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ORIGINAL ARTICLE

Relationship between toothpastes properties and patient-reported discomfort: crossover study

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Abstract
Objectives This study aims to correlate patient-reported reactions with in vitro analyses of the pH, abrasive quality, and cytotoxicity of four toothpastes.
Materials and Methods One hundred twenty-one patients received non-identified samples of toothpaste to be used for 6 days and answered a questionnaire about their sensations. In vitro analysis: the pH of toothpastes was measured with a pH meter. The abrasivity of toothpastes was evaluated against composite resin specimens (n=10). A toothbrushing machine was used to simulate wear, which was indirectly measured by mass loss using a scale. Cell culture media conditioned with toothpaste were used to assess the cytotoxicity. Confluent cells were kept in contact with the conditioned media or control for 24 h. The cell viability was measured using the 3-(bromide, 4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium (MTT)-reduction assay. The obtained data on the pH, weight loss, and cell viability were compared by ANOVA/Tukey's tests ($p<0.05$).
Results With the exception of the bleaching effect paste, the Oral B® paste produced the highest frequencies of irritation reports, tooth sensitivity, taste discomfort, and texture discomfort in the clinical study; patients also reported rougher teeth, soft tissue peeling, dry mouth, thrush, tingling, and taste changes in response to this paste. The in vitro analysis demonstrated that Oral B® had the lowest pH, the highest abrasivity, and produced the lowest cell viability ($p<0.01$).
Conclusion Results suggest that low pH toothpastes that are highly abrasive and cytotoxic may cause undesirable reactions in patients.
Clinical relevance Toothpaste's properties should be well known for indication to patient therefore minimizing discomfort reports.

Keywords Toothpaste · Cytotoxicity · Abrasivity · Discomfort · Crossover study

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Introduction

Fluoridated toothpastes have complex formulations that provide preventive and therapeutic treatment of great relevance [1, 2]. Their composition includes antimicrobial agents, abrasive components, detergents, flavors, and others. Each component has its specific function and provides different characteristics for toothpastes [3–8]. Clinically, some patients have reported shortcomings that affect the oral mucous membrane (burning sensations and desquamation) and hard tissues (dentin hypersensitivity or harshness feeling). Some studies have shown allergic reactions and ulcerated lesions in the oral cavity associated with components present in commercial toothpaste [9–11]. The number of recent studies assessing cell viability, cytotoxicity, and genotoxicity has shown the greater concern about the possible adverse effects caused by components of toothpastes [12–16]. A recent study showed that whitening toothpastes were more genotoxic to cells in vitro than the common toothpastes [12].

These adverse reactions may be associated with artificial chemical additives, i.e., sodium lauryl sulfate (SLS). SLS is considered to be irritating but used in dentifrices formulation [9]. It is the most commonly used detergent [8, 17]. Its concentration ranges from 1 to 3 % [18]. The frequent use of SLS may result in allergic and toxic reactions [19–22]. Clinical studies have reported irritation, burning mouth, epithelial desquamation, and recurrent aphthous ulceration related to SLS [19, 7, 23, 24]. Toothpastes also contain flavoring agents (essential or aromatic oils) that can cause burning sensations of the mouth and a bitter taste [3].

Tooth sensitivity and rough sensation may be partially related to toothpastes' pH and abrasive qualities. Allowed pH for toothpastes ranges from acid and probably more aggressive (pH=4) to alkaline (10) [25]. A study has reported that slightly alkaline solution may lead to adverse oral manifestations, and it was implicated as the causative factor in hypersensitivity reactions [26]. The incorporation of abrasives in the toothpaste formulation improved cleaning efficacy [27]. A wide variety of abrasive systems are present in commercially available toothpastes, including precipitated silica of various sized particles [27]. Toothpaste can provide low, medium, or high abrasiveness [4]. There is a wide variation in the abrasiveness (i.e., recommended dietary allowance (RDA) range between 36 and 269) [28, 29]. In general, it has been demonstrated that smaller and less irregular particles result in low abrasiveness dentifrice [30–32] and that majority of dentifrices contained hydrated silica have higher abrasiveness [28]. The abrasion caused by brushing may result in gradual loss of restorative, dentin, and enamel surface [33], thereby causing recession in the cervical margin while increasing the roughness of the teeth [34, 35]. Manufacturers are investigating formulations that maximize cleaning and minimize abrasivity, thus reducing the undesirable effects [36].

