Cytotoxic effects of different detergent containing children’s toothpastes on human gingival epithelial cells

Sinem Birant1*, Yazgul Duran2, Tunc Akkoc2 and Figen Seymen3

Abstract
Background: This study aimed to evaluate possible cytotoxic effects to gingival epithelial cells exposed to children toothpastes containing different detergent.

Methods: Tissues required for the isolation of human gingival epithelial cells were obtained by biopsy during the extraction of the impacted third molar tooth. Toothpaste solutions of different concentrations were prepared from five different children’s toothpastes with different detergent contents. Isolated gingival epithelial cells were stimulated with experimental groups consisting of toothpaste solutions (Colgate, Sensodyne, Splat, Nenedent, Perlodent) at different concentrations and a control group consisting of complete Dulbecco’s modified eagle medium. After the experiments, cell viability was evaluated using flow cytometry. 2 Way ANOVA was used to see the interaction effect of the main effects of toothpaste solution and concentration factors. Pairwise comparisons were made by Tukey post hoc tests. In the study, the significance level was taken as 0.05.

Results: As a result of the analysis, it was seen that the toothpaste solution and concentration factors and the interactions of these 2 factors were effective on the viable, early apoptotic, late apoptotic and necrotic cell rates. The statistically highest live cell ratios were detected in Splat’s toothpaste solutions (90.14% at 0.4% concentration) after the control group (90.82%) and the group with the lowest viability values was determined in Colgate group (75.74% at 0.4% concentration) (p<0.05).

Conclusions: According to the results of the study, it was observed that toothpastes containing SLS affected the viability of cells more negatively than toothpastes with other detergent contents.

Keywords: Toothpaste, Stem cell, Annexin V, Detergent, SLS

Introduction
Dental plaque is defined as a dynamic biofilm ecosystem consisting of more than 100 bacterial species, desquamated epithelial cells, salivary glycoproteins, leukocytes, macrophages and food residues that accumulate on tooth surfaces [1, 2].

Tooth decay and periodontal diseases are among the most common bacterial infections. It is reported that the most important factor of these diseases is dental plaque deposited on the tooth surface [3]. For this reason, it is critical to remove dental plaque from the tooth surface and provide oral hygiene in the prevention of tooth decay and gingival diseases. The simplest method to apply in this regard is to give individuals the habit of brushing their teeth. The most commonly used toothpastes during tooth brushing are among the most effective cosmetic and therapeutic agents in routine use, and among all
dental products, they are among the most widely used by consumers [4–7].

There are many components in toothpaste, whose activities and functions are different from each other. Among these components, abrasives, water and moisturizers are present in toothpastes by 20–40%, detergents 1–2%, binding agents and sweeteners 2%, therapeutic agents 5%, colorants and preservatives 1%. The presence of these components or their concentration in toothpaste can cause undesirable side effects (such as dry mouth, recurrent aphthous and ulcers) [8, 9].

Detergents are substances that reduce surface tension known as surfactants. They have two groups, hydrophilic and hydrophobic [10, 11]. While the long hydrocarbon chain forms the water-repellent (hydrophobic) part of the molecule, it also provides the molecule with surface active properties. The polar group forms the water-loving (hydrophilic) part of the molecule and enables it to dissolve in water. The combination of these polar and apolar groups is defined as the amphiphilic structure. Thanks to the amphiphilic structure, surfactants can be dissolved in both polar and apolar solvents. While detergents adhere to water molecules with their polar parts due to these chemical properties, they ensure the removal of dirt from the environment by holding on to the dirt with their apolar parts [10–13].

Detergents are classified as anionic, cationic, amphoteric and nonionic detergents according to the ionic charge of the hydrophilic group they contain. Anionic and amphoteric detergents are frequently used in toothpaste. Sodium lauryl sulfate (SLS), sodium methy cocoyl taurate (addinol), sodium streate (sodium octadecanoate), sodium lauryle sarcosinate, sodium CI2-14 olefin sulfonate, sodium CI4-16 olefin sulfonate from anionic detergents and cocamidopropyl betaine (CABP) among amphoteric detergents are surfactants used frequently in toothpastes. In addition to their foaming and cleansing properties, they are routinely added to toothpastes due to their antibacterial and plaque inhibition properties [14–17].

