each toothpaste, such as Pro-Argin,

Calcium carbonate, Strontium acetate, Stannous fluoride, Zinc-carbonate hydroxyapatite, New silica, Tetrapotassium pyrophosphate and Hydroxyapatite (4). On the other hand, Potassium based toothpastes have been used to block neural transmission. In clinical trials, these toothpastes have spent at least two weeks of twice daily use to promote considerable reductions in the dentin sensitivity (3).

Some studies have investigated the cytotoxicity (5-7), genotoxicity (7), antimicrobial activity (6,8,9) and changes in the surface roughness (10) caused by different toothpastes, which have shown different and interesting results. This kind of study is important once there are many types of toothpaste available to use currently, which have different functions and consequently a wide range of components, including different active ingredients.

Based on this idea, the aim of this study is to evaluate if the toothpastes with desensitizing action can be cytotoxicity and/or genotoxicity for human gingival fibroblasts, and assess its antimicrobial activity, as well as, possible alterations on the enamel teeth after use of these toothpastes. The hypotheses of this study were (1) the

toothpastes, in general, could cause toxicity; (2) common toothpaste could present higher antimicrobial activity and (3) common toothpaste would not induce an increase in the surface roughness compared to desensitizing ones.

Material and Methods

This project was developed in accordance with the Research Ethics Committee of the Institute of Science and Technology, UNESP - Univ Estadual Paulista, São Jose dos Campos, School of Dentistry (Approval no. 21806313.4.0000.0077).

Experimental Groups

Four groups were established: Colgate Sensitive (Colgate Sensitive Pró-Alívio, Colgate-Palmolive); Sensodyne (Sensodyne Rapido Alivio, Sensodyne); Oral B Sensitive (Oral B Pro Sensitive, Oral-B) and Colgate (Colgate Total 12 Professional Clean, Colgate-Palmolive). The main components of the tested toothpastes are showed in Table 1.

The toothpaste samples were placed in 24-well plates (0.2 g/mL) and covered with 3 mL of Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, penicillin, and streptomycin and incubated in the dark for 24 h at 37 °C. After this period, these original extracts (1:1) were then serially diluted in cell culture medium before testing. The biocompatibility tests were performed after 24h of indirect contact, once we have intended to evaluate the long-term contact of the cells with different concentrations of toothpastes to simulate the action of residual toothpaste, after brushing, in the oral cavity.

Analysis of Cytotoxicity (MTT Test)

Gingival human fibroblasts (FMM-1, Cell Bank, São

Paulo State University, SP, Brazil) were routinely cultivated in DMEM supplemented with 10% fetal bovine serum, penicillin, and streptomycin at 37°C and 5% CO₂. The cells were plated at 8×10^3 cells/well in 96-well plates and incubated for 24 h at 37°C and 5% CO₂. After, the medium was removed and the cells were exposed to 200 μL of the toothpastes extracts at different dilutions (1:1, 1:2, 1:4, 1:8, 1:16, 1:32) for 24 h. The cells of the untreated control group were maintained with 200 µL of pure DMEM for this same period. Then, the pure and conditioned medium was removed from the plates and the cell survival was measured using the MTT test, which is based on the activity of succinyl dehydrogenase (SDH). For this, 100 μL of MTT solution 90.5 mg/mL PBS) was added to each well, and the cells were incubated for 1 h. Then, 100 μL of dimethyl sulfoxide solvent (DMSO) was also added to each well and the plates were shaken at room temperature for 10 min. The resulting optical density was measured in a spectrophotometer (Bio-Tek, Winooski, Vermont, USA) at 570 nm. Four replicate cell cultures were exposed to each concentration of the toothpastes extracts in two independent experiments. The cytotoxicity was expressed as a percentage of the untreated control group (= 100%), as previously published (7), and the difference between the values was statistically analyzed by ANOVA and Z test (p<0.05).