Due to these complaints, the objective of this study was to use in vitro methods to evaluate the pH, abrasive potential and cytotoxicity of four commercial toothpaste brands and to relate these factors to the reactions and sensations reported by patients through questionnaires after using each toothpaste. The hypothesis of this work is that toothpastes with lower pH and higher abrasivity cause higher discomfort to users and produce a higher level of cytotoxicity.

Materials and methods

This study was approved by the Ethical Committee in Research of the Ibirapuera University (protocol 367,763). Four toothpastes were used (Table 1): Colgate Total® 12 Clean Mint (reference control toothpaste), Oral B® Limpeza Profunda (complaining patients' toothpaste), Colgate® Luminous White (LW; high abrasiveness toothpaste), and Closeup® Ação Profunda (different manufacturer toothpastes).

In vitro phase

Toothpaste abrasivity

Composite cylinder specimens (Ø=5 mm, h=1.7 mm; shade A3, Charisma, Kulzer, Harnau, Germany) were prepared in single increment and photo-polymerized for 20 s with 800 mw/cm^2 (Radii-CAL, SDI, São Paulo, SP, Brazil) to evaluate the abrasive effect of each toothpaste (n=10). Specimens were kept immersed in distilled water (37 °C) for 7 days then

Table 1 Information on the toothpastes used in this study based on the manufacturers' information

Commercial brand (manufacture)	Active ingredients	Composition
Oral B® Pró-Saúde Limpeza Profunda (Procter & Gamble)	Stannous fluoride (1100 ppm) Sodium fluoride (350 ppm)	Carnauba wax/CL 74160, carrageenans, flavor, glycerin, hydrated silica, PEG-6, propylene glycol, sodium gluconate, sodium hexametaphosphate, sodium lauryl sulfate, sodium saccharin, trisodium phosphate, water, xanthan gum, zinc lactate
Colgate® Luminous White (Colgate-Palmolive)	Sodium fluoride 0.32 % (1450 ppm)	Cellulose gum, cocamidopropyl betaine, FD&C Blue no. 1 (CL42090), flavor, glycerin, hydrated silica, PEG-12, polyethylene, sodium hydroxide, sodium lauryl sulfate, sodium saccharin, sodium triphosphate, sorbitol, tetrapotassium pyrophosphate, water, xanthan gum
Closeup Ação Profunda® (Unilever)	Sodium fluoride (1400 ppm)	Cellulose gum, CL42090, flavor, hydrated silica, mica (micro-shine crystal), PEG-32, sodium hydroxide, sodium lauryl sulfate, sodium saccharin, sorbitol, water, zinc sulfate
Colgate Total 12 Clean Mint® (Colgate-Palmolive)	Sodium fluoride 0.32 % (1450 ppm) Triclosan 0.3 %	Carrageenans, copolymer PVM/MA, flavor, hydrated silica, sodium hydroxide, sodium lauryl sulfate, sodium saccharin, sorbitol, titanium dioxide (CL778910), triclosan, water

dried in a desiccator. They were weighted using a precision balance (Adventurer-NJ, China) every 24 h until a constant value was achieved (three consecutive measurements) with a maximum variation of 0.0002 g [37]. The average of the last three weightings was calculated and adopted as the value of the initial mass of each specimen (MI). After brushing (next item), the specimens were dehydrated for the final analysis with the same method used to initial weighting. The difference between initial and final values represents the change in the mass. A brushing machine was used to the brushing simulation of composite samples. The heads of Smile Original® toothbrushes (Colgate-Palmolive Company, NJ, USA) were fixed at the arms of the brushing machine; 30,000 brushing cycles were performed (1.5 hertz and 210 g load) for each specimen.