SLS is a detergent that is often used in toothpastes with a ratio of 0.5% to 2%. SLS prevents the growth of some microorganisms by adsorption to the cell wall, penetration through the cell wall, interaction with the cell membrane, lipids and proteins, leakage of intracellular components with an increase in cell permeability and lysis in the cell [18, 19]. It has been reported in studies that SLS increases plaque inhibition, decreases Streptococcus mutans penetration, decreases lactate production, glucosyltransferase activity and the amount of extracellular polysaccharide created by S. mutans [20–22]. Despite these positive features, some toxic effects of SLS have also been reported. Oral epithelial destruction, ulcerations and inflammations caused by SLS have been observed in clinical studies. It has been reported that SLS in the toothpaste denatures the glycoproteins of the mucin layer, causing the barrier function of the oral mucosa to deteriorate, and the gingiva and buccal mucosa to be more sensitive to irritants such as exogenous antigens [23, 24]. It has also been stated that SLS may be responsible for a decrease in the keratinization level of the human oral epithelium. Sodium lauryl sulfate has also been reported to cause irritation of the oral mucosa in patients with dry mouth and the use of SLS is also associated with recurrent aphthous ulcers [23–26]. Although SLS is the most commonly used surfactant among toothpastes, surfactants with less side effects such as betaines are also used in toothpastes. Cocamidopropyl betaine, an amphoteric detergent, has been reported to have less mucosal irritation and foaming effect than SLS, and it is more biocompatible [27, 28].

There are different evaluation methods in studies conducted to determine the toxic effects of materials on cells or to investigate their biocompatibility. These tests are classified as clinical use tests, in vivo animal experiments, and in vitro cell culture tests. Cell culture tests are frequently used in cytotoxicity studies due to their ability to mimic the physiological states of living tissues. In addition, cell culture studies have many advantages such as rapid application, repetition, standardization, low cost, easy control of the experimental environment during the experiment and not being affected by different individual factors [29]. In this study, in vitro cell culture tests were preferred to determine the effects of toothpastes on cells. For this reason, in this study, it was preferred to create a primary cell culture instead of cell lines, considering the creation of experimental conditions closer to in vivo conditions. In addition, unlike other studies, not only cell viability but also apoptosis and necrosis rates were included in the study. The aim of this study was to investigate the effects of different detergent-containing children’s toothpastes on the viability of human primary gingival epithelial cells.

Materials and Methods

The study was approved by the ethics committee of Istanbul University, Faculty of Dentistry (170/2017) according to Helsinki Declaration guidelines.

Isolation and characterization of gingival epithelial cells (GECs)

5 fully impacted human third molars, which were removed from systemically healthy patients (aged 18–25 years) were used for tissue biopsy. Gingival tissues surrounding the tooth sockets were collected immediately after tooth extraction. For the isolation of gingival
epithelial cells, the gingival tissue was incubated at 4 °C in 0.4% dispase overnight. The epithelium strips were then mechanically separated and trypsinized in 0.05% trypsin/0.53 mM EDTA (Gibco, Grand Island, NY, USA) at 37 °C for 10–15 min. After strong pipetting, the cell suspension was centrifuged at 700 g for 5 min and the cell pellet was resuspended in keratinocyte growth medium (Dermalife Basal Medium; Lifeline, Walkersville, MD, USA). The cells were transferred to T-25 cm² flask and were placed in the incubator which provided 5% CO₂ environment at 37 °C. The keratinocyte growth medium was changed every 2 days and the proliferation and spreading of the cells on the flask was monitored at regular intervals by inverted microscope (EVOS-AMG, Thermo Fisher Scientific, Waltham, MA, USA).