Analysis of Genotoxicity (MNT Test)

For the genotoxicity, FMM-1 were routinely cultivated in DMEM supplemented with 10% fetal bovine serum, penicillin, and streptomycin at 37°C and 5% $\rm CO_2$. Thus, the cells were plated at a density of $\rm 3 \times 10^5$ on microscopic glass slides and incubated for 24 h at 37°C and 5% $\rm CO_2$. After this period, the medium was removed and the

Table 1. Ingredients of the toothpastes tested in this study

Toothpastes and ingredients

Colgate Sensitive Pró-alívio (Colgate-Palmolive)

Active Ingredients: 8% arginine and 1.10% Sodium monofluorophosphate (1450 ppm fluoride ion)

Inactive Ingredients: calcium carbonate, water, sorbitol, arginine, bicarbonate, sodium lauryl sulfate, sodium

monofluorophosphate, flavor, cellulose, sodium bicarbonate, acesulfame potassium, sodium, sucralose, titanium dioxide.

Sensodyne Rápido Alívio, Sensodyne

Active Ingredients: 10% strontium chloride

Inactive Ingredients: water, glycerin, sorbitol, calcium carbonate, hydroxyethyl cellulose, silicon dioxide, taurate fatty acid Coco-N-Methyl-N sodium, flavor, polyoxyl stearate, titanium dioxide, sodium saccharin, red dye. This product contains no sugar.

Oral B Pro Sensitive, Oral-B

Active Ingredients: Sodium fluoride (1450 ppm fluoride ion) and Stannous chloride

Inactive Ingredients: water, sorbitol, silica, sodium gluconate, sodium lauryl sulfate, cellulose, flavor, carrageenan, zinc citrate, titanium dioxide, hydroxyethyl cellulose, sodium hydroxide, phytic acid, sodium saccharin.

ColgateTotal 12 Professional Clean, Colgate-Palmolive

Active Ingredients: Sodium fluoride and 0.3% Triclosan (1450 ppm fluoride ion)

Inactive Ingredients: sodium lauryl sulfate, sorbitol, hydrated silica, Gantrez, sodium saccharin, flavor, dyes, water,

fluoride, carrageenan, sodium hydroxide, titanium dioxide, artificial dyes CI 77891, CI 77019 and CI 42090.

cells were exposed to 3 mL of the toothpastes extracts at different dilutions (1:8 and 1:16). The cells were also exposed to 3 mL of Ethyl methanesulfonate (EMS), which was considered a positive (genotoxic) control and pure DMEM, used as an untreated control group. After 24 h, the medium of all groups was removed and the cells were fixed in 100% ethanol for 30 min and stained with Schiff reagent (Sigma) for 30 min. The microscopic glass slides were observed under a light microscope (100 X) and the number of micronuclei was determined in 1.000 cells/slide of two parallel cultures (slides) per concentration, in two independent experiments, as previously described (7). The differences between the values were statistically analyzed using Mann-Whitney U test (P<0.05).

Analysis of Antimicrobial Activity

Reference strains (ATCC) of *C. albicans* (ATCC 18804), *S. mutans* (ATCC 35688) and *S. aureus* (ATCC 6538) obtained from the Laboratory of Microbiology and Immunology, Institute of Science and Technology/UNESP, São José dos Campos, SP, Brazil, were used. *C. albicans* were cultured in Sabouraud-dextrose broth (Himedia) for 24 h at 37°C and bacteria were cultured in BHI (Himedia), *S. mutans* was incubated under microaerophilic conditions (5% CO₂). The microbial suspensions were prepared in sterile saline (0.9% NaCl) at a standard concentration of 5 x 10² to 2.5 x 10³ CFU/mL for *C. albicans* and 5 x 10⁵ CFU/mL for bacteria.

The microdilution method was carried out according to CLSI guidelines (11, 12). For this purpose, 10 dilutions of the toothpaste extracts (0.16 to 0.0003125 mg/mL⁻¹) were evaluated. In 96-well plates, 100 µL of culture medium, being RPMI 1640 broth (Himedia) for C. albicans and BHI broth (Himedia) for bacteria, were added in 10 wells and 100 µL of toothpaste extract only in the first well, where serial dilutions started till the tenth dilution. Then, 100 μL of the standardized microbial suspension were added in all the wells. After 24 h of incubation, the minimum inhibitory concentration (MIC) was determined on well without turbidity, and 100 µL of this concentration and two subsequent concentrations, one higher and one lower, was seeded onto Sabouraud-dextrose or BHI agar plates. After 48 h of incubation, the minimum microbicidal concentration (MMC) of the toothpastes extracts was determined by the absence of colonies on plates. The results are reported as MIC and MMC of the toothpaste extract in contact with the microorganisms, analyzed in two independent experiments.