Dentifrices were diluted at a ratio of 2:1 (ISO 14569-1). This preparation was replaced every 10,000 cycles. After that, the samples were put into an ultrasonic tank (Thorthon, Vinhedo, Brasil) for 10 min, washed, and stored for 7 days at 37 °C.

pH measurements

The pH measurements for the four toothpastes (n=3) were carried out by a pH meter (pH Meter E-520, Switzerland). The pH meter was calibrated before each measurement.

Cytotoxicity analysis

Fibroblasts obtained from fragments of human gingiva (FMM1 cell line) [38] grown between the sixth and tenth passages were used. They were obtained at the cell bank of the Department of Dentistry of the University of São Paulo. The cells were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM; LGC Biotecnologia, Cotia, SP, Brazil) supplemented with 10 % fetal bovine serum (FBS; Cultilab, Campinas, SP, Brazil) and 1 % antibiotic-antimycotic solution (Sigma Aldrich, St. Louis, MO, USA) and maintained in an incubator at 37 °C in a humid atmosphere containing 5 % CO_2 and 95 % air. The cell growth was monitored daily under a phase contrast microscope; the medium was changed every day. For the experiments, the cells were harvested and plated onto 24-well culture plates.

The toothpastes' cytotoxicity was analyzed by using conditioned media (e.g., media containing substances leached or dissolved from each toothpaste). The conditioned media were obtained as recommended by the protocol published by the American Society for Testing Materials (ASTM) [39]. The determination of concentration of the toothpastes, as well the conditioning time, followed this protocol.

Briefly, test tubes containing the toothpastes were filled with Dulbecco's modified Eagle's culture medium. Conditioning was performed for 1 h at 37 °C, using 0.2 g of each toothpaste/ml of fresh medium. For the cytotoxic analysis model, the experimental groups were as follows: control-fresh medium; medium conditioned with Oral B® or Colgate® LW or Closeup or Colgate Total®.

For all experiments, the cells were plated (2×10^3 cells/well) in 24-well culture plates and maintained in a humidified chamber at 37 °C. Twenty-four hours later, the culture medium was exchanged with the experimental conditioned medium, which remained in contact with the cells for 5 min to simulate brushing length. The control group received fresh culture medium. Then, the conditioned medium was exchanged for fresh medium, and the cultures were incubated in a humidified chamber at 37 °C for 24 h. All experiments were performed in triplicate.

Twenty-four hours after the cells exposure to the conditioned medium, cell viability of all groups was measured, also observing the survival of the cells over time. This analysis was based on the mitochondrial activity as measured by the 3-(bromide, 4,5-dimethylthiazol-2yl)-2,5-diphenyltetrazolium (MTT)-based cytotoxicity assay [40]. This test is based on the ability of mitochondrial enzymes produced by metabolically active cells to reduce MTT (Invitrogen, Eugene, OR, USA) molecules to an insoluble salt of formazan, which can be detected at 562 nm of absorbance using a spectrophotometer (Amersham Biosciences, Biochrom Ltd., Cambridge, England). According to a recent study [41], this assay indirectly determines the cell viability. Thus, the absorbance data of the control group grown in ideal cell culture conditions indicates the maximum cell number in one well. The comparison of these data with those of the experimental groups indirectly indicates the number of viable cells in the experimental wells. The mean optical density of the control groups was considered to be 100 % cell viability. Then, the cytotoxicity of all other experimental groups was classified as follows: >90 %=no cytotoxic; 60–89 %=low cytotoxicity; <59 %=high cytotoxicity.