Cells were fixed on the slide using 95%, 70% and 50% alcohol, respectively, at room temperature. Then, the fixation process was completed by dipping the slide into distilled water. Staining was performed with hematoxylin (Sigma-Aldrich, St. Louis, MO, USA) for 8 min. After staining, it was washed with distilled water and the second staining process was started with Eosin (Sigma-Aldrich). After staining with eosin for 90 s, the cells were dehydrated with 95% alcohol and then dipped in xylol 20 times. Microscope slides were fixed using Permount (Fisher Scientific, Pittsburgh, PA, USA) and epithelial cells were analyzed by Binocular Research Microscope (Olympus BH2-RFCA) for characterization [30].

**Preparation of toothpaste solutions**
The toothpastes used in this study were Colgate 6+, Sensodyne Pronamel 6+, Nenedent (4–9 aged), Perlodent Junior 6+, Splat Juicy. The different detergent contents and other properties of these toothpastes can be seen in Table 1. Toothpaste solutions of 80%, 50%, 20% and 0.4% concentrations of these toothpastes used in the study were prepared by the method in our previous study and homogenized extraction liquids were obtained from toothpastes for cell viability experiments [31].

### Evaluation of cell viability by flow cytometry
Gingival epithelial cells (5 × 10⁵ cells) were plated into 48-well plates separately to perform viability experiments in each concentration of toothpaste solution. The viability experiments were carried out following the method used in our previous study [31]. Gingival epithelial cells were exposed to toothpaste solutions for 2 min, washed with DPBS (Dulbecco's phosphate buffered saline) (Gibco, Grand Island, NY, USA) and suspended in serum-free medium. 4 µL of Annexin V (BD Biosciences, CA, USA) was added to the tubes and the tubes were kept in a dark environment for 10 min. 200 µL of binding buffer was added and centrifuged at 1500 rpm for 5 min. Tubes were vortexed by adding 200 µL binding buffer. Then, 10 µL propidium iodide was added to the tubes to read the rates of viable, necrosis, early and late apoptotic cells in cells exposed to toothpaste solutions. The experiments with flow cytometry were repeated 5 times, and the average of the results obtained was calculated to determine the rates of viable, early apoptotic, late apoptotic and necrotic cells [31].

| Table 1 Composition of materials evaluated |
|-------------------------------------------|
| **Materials**                              | **Composition**                                                                 | **Manufacturer**                                      |
| Colgate 6+                                 | Sorbitol, aqua, hydrated silica, PEG-12, Sodium Lauryl Sulfate, cellulose gum, sodium saccharin, sodium fluoride (1450 ppm F⁻), aroma, hydroxypropyl methylcellulose, menthol, glycerin, cinnamal, eugenol, limonene, CI 77,891, CI 42,090 | Colgate Palmolive Company, Belgium                   |
| Nenedent Kids (4–9 aged)                  | Aqua, hydrated silica, glycerin, xylitol, propylene glycol, xanthan gum, titanium dioxide, aroma, Sodium Lauryl/Sarcosinate, disodium EDTA, sodium monofluorophosphate (500 ppm F⁻), sodium chloride | Dentinox, Berlin, Germany                            |
| Perlodent Junior 6+                        | Aqua, sorbitol, hydrated silica, propylene glycol, tetrapotassium pyrophosphate, xanthan gum, Sodium C14-16 Olefin Sulfonate, aroma, titanium dioxide, sodium fluoride (1450 ppm F⁻), sodium saccharin, phenoxyethanol, ethylhexyl glycerin | Rossmann, Germany                                    |
| Sensodyne Pronamel 6+                     | Aqua, sorbitol, hydrated silica, glycerin, PEG-6, Cocamidopropyl Betaine, xanthan gum, aroma, sodium fluoride (1450 ppm F⁻), sodium saccharin, sucralose, titanium dioxide, sodium hydroxide, limonene | Glaxo Smith Kline, ABD                               |
| Splat Juicy                               | Aqua*, dicalcium phosphate dihydrate*, hydrogenated starch hydrolysate*, glycерин*, hydroxyapatite, cellulose gum*, aroma, xanthan gum*, potassium thiocyanate, laurotetra sodium, lactoperoxidase*, glucose oxidase*, glucose pentaacetate, aloe barbadensis leaf extract*, sodium metylparaben, hydrolyzed casein*, glycyrrhiza glabra root extract* (*natural origins) | SIA Splat Trading, Okulovka, Russia                  |
| Complete DMEM (CDMEM)                     | 10% FBS (Fetal bovine serum), DMEM (Dulbecco’s Modified Eagles Medium) supplemented with 1% penicillin/streptomycin | Gibco, Grand Island, USA                             |
Statistical analysis
The experiments were repeated 5 times. The average of the test results obtained was taken. The obtained data were analyzed using the IBM SPSS V23 statistical program. 2 Way ANOVA was used to see the interaction effect of the main effects of toothpaste solution and concentration factors. Pairwise comparisons were made by Tukey post hoc tests. In the study, the significance level was taken as 0.05.