Analysis of Surface Roughness

The procedures of this test were performed as previously described (7). For this, twenty bovine teeth (Frigorifico Mantiqueira, Sao Jose dos Campos, SP, Brazil) were used and

divided into four groups (n= 5). The crowns were sectioned and then the vestibular and lingual surfaces of each tooth crown, containing enamel and dentin, were cut into a cylindrical shape (3 mm diameter). After, the samples were embedded in chemically activated acrylic resin blocks. These blocks were polished with water abrasive papers of three granulations: 120, 300 and 600 grit, in a polishing machine (Polipan 2 Pantec, Sao Bernardo do Campo, Brazil) and the enamel surface roughness was measured in a rugosimeter, model FJ 400 (Mitutoyo, Shinagawa-ku, Japan).

Before the brushing, solutions containing the toothpastes were prepared. For this purpose, 6 g of each toothpaste was diluted in 6 mL of distilled water (1:1). Thus, the solutions were placed in four syringes (10 mL), which were attached to the brushing machine type MEV-2 (Odeme, Luzerna, Brasil), with the function to inject solutions throughout the brushing cycle. This brushing machine, which had toothbrushes with medium bristles, was used to work in all groups simultaneously. The samples were submitted to brushing for 20 minutes by 10,000 cycles, which correspond to 1 year of tooth brushing (13). After this procedure, the new surface roughness was measured. Two independent experiments were performed. The roughness values of initial and final measurements, and the differences between them (delta roughness) were statistically analyzed by ANOVA (p<0.05).

Results

Analysis of Cytotoxicity

The results of cytotoxicity test may be observed in Figure 1. Colgate Sensitive showed cell survival rate statistically different from the untreated group (p<0.05) at 1:2, 1:4, 1:16 and 1:32. At the lower concentrations, the cell survival was higher than the one observed by the untreated group. The cell survival rate was below 50% in none of the dilutions. Colgate Sensitive was statistically different from Colgate (p<0.05) at all concentrations.

Cell exposure to Sensodyne resulted in cell survival rate statistically different from the untreated group (p<0.001) at all dilutions, showing rates below 50% at 1:1 and 1:2. Sensodyne was statistically different from Colgate (p<0.05) at 1:8, 1:16 and 1:32.

Oral B Sensitive showed cell survival rate statistically different from the untreated group (p<0.001) at all dilutions. At 1:16 and 1:32, the cell survival was higher than 100% (untreated group). The cell survival rate was below 50% at 1:2, and equal to 50% at 1:4 and 1:8. This toothpaste was statistically different from Colgate (p<0.05) at 1:4, 1:16 and 1:32.

Colgate exhibited cell survival rate statistically different from the untreated group (p<0.001) at all dilutions. At 1:32, the cell survival was higher than the one presented by the

untreated group. At 1:1, 1:8 and 1:16 the cell survival rate was below 50%.

Analysis of Genotoxicity

All toothpaste extracts were not able to increase the number of micronuclei compared to the untreated group. The results of the desensitizing toothpaste extracts were statistically similar to that presented by Colgate. Only the EMS, which was used as positive control, has increased the number of micronuclei in the treated cultures by approximately 3 folds compared to the number of micronuclei observed in the untreated group (p<0.001) (Fig. 2).

Analysis of Antimicrobial Activity

The MIC and the MMC of the toothpaste concentrations are shown in Table 2. Colgate Sensitive at concentration of 0.16 mg/mL⁻¹ has eliminated *C. albicans* and *S. mutans* however *S. aureus* was not eliminated by any of the tested concentrations.