Clinical phase

One hundred twenty-one patients (85 women and 36 men) aged between 20 and 50 years old were selected. Symptoms of thrush, previous dentin hypersensitivity, severe or generalized cervical erosion, dry mouth, less than 20 vital teeth, individuals under 18 or over 50 years old, and individuals with any disabilities, which could compromise oral hygiene were excluding factors. All patients (selected or not) received oral hygiene orientation and prophylaxis. The recommended amount of toothpaste was shown to the participants (Electronic supplementary material (ESM) 1), followed by brushing supervised by a dentist. Patient brushing time was recorded each week (n=4). The patients received one toothpaste sample per week without any identification (double-blinded study design) and answered two questionnaires: after using each product (ESM 2) and after using all toothpastes (over a 1-month period) (ESM 3).

Results analysis

The data on pH, mass loss, and cell viability were analyzed using one-way ANOVA test followed by the Tukey's test. Student's *t* test was used to compare the brushing time of patients with and without complaints about the Oral B® toothpaste. In all tests, the level of significance was set at 5 % ($p<0.05$).

The clinical data obtained from the questionnaires were assessed using a descriptive analysis.

A correlation test between the outcomes of the questionnaire and the outcomes of the in vitro measurements was performed.

Results

Clinical phase

The results obtained from the questionnaires are described in Table 2.

The average brushing time for the 121 patients was 101 s, with a standard deviation of 35 s. The average brushing time for the group with complaints about the Oral B® toothpaste (a total of 104 patients) was 103 s, and the average brushing time of the group without complaints (17 patients) was 87 s. This 16 s longer was not statistically significant ($p>0.05$).

In vitro phase

Toothpaste pH The pH results are described in Table 3. The pH values ranged from 5.8 to 8.1 for Oral B® Pró-Saúde Limpeza Profunda and Colgate® Luminous White, respectively.

Mass loss The results of the mass loss are described in Table 4. The values of mass loss ranged from 0.9 to 4.0 % for Closeup® Ação Profunda and Oral B® Pró-Saúde Limpeza Profunda, respectively.

Cytotoxicity Figure 1 illustrates the percentages of cell viability in all experimental groups 24 h after contact with culture medium conditioned by the toothpastes. The mean optical density of the control group (CG) was considered to be 100 %. All other groups presented percentages of viable cells ranging from 16 to 21 %, which were considered highly

Table 2 Absolute and relative frequencies for each of the items evaluated on the questionnaire

Items evaluated	Oral B® Pró-Saúde Limpeza Profunda	Colgate® Luminous White	Closeup Ação Profunda®	Colgate Total 12 Clean Mint®
Liked the toothpaste	64	103	111	96
	53 %	85 %	92 %	79 %
Burning sensation/irritation//swelling/sensitivity (cheek, tongue, lips, gum, palate, or papillae)	38	15	11	8
	31 %	12 %	9 %	7 %
Tooth sensitivity	21	8	3	7
	17 %	7 %	2 %	6 %
Unpleasant taste	62	20	21	43
	51 %	17 %	17 %	36 %
Unpleasant texture (sandy, rough, sticky)	77	33	14	14
	64 %	27 %	12 %	12 %
Rougher teeth	19	6	3	3
	16 %	5 %	2 %	2 %
Peeling/exfoliation/roughness (cheek, tongue, lips, or gum)	22	5	2	2
	18 %	4 %	2 %	2 %
Dry mouth/thirst	36	10	10	11
	30 %	8 %	8 %	9 %
Presence of aphthous ulcer/wounds	6	1	0	1
	5 %	1 %	0 %	1 %
Itching/tingling/taste changes (cheek, tongue, or lips)	4	3	0	1
	3 %	2 %	0 %	1 %
Whitening effect on teeth	2	13	0	1
	2 %	11 %	0 %	1 %

Table 3 Means (standard deviations) for pH values

Toothpaste	pH
Oral B® Pró-Saúde Limpeza Profunda	5.8 (0.20) C
Colgate® Luminous White	8.1 (0.03) A
Closeup® Ação Profunda	6.8 (0.03) B
Colgate Total® 12 Clean Mint	6.9 (0.03) B