Results
Isolation and characterization of cells
It was observed that the isolated gingival epithelial cells had a cylindrical and cubic morphology by following their proliferation and reached a confluent structure from the 0th to the 3rd passage. The microscope image obtained as a result of staining with hematoxylin and eosin for the characterization of isolated gingival epithelial cells showed that the cells exhibited a cubic morphology (Fig. 1).

Cell viability in cells cultured exposed to the children’s toothpaste containing the different detergent content
After exposure to the different toothpaste solutions at different toothpaste concentrations, viable and dead cell ratios were determined graphically according to Annexin-V/PI positive and negativity. Annexin V (−) and PI (−) live, Annexin V (+) and PI (−) early apoptotic cell, Annexin V (+) and PI (+) late apoptotic cell, Annexin V (−) and PI (+) considered as a necrotic cell. The flow cytometry graphs of the control group (CDMEM) in Fig. 2, the Splat group in Fig. 3, and the Sensodyne group in Fig. 4, the Nenedent group in Fig. 5, the Perlodent group in Fig. 6 and the Colgate group in Fig. 7 show the average viable, early apoptotic, late apoptotic and necrotic cell ratios.

When Table 2 is examined, it is seen that the effect of the toothpaste on cell viability is statistically significant (F = 12.781, p = 0.00 < 0.05). The main effect of the toothpaste on cell viability can explain 81% of the variance in viability measurements. The effect of the second factor, concentration, on cell viability is again statistically significant (F = 9.416, p = 0.00 < 0.05). The main effect of concentration can explain about 65% of the variance in viability measurements. In addition, the effect of the interaction of these 2 factors on cell viability was found to be statistically significant (F = 135.463, p = 0.00 < 0.05). The interaction effect can explain about 95% of the variance in viability measurements. In the results obtained for early apoptotic cell rates, it is seen that the effect of the toothpaste on early apoptosis is statistically significant (F = 3.063, p = 0.04 < 0.05). The main effect of the toothpaste could explain half of the variance in early apoptotic cell rates. The effect of the second factor, the concentration, on early apoptosis was also statistically significant (F = 3.567, p = 0.40 < 0.05). The main effect of concentration can explain about 42% of the variance in early apoptotic cell ratios. In addition, the effect of the interaction of these 2 factors on early apoptosis was found to be statistically significant (F = 103.589, p = 0.00 < 0.05). The interaction effect can explain a large part of the variance in early apoptotic cell ratios, about 94%. In the results obtained for late apoptotic cell ratios, it is seen that the effect of the toothpaste on late apoptosis
is statistically significant ($F = 2.966, p = 0.047 < 0.05$). The main effect of the toothpaste could explain half of the variance in late apoptotic cell ratios. The effect of the second factor, the concentration, on late apoptosis was again statistically significant ($F = 3.740, p = 0.04 < 0.05$). The main effect of concentration can explain about 65% of the variance in late apoptotic cell ratios. In addition, the effect of the interaction of these 2 factors on late apoptosis was found to be statistically significant ($F = 65.969, p = 0.00 < 0.05$). The interaction effect can explain a large part of the variance in late apoptotic cell ratios, about 91%. In the results obtained for nectoric cell ratios, it is seen that the effect of the toothpaste on necrosis is statistically significant ($F = 14.286, p = 0.00 < 0.05$). The main effect of the toothpaste on necrosis can explain 83% of the variance in necrotic cell ratios. The effect of the second factor, the concentration, on necrosis was statistically significant again ($F = 3.819, p = 0.03 < 0.05$). The main effect of concentration can explain about 43% of the variance in necrotic cell ratios. In addition, the effect of the interaction of these 2 factors on necrosis was found to be statistically significant ($F = 31.576, p = 0.00 < 0.05$). The interaction effect can explain a large part of the variance in necrotic cell ratios, about 83%.