For Sensodyne, the MMC was not reached by any of

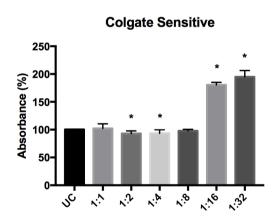
the tested concentrations, although we have observed reduction in the microbial activity of *S. aureus* and *C. albicans* at concentrations of 0.16 mg/mL⁻¹ and 0.08 mg/mL⁻¹, respectively. There was no reduction of microbial activity of *S. mutans*.

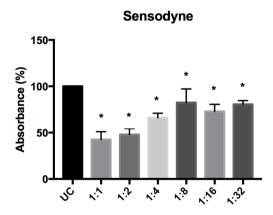
Oral B Sensitive at concentration of 0.04 mg/mL⁻¹ has eliminated *C. albicans*, and *S. mutans* was eliminated at concentracion of 0.08 mg/mL⁻¹ while *S. aureus* was not eliminated by any of the tested concentrations.

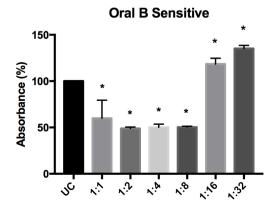
Colgate was able to eliminate all the microorganisms. The CMM of *C. albicans* and *S. aureus* was 0.01 mg/mL⁻¹ and the CMM of *S. mutans* was 0.02 mg/mL⁻¹.

Analysis of Surface Roughness

After the brushing, the values of initial and final measurements were statistically different only in the groups Colgate Sensitive and Oral B Sensitive (p<0.05). Furthermore, the values of final readings minus the values of initial ones (delta roughness) were obtained. Colgate Sensitive, Sensodyne, Oral B Sensitive, and Colgate showed a mean roughness increase (delta roughness) of 0.032,







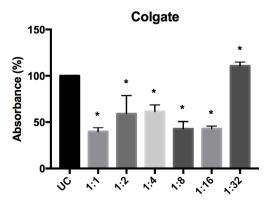


Figure 1. Cytotoxicity of the toothpastes in FMM-1 cells after exposure to extracts (1:1) and its dilutions. Statistically significant differences between untreated and treated cell cultures are indicated by asterisks. UC: untreated group.

0.036, 0.04 and $0.05~\mu m$, respectively (Fig. 3). The values of medians and standard deviation (SD) of the roughness data are shown in Table 3. The statistical analysis of delta roughness showed that there were no differences between the groups.

Discussion

The hypotheses of this study were accepted once most of the toothpastes have caused toxicity and common toothpaste has presented higher antimicrobial activity,

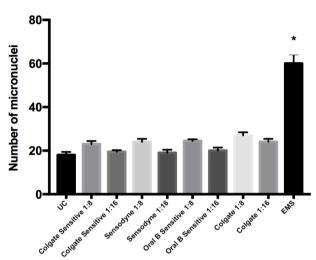


Figure 2. Induction of micronuclei in FMM-1 cells after exposure to toothpastes dilutions (1:8 and 1:16). Statistically significant differences between untreated and treated cell cultures are indicated by asterisks. UC: untreated group.

as hypothesized. Moreover, common toothpaste has not induced an increase in the surface roughness compared to desensitizing ones.

The active ingredients of the desensitizing toothpastes investigated in this study are 10% Strontium chloride

Initial Roughness

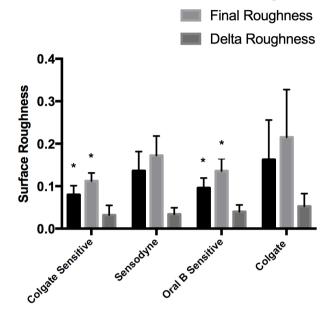


Figure 3. Initial and final roughness means values and the difference between them (delta roughness), after brushing cycles of bovine teeth. Statistically significant differences between final and initial measurements are indicated by asterisks.

Table 2. Values of MIC and MMC (mg/mL-1) of the toothpaste extracts for all microorganisms evaluated

Microorganism	Colgate Sensitive		Sensodyne		Oral B Sensitive		Colgate	
	CIM	MMC	CIM	MMC	CIM	MMC	CIM	MMC
C. albicans	0.02	0.16	0.08	*	0.01	0.04	0.005	0.01
S. aureus	0.04	*	0.16	*	0.04	*	0.005	0.01
S. mutans	0.04	0.16	*	*	0.04	0.08	0.01	0.02

^{*} MMC or MIC was not achieved.