Means followed by the same letter are statistically similar (p<0.05)

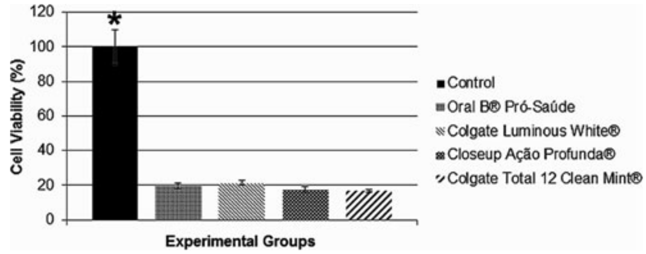

Fig. 1 Percentages of cell viability in all experimental groups

cytotoxic. The percentages of cell viability were similar in all experimental groups and significantly lower than that of the control cultures.

Regression analyses Table 5 shows a good correlation between five clinical outcomes and mass loss values (R^2>75 %).

Discussion

The study hypothesis was partially accepted: the dentifrice with lower pH value and increased abrasivity caused increased patient discomfort.

The Oral B® toothpaste had the lowest pH (5.8) and the largest mass loss (4 %). This result confirms previous findings, which showed that acidic pH produced greater changes in tooth surfaces [42, 43]. The pH found for this toothpaste is actually somewhat alarming as it is very close to the critical pH value needed for tooth demineralization (5.5). According to Addy and Hunter [25], the pH variation allowed for toothpastes is 4–10. This causes some concern about the possible dentine wear due to chemical erosion where the dentifrice has low pH. It is well known that acid dentifrices have higher anticaries efficacy compared with neutral dentifrices [44]. However, few studies have evaluated the pH of commercial toothpastes itself and its possible side effects [45]. Andrade Júnior et al. [46] reported a pH dentifrice for Oral B® teeth and gum (6.1) that was similar to the one found in this study (5.8). Other authors have demonstrated that eight different Brazilian commercial toothpastes have acidic pH values [45]. Even so, there are no data in the literature to assess whether the pH of a dentifrice could potentiate undesirable effects, such as the formation of non-carious lesions. In the same way, there are no studies correlating pH values of different commercial toothpastes and clinical outcomes. Most of the papers refer to the pH-cycling test (with evaluation of demineralization/re-mineralization) and acidic challenge that were not the focus of this work [47, 48, 33].

Table 4 Means (standard deviations) for the mass loss in percent

Toothpaste	Mass loss (%)
Oral B® Pró-Saúde Limpeza Profunda	4.0 (0.9) A
Colgate® Luminous White	3.0 (0.3) B
Closeup® Ação Profunda	0.9 (0.3) C
Colgate Total® 12 Clean Mint	1.2 (0.3) C

Means followed by the same letter are statistically similar (p<0.05)

Colgate Total® and the Closeup® Ação Profunda toothpastes had statistically similar pH values (more neutral, ≅7.0). Colgate® LW pH was the highest among the studied toothpastes (8.1).

The abrasivity of toothpastes has been studied for many years [12, 36, 49, 27, 28]. There are different methods to determine the degree of abrasion of toothpastes. Some authors emphasize the importance of not only considering the RDA values but to consider both a qualitative (roughness) and a quantitative (volume loss) value when describing toothpaste abrasivity [49–52]. In this study, weight changes in composite specimens were evaluated [34]. Composite was used instead of extracted human teeth for standardization because natural teeth are variable (e.g., donor race and age, the degree of maturation of the tooth, the amount of fluoride absorbed), which could hinder the actual determination of the abrasivity of toothpaste. Other studies in literature used acrylic plates to measure toothpaste abrasivity [29, 53, 49].