When cell viability rates between toothpastes were compared, the difference between the means of viability
at all 4 different concentration levels was statistically significant ($p<0.05$). When comparing early apoptotic cell rates between toothpastes, the difference between the early apoptotic means for the 0.40% concentration was not statistically significant ($p>0.05$). Accordingly, when the toothpastes are used with 0.40% concentration, the early apoptotic cell rates is independent of the toothpaste used. However, at the other 3 concentration levels (20%, 50%, 80%), the effect of the toothpaste on early apoptotic cell rates was statistically significant ($p<0.05$). When late apoptotic cell rates between toothpastes were compared, the difference between the means of late apoptotic cell rates at all 4 different concentration levels was statistically significant ($p<0.05$). When necrotic cell rates between toothpastes were compared, the difference between the means of necrotic cell at all 4 different concentration levels was statistically significant ($p<0.05$) (Table 3, Fig. 8).

When the viable, early apoptotic, late apoptotic, necrotic cell rates of the Colgate group were compared between 4 different concentration levels, at least 1 of the differences between the means was statistically significant ($p<0.05$). Accordingly, when Colgate is used, all the measured variables are dependent on the concentration level. When the viable and necrotic cell rates of the
Splat Juicy group were compared between 4 different concentration levels, at least 1 of the differences between the means was statistically significant ($p < 0.05$). Accordingly, these variables are dependent on the concentration level when Splat Juicy is used. However, on the other hand, when this material was applied with different concentrations, no significant difference was found in early and late apoptotic cell rates ($p > 0.05$). That is, apoptosis for Splat Juicy group are independent of the concentration. When the viable, early apoptotic, late apoptotic, necrotic cell rates of Sensodyne group were compared between 4 different concentration levels, at least 1 of the differences between the means was statistically significant ($p < 0.05$). Accordingly, all the variables measured when Sensodyne is used are a variable dependent on the concentration level. When the viable, early apoptotic, late apoptotic, necrotic cell rates of NeNedent group were compared between 4 different concentration levels, at least 1 of the differences between the means was statistically significant ($p < 0.05$). Accordingly, when NeNedent is used, all of the measured variables are dependent on the concentration level. When the viable, early apoptotic, late apoptotic, necrotic cell rates of Perlodent group were compared between 4 different concentration levels, at least 1 of the differences between the means was statistically significant ($p < 0.05$). Accordingly, the variables measured when Perlodent is used are dependent on the concentration level.
least 1 of the differences between the means was statistically significant ($p < 0.05$). Accordingly, when Perlodent is used, all of the measured variables are dependent on the concentration level (Table 4, Fig. 9).

**Discussion**

Detergents, one of the toothpaste components, are frequently used in removing plaque, due to their antimicrobial properties. However, it is stated that besides these positive properties, they also have the potential to adversely affect the oral mucosa [32]. In this study, when the viability rates of different detergent-containing children's toothpaste solutions on cells were evaluated, it was observed that the lowest viable cell rates were in SLS-containing toothpaste solutions. After the control group, the highest vitality