Table 3. Values of medians and SD of the roughness data

	Colgate Sensitive		Sensodyne		Oral B Sensitive		Colgate	
	Medians	SD	Medians	SD	Medians	SD	Medians	SD
Initial roughness	0.08	0.0212	0.15	0.0456	0.1	0.023	0.22	0.0932
Final roughness	0.12	0.0192	0.18	0.046	0.12	0.0288	0.28	0.1124
Delta roughness	0.03	0.0228	0.04	0.0167	0.04	0.0158	0.06	0.0299

(Sensodyne), arginine + 8% sodium monofluorphosphate (Colgate Sensitive), and sodium fluoride (Oral B Sensitive). Strontium chloride has been used for 50 years in toothpastes as desensitizing (14). This component can be absorbed by enamel and dentin once it has biological and chemical properties that are similar to those found in the calcium (15). Generally, the studies report a relief in the dentin hypersensitivity, after using desensitizing toothpastes with Strontium salts (2). Nevertheless, a recent meta-analysis study indicated that strontium-containing toothpaste does not have a desensitizing effect (16).

Techniques of confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM) and atomic force microscopy (AFN) showed that arginine-calcium carbonate technology promotes perfect occlusion of dentinal tubules (17). Sodium fluoride has also promoted obliteration of dentinal tubules and subsequent relief in the dentin hypersensitivity (2). After topical application, fluoride produces a barrier by precipitating CaF₂ on the dentin surface (18).

The cytotoxic effects of toothpastes have been investigated in vitro (5-7). Some authors have suggested that detergents, specifically sodium lauryl sulfate, may induce cytotoxic effects in vitro (5,19). According to Cvikl et al. (5) toothpastes conditioned medium containing sodium lauryl sulfate and amine fluoride are significantly more cytotoxic to fibroblasts and epithelial cells than toothpastes conditioned medium containing cocamidopropyl betaine and Steareth-20. In this study, most of the toothpastes have presented sodium lauryl sulfate, except Sensodyne. The only toothpaste that did not promote cytotoxic effects in vitro, at any concentration, was Colgate sensitive, which additionally promoted a high proliferation of viable cells at low dilutions. The toothpaste most cytotoxic to the cells was Colgate, which not present a desensitizing action. In the highest dilution (1:32), all the toothpastes have not caused cytotoxic effects to the cells.

The toothpastes, in general, were cytotoxic to the cells *in vitro*. In spite of this, the toxic effects *in vivo* can be different, once oral cavity environment differs from *in vitro* conditions in many features such as saliva, blood flow, creatine levels, mucus layer and microbiota, which may protect the oral site from injurious (6).

There is a lack in the literature of studies that assess the genotoxicity of toothpastes. Camargo et al. (7) evaluated the genotoxicity of *Chinese hamster* fibroblasts (V79) after contact with common and whitening toothpastes and noticed that some whitening toothpastes were genotoxicity for the cells. In this study toothpastes with and without desensitizing components induced no significant micronucleus formation in FMM-1 cells, similar to the untreated group and statistically different from the EMS,

which is known as a genotoxic substance (20).

Regard to antimicrobial activity, the main purpose of the toothpaste is to reduce, control and prevent dental caries and periodontal disease by suppressing opportunistic pathogens, as S. mutans, S. aureus and C. albicans. Toothpastes with evident in vitro antimicrobial activity may be effective against the same microorganisms in vivo, whilst vehicles without clear in vitro antimicrobial activity usually not show effectiveness against the pathogens in vivo (8). We have observed in this study that Colgate was the most effective dentifrice against the evaluated bacteria and yeast, showing in general the lowest values of MMC. This excellent antimicrobial activity, also observed in a previous study (8), may be related to the triclosan, present in the formulation of Colgate. A previous study (21) concluded that triclosan is a multi-target inhibitor for S. mutans. Also, Prasant et al. (9) have showed that triclosan containing toothpastes formulations are more effective in control of oral microflora compared to non-triclosan containing synthetic toothpastes.