Although still contradictory in the literature, some studies show that dentifrices marketed as "whitening" products were generally more abrasive to the enamel and dentin surface [12], especially for those containing silica [28]. Besides, whitening toothpastes may contain additional chemical agents, which augment the abrasive cleaning [54]. The present study choose Colgate® LW as representative of highly abrasive toothpaste (RDA=240) and Colgate Total® as a reference control and low abrasive toothpaste (RDA=70) [27]. Surprisingly, this work showed that Oral B® Pró-Saúde Limpeza Profunda toothpaste has the highest abrasivity of all toothpastes used in this study. At the second position was the "whitening" product (Colgate® LW). Colgate Total® and the Closeup® Ação Profunda toothpastes had statistically similar mass loss values (≅1.0). All dentifrices analyzed at the present study contain hydrated silica. Differences in amount, form, or regularity of particles could probably explain the results. The presence of carnauba wax in Oral B® Pró-Saúde Limpeza Profunda may be a possible explanation for the higher

Table 5 *R* square from the regression analyses between the clinical outcomes and in vitro results

Items evaluated with the questionnaire	pH	Mass loss
Liked the toothpaste	R^2=0.515	R^2=0.599
Burning sensation/irritation/swelling/sensitivity (cheek, tongue, lips, gum, palate, or papillae)	R^2=0.437	R^2=0.748*
Tooth sensitivity	R^2=0.340	R^2=0.795*
Unpleasant taste	R^2=0.645	R^2=0.274
Unpleasant texture (sandy, rough, sticky)	R^2=0.320	R^2=0.855*
Rougher teeth	R^2=0.394	R^2=0.797*
Peeling/exfoliation/roughness (cheek, tongue, lips, or gum)	R^2=0.485	R^2=0.720
Dry mouth/thirst	R^2=0.624	R^2=0.589
Presence of aphthous ulcer/wounds	R^2=0.471	R^2=0.709
Itching/tingling/taste changes (cheek, tongue, or lips)	R^2=0.061	R^2=0.941*
Whitening effect on teeth	R^2=0.591	R^2=0.212

*R^2 ≥0.75—a strong relationship between variables

abrasiveness observed since this component has a very high hardness.

In the clinical phase, Oral B® toothpaste was also associated with the highest frequency of reported irritation (31 %), tooth sensitivity (17 %), discomfort with taste (51 %), and texture (64 %), rougher teeth (16 %), soft tissue peeling (18 %), dry mouth (30 %), presence of aphthous ulcers (5 %), and tingling and taste changes (3 %). Thereby, the present study demonstrates the relationship between high pH and high abrasivity and the discomfort to soft and hard tissues reported by the patients. Another interesting fact is that this dentifrice was the one that had a lower acceptance rate among patients: only 53 % of the patients said that they enjoyed it the same, whereas for the other toothpastes, the acceptance rate was 79–92 %.

The pH and mass loss similarity found by Colgate Total® and the Closeup® Ação Profunda toothpastes was also reflected in the clinical study, as seven of the nine items associated with discomfort showed similar percentage values (burning, 7 and 9 %; discomfort with texture, 12 and 12 %; rougher teeth, 2 and 2 %; peeling, 2 and 2 %; dry mouth, 9 and 8 %; presence of aphthous, 1 and 0 %; and itching, 1 and 0 %, for Colgate Total® and Closeup®, respectively).

According to informal communication by manufacturers, the zinc present in toothpastes can be related to symptoms of dry mouth. This may explain the discomfort from Oral B® (30 %) as it contains zinc lactate. However, the Closeup® toothpaste also contains zinc but produced a low dry mouth index (8 %). However, in Closeup®, the zinc occurs in the form of zinc sulfate. More detailed chemical studies are needed to determine whether there are differences between these forms of zinc in terms of its solubility and dissociation in the saliva. Further studies could also determine the actual concentrations of this element in the products and might explain the different percentages of dry mouth reported. The presence of zinc sulfate reduces bad breath from bacteria within the oral mucosa [55], which may be related to these toothpastes' greater acceptance (92 %) by patients, who presumably appreciated the sense of having fresh breath.

The excessive amount of aromatic oils in some toothpastes formulations has been linked to burning sensations in the oral mucosa [3]. Peppermint oil is related to many intraoral disorders [56]. This flavoring is found in the Colgate Total®, Oral B®, and Colgate® LW used in this study. Again, little can be said because there is no information about concentrations of components for the studied products. Therefore, it is difficult to determine whether the burning discomfort reported by patients (31 %) with Oral B® toothpaste is related to the present aromatic oils.

It should be noted that although Colgate® LW pH was the highest among the studied toothpastes (8.1), its mass loss was the second highest (3 %). So, high abrasiveness and a high pH may be responsible for the second highest rate in four of the nine patient discomfort items (burning (12 %), discomfort in response to the texture (27 %), rougher teeth (5 %), and mucosal peeling (4 %)). Some authors have reported that pyrophosphates (present only in Colgate® LW) along with the increased concentrations of flavorings and detergents and their higher intraoral alkalinity are strongly implicated as the causative factor in certain hypersensitivity reactions [26].

These frequencies were all lower than those observed for Oral B® toothpaste. It appears that an alkaline pH did not decrease the influence of abrasiveness on clinical feelings of discomfort.

Colgate® LW was the only toothpaste claiming to have whitening effect. Consistently, the patients reported a higher frequency of bleached teeth after using this product (11 %) compared with the other toothpastes. It should be noted that in this clinical study, tooth color change has not been analyzed with Vita shade guides, colorimeters, or digital photograph analysis as other clinical studies. Therefore, these clinical results were only subjective.

Regression analyses (Table 5) showed a high correlation (R^2 higher than 0.75 which means that 75 % of the two variables data could be adjusted in a linear regression) between mass loss values and five clinical outcomes (i.e., itching/tingling/taste changes, R^2=0.941; rougher teeth, R^2=0.797; burning sensation/irritation/swelling/sensitivity, R^2=0.748).

Reports of irritation and soft tissues peeling may also be related to the presence of SLS in Oral B® toothpaste, which has been reported to be irritant in previous studies [8, 57]. SLS is the most toxic agent on mucosal cells and cause epithelial desquamation [14, 57]. Considering the cytotoxicity, this anionic detergent may cause adverse effects on cells due to their ionic properties, which can promote cell death [58]. However, all dentifrices used in this study contain SLS. Recently, a lot of studies have been done to analyze the cytotoxicity, genotoxicity, and antibacterial effects of commercial toothpastes [13, 59, 52]. At the present study, it has been shown that all dentifrices studied were highly toxic to human cells. Some authors have reported that whitening toothpastes were more cytotoxic and more genotoxic to cells in vitro than the common toothpastes. However, this fact that has not been observed in this study [52].

It is known that higher concentrations of SLS can cause more destructive epithelial lesions [58]. This may suggest that concentration of SLS in Oral B® toothpaste is higher than in other toothpastes, but this information could not be obtained from the manufacturers or from literature. Tissues exposed to SLS concentrations as low as 0.015 % show changes in the epithelial structure and architecture, and concentrations of 1.5 % are capable of almost completely destroying the epithelium, thus causing atrophy and cell death [58]. These data show that the concentrations allowed for use in toothpastes (1–3 %) can cause serious damage to users [18].

Although some authors have reported that triclosan can reduce SLS-caused irritation [6], this was not confirmed as Colgate Total®, which contains triclosan, showed similar percentages for the four items related to patient discomfort as did toothpastes without triclosan (Closeup and Colgate® LW).

Patients with gingival recession or abfraction were not included in this study. Recent studies showed that toothpaste plays an important role in non-carious lesions formation and that greater amount of dentin wear was correlated with higher abrasive index for some dentifrices [60, 61]. So, patients with severe tooth wear and exposed and/or eroded dentin surfaces should be more careful with their oral hygiene. Some recommendations as use of less abrasive products [60, 62] and use of sonic toothbrushes (less brushing force) are important actions to reduce abrasion [63]. In these cases, the dentist should be very careful in indicating toothpastes with potentially abrasive because the patients' discomfort would likely be even greater and the effects of surface wear on their teeth would likely be more exaggerated.