Oral B Sensitive and Colgate sensitive presented antimicrobial activity against *C. albicans* and *S. mutans*, although they were not effective against *S. aureus*. A previous study (22) showed that desensitizing paste containing 8% arginine and calcium carbonate (Ar-Ca) onto hypersensitive surfaces of teeth can significantly suppress *S. mutans* biofilm formation and maturation, which is consistent with our findings.

Sensodyne not presented an effective antimicrobial activity, especially for *S. mutans*, once there was observed no reduction of this bacteria activity, even at the highest concentration used in this study. Regarding the microorganisms, *C. albicans* was the less resistant to the toothpastes. *S. mutans* was the most resistant microorganisms to Colgate and Sensodyne, once the MIC was not reached for this bacteria. On the other hand, *S. aureus*, in general, was the most resistant microorganisms for the desensitizing toothpastes.

Some studies have evaluated the relationship between the abrasive potential of toothpastes and changes on the enamel surface (7,10,23). The frequency and intensity that individuals make use of toothpastes varies due to hygiene habits and personal characteristics of each individual, as well as, the force applied during the brushing, period of each brushing and hardness of the toothbrushes.

In vitro models, as in the present study, permit the investigation and comparison of different treatments, under standardized operating conditions and reasonable cost, besides to be relatively easy to execute (24).

It is commonly accepted that toothpastes necessitate of abrasivity to preventing or decreasing extrinsic stains (25). Nevertheless, their abrasivity needs to be moderated in order to prevent underlying enamel remotion and consequently exposed dentine (24). International Standards Organization (ISO) determinated that the abrasivity of toothpastes formulation may be designated as Relative Enamel Abrasivity (REA) and Relative Dentine Abrasivity (RDA). The reference dentifrice is considered to present an RDA value of 100 and an REA value of 10 (26).

The abrasives present in toothpastes usually are hydrated silica, calcium carbonate, dicalcium phosphate dihydrate, calcium pyrophosphate, sodium bicarbonate and alumina (27). In the present study, the abrasives composites of the toothpastes were hydrated silica (Colgate), silica (Oral B Sensitive), calcium carbonate (Sensodyne) and calcium carbonate + sodium bicarbonate (Colgate Sensitive).

Schemehorn et al. (28) observed that toothpastes known as "whitening" were generally more abrasive to dentin, especially ones containing silica. Their results indicated that different abrasives may indicate different outcomes for the dentin. A previous study has demonstrated that Colgate Total 12 present higher RDA than Sensodyne Repair, which was followed by Colgate Sensitive Pro-Relief. Despite this, the desensitizing toothpastes tested by these authors has produced a similar rate of erosive dentin wear compared to the conventional one (29). Garcia-Godoy et al. (23) analyzed the effect of enamel roughness after the use of a desensitizing paste containing 8% arginine and calcium carbonate and concluded that this paste did not have a significant effect on the enamel surface roughness of the tested substrates.

In this study, surface roughness of tooth enamel increased in the groups Colgate Sensitive and Oral B Sensitive. However, the comparisons between groups showed that there were no differences between them. In this way, although calcium carbonate associated with sodium bicarbonate (Colgate Sensitive) and silica (Oral B Sensitive) have shown the most abrasive tendency, ours results indicated that brushing teeth with these toothpastes is not different for the enamel from brushing teeth with toothpastes containing hydrated silica and calcium carbonate (Colgate and Sensodyne, respectively).

Thus, this in vitro study demonstrated that Sensodyne and Oral B Sensitive cause *in vitro* cytotoxicity to FMM-1 cells, as well as, Colgate. The desensitizing and common toothpastes tested here were not genotoxic to FMM-1 cells. Colgate presented higher antimicrobial activity than desensitizing toothpastes, although Oral B Sensitive and Colgate Sensitive have also shown a good one. There were no differences in the surface roughness presented by desensitizing toothpastes when compared with the common one.

Based on our findings, Colgate Sensitive toothpaste can be a good clinical choice, once it is biocompatible and

effective against microorganisms. Additionally, brushing teeth with desensitizing toothpastes does not promote differences on the enamel surface roughness compared to brushing teeth with Colgate.

Resumo

Os objetivos desse estudo foram avaliar a citotoxicidade, genotoxicidade, atividade antimicrobiana de dentifrícios dessensibilizantes em comparação com um comum e também a rugosidade superficial do esmalte dentário submetido à escovação com esses dentifrícios. Amostras de três dentifrícios dessensibilizantes (Colgate Sensitive, Sensodyne e Oral B Sensitive) e um dentifrício comum (Colgate) foram colocadas em contato com fibroblastos gengivais humanos e a citotoxicidade e genotoxidade foram mensuradas pelo ensajo MTT e teste do micronúcleo. A atividade antimicrobiana dos extratos dos dentifrícios contra C. albicans, S. mutans e S. aureus foi determinada. Para a avaliação da rugosidade superficial, espécimes de dentes bovinos foram submetidas à 10.000 ciclos de escovação. Os resultados foram analisados estatisticamente usando os testes Mann-Whitney U. ANOVA e Teste Z (P<0.05), Todos os dentifrícios causaram efeito citotóxico às células (P<0,05), exceto o Colgate Sensitive. Os dentifrícios não aumentaram o número de micronúcleos em comparação com o grupo não tratado. O Colgate foi capaz de eliminar todos os microorganismos avaliados em concentrações mais baixas em comparação com Colgate Sensitive e Oral B Sensitive, que não foram capazes de eliminar os S. aureus. O Sensodyne não atingiu a concentração microbicida mínima para qualquer microorganismo. A rugosidade superficial do esmalte dentário aumentou após a escovação com Colgate Sensitive e Oral B Sensitive, porém a comparação entre os grupos não mostrou diferença na rugosidade superficial do esmalte apresentada por dentifrícios dessensibilizantes quando comparados ao comum (p>0,05). Com base nesses resultados, podemos concluir que, embora nenhum dentifrício tenha induzido genotoxicidade, o Colgate Sensitive também não foi citotóxico. O Colgate foi o mais eficaz contra os microorganismos, e não houve diferença na rugosidade superficial do esmalte entre os grupos.

Acknowledgements

The authors acknowledge support from The State of São Paulo Research Foundation – FAPESP (Grant #2012/00574-4).

References

- Mantzourani M, Sharma D. Dentine sensitivity: past, present and future. J Dent 2013;41:S3-S17.
- Shiau HJ. Dentin hypersensitivity. J Evid Based Dent Pract 2012;12:220-228
- Davies R, Scully C, Preston AJ. Dentifrices-an update. Med Oral Patol Oral Cir Bucal 2010;15:e976-982.
- Arnold WH, Prange M, Naumova EA. Effectiveness of various toothpastes on dentine tubule occlusion. J Dent 2015;43:440-449.
- Cvikl B, Lussi A, Gruber R. The in vitro impact of toothpaste extracts on cell viability. Eur J Oral Sci 2015;123:179-185.
- Ghapanchi J, Kamali F, Moattari A, Poorshahidi S, Shahin E, Rezazadeh F, et al. In vitro comparison of cytotoxic and antibacterial effects of 16 commercial toothpastes. J Int Oral Health 2015;7:39-43.
- Camargo SE, Jóias RP, Santana-Melo GF, Ferreira LT, El Achkar VN, Rode SeM. Conventional and whitening toothpastes: cytotoxicity, genotoxicity and effect on the enamel surface. Am J Dent 2014;27:307– 311.
- Haraszthy VI, Zambon JJ, Sreenivasan PK. Evaluation of the antimicrobial activity of dentifrices on human oral bacteria. J Clin Dent 2010;21:96-100.
- Prasanth M. Antimicrobial efficacy of different toothpastes and mouthrinses: an in vitro study. Dent Res J 2011;8:85-94.
- 10. Hilgenberg SP, Pinto SC, Farago PV, Santos FA, Wambier DS. Physical-