The results demonstrated that toothpastes with lower pH and higher abrasivity caused major discomfort to patients. It should be clear that some factors, such as the plaque index and smoking status, were not evaluated in these patients. Thus, for these normal-reactive patients, the in vitro pH data, abrasivity and cytotoxicity are perhaps less relevant compared with those from patients with poor oral hygiene or a history of smoking. For the average patient (normal-reactive), most toothpaste on the market are good, with excellent user acceptance and no reports of adverse effects.

To observe the direct effects of substances leached from the toothpastes on the oral soft tissues, the effects of these materials in cultured oral mucosa fibroblasts were studied. Although in vitro tests ideally should be performed in conditions close to those of the clinical situation, sometimes it is not feasible. In these cases, there are recommendations to be followed for performing such tests. This is the case when testing cytotoxicity of medical devices. Then, this work followed the protocol published by the American Society for Testing and Materials [39]. The determination of concentration of the toothpastes, as well as the conditioning time, followed this protocol. For the in vitro cytotoxicity assay, the type of the cell is not relevant. There are studies using different types of cells. However, aiming at being as close to the clinical situation as possible, it was decided to use fibroblasts obtained from gingiva, as the response to substances leached or dissolved from the toothpastes during tooth brushing would permeate the epithelium of the oral mucosa reaching the underneath connective tissue most composed by fibroblasts. Also, it was decided to use a contacting time higher than that of the actual toothbrushing, because the fibroblasts in the oral mucosa will be in contact with substances leached or dissolved from the toothpastes that would permeate the epithelia during toothbrushing. Then, these substances would rest in contact with the fibroblasts of the connective tissue underneath the epithelia longer after the toothbrushing.

All toothpastes evaluated were classified as highly cytotoxic, as the resulting cell viabilities were lower than 50 %. This decrease in cell viability can be attributed to the effect of various toothpaste components, either alone or combined. In fact, some studies have demonstrated that certain toothpaste components were responsible for the cultured cell death [64, 65]. As described above, the main components associated with the adverse effects of the toothpastes studied was SLS and triclosan [5].

In combination with the results of the in vitro study, these results show that a combination of characteristics, such as low pH, high abrasive capacity, and high cytotoxicity seem to be related to the undesirable reactions reported by patients. Therefore, it is extremely important to know the characteristics of each toothpaste and to have detailed information on the composition and concentration (%) of each product. It is currently very difficult to obtain this information because of the secrecy of the companies, which is challenging for dentists, users and researchers.

The main objective of this study was to obtain more information about the complaints that healthy patients have about toothpastes. It should be kept in mind that the clinical results would have been different if ill patients with systemic or local changes had been included in this study. Further research is needed to better understand which components and properties are related to the patients' reports. This would provide dentists the required information to correctly and safely indicate specific products for their patients, reducing the levels of discomfort following the use of specific toothpastes. The dentist must be aware of the effects and possible adverse reactions of the listed products.

Conclusions

Based on the results obtained here and considering the possible limitations of the study, the following conclusions can be drawn:

- Oral B® Pró-Saúde Limpeza Profunda toothpaste has the lowest pH and causes the highest abrasivity of all toothpastes used in this study.
- Oral B® Pró-Saúde Limpeza Profunda toothpaste also resulted in major discomfort, as reported in the patient questionnaires.
- Colgate® Luminous White toothpaste produced the second highest mass loss and discomfort reported by patients, even with its higher pH (8.1).
- All dentifrices studied were highly toxic to human cells.
- Toothpastes with lower pH values and greater abrasivity generated increased discomfort in users.

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Ethical standards This study was approved by the Ethical Committee in Research of the Ibirapuera University (protocol 367 763). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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