- chemical characteristics of whitening toothpaste and evaluation of its effects on enamel roughness. Braz Oral Res 2011;25:288-294.
- NCCLS (National Committee for Clinical Laboratory Standards): Reference method for broth dilution antifungal susceptibility testing of yeasts. Approved standard - second edition. M27-A2. Wayne, PA, USA; 2002. ISBN 1-56238-469-4.
- NCCLS (National Committee for Clinical Laboratory Standards): Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard - sixth edition. M7-A6. Wayne, PA, USA; 2003. ISBN 1- 56238-486-4.
- Vicentini BC, Braga SR, Sobral MA. The measurement in vitro of dentine abrasion by toothpastes. Int Dent J 2007;57:314–318.
- Li Y, Lee S, Zhang YP, Delgado E, DeVizio W, Mateo LR. Comparison of clinical efficacy of three toothpastes in reducing dentin hypersensitivity. J Clin Dent 2011;22:113-120.
- Liu H, Hu D. Efficacy of a commercial dentifrice containing 2% strontium chloride and 5% potassium nitrate for dentin hypersensitivity: a 3-day clinical study in adults in China. Clin Ther 2012;34:614-622.
- Bae JH, Kim YK, Myung SK. Desensitizing toothpaste versus placebo for dentin hypersensitivity: a systematic review and meta-analysis. J Clin Periodontol 2015;42:131-141.
- Petrou I, Heu R, Stranick M, Lavender S, Zaidel L, Cummins D, et al. A breakthrough therapy for dentin hypersensitivity: how dental products containing 8% arginine and calcium carbonate work to deliver effective relief of sensitive teeth. J Clin Dent 2009;20:23-31.
- Ehrlich J, Hochman N, Gedalia I, Tal M. Residual fluoride concentrations and scanning electron microscopic examination of root surfaces of human teeth after topical application of fluoride in vivo. J Dent Res 1975;54:897-900.
- Babich H, Babich JP. Sodium lauryl sulfate and triclosan: in vitro cytotoxicity studies with gingival cells. Toxicol Lett 1997;91:189-196.
- 20. Miller B, Pötter-Locher F, Seelbach A, Stopper H, Utesch D, Madle S.

- Evaluation of the in vitro micronucleus test as an alternative to the in vitro chromosomal aberration assay: position of the GUM Working Group on the in vitro micronucleus test. Gesellschaft für Umwelt-Mutations-forschung. Mutat Res 1998;410:81–116.
- Phan TN, Marquis RE. Triclosan inhibition of membrane enzymes and glycolysis of Streptococcus mutans in suspensions and biofilms. Can J Microbiol 2006;52:977-983.
- Fu D, Pei D, Huang C, Liu Y, Du X, Sun H. Effect of desensitising paste containing 8% arginine and calcium carbonate on biofilm formation of Streptococcus mutans in vitro. J Dent 2013;41:619-627.
- Garcia-Godoy F, Garcia-Godoy A, Garcia-Godoy C. Effect of a desensitizing paste containing 8% arginine and calcium carbonate on the surface roughness of dental materials and human dental enamel. Am J Dent 2009;22:21A-24A.
- Philpotts CJ, Weader E, Joiner A. The measurement in vitro of enamel and dentine wear by toothpastes of different abrasivity. Int Dent J 2005;55:183-187.
- 25. Stookey GK, Burkhard TA, Schemehorn BR. In vitro removal of stain with dentifrices. J Dent Res 1982;61: 1236-1239.
- International Standards Organization Dentistry toothpastesrequirements, test methods and marking. Geneva: International Organization for Standardization; 2010. ISO 11609.
- 27. Hefferren JJ. Historical view of dentifrice functionality methods. J Clin Dent 1998;9:53-56.
- Schemehorn BR, Moore MH, Putt MS. Abrasion, polishing, and stain removal characteristics of various commercial dentifrices in vitro. J Clin Dent. 2011;22:11-18.
- Lopes RM, Turbino ML, Zezell DM, Scaramucci T, Aranha AC. The effect
 of desensitizing dentifrices on dentin wear and tubule occlusion. Am
 J Dent 2015;28:297-302.

Received January 10, 2017 Accepted June 24, 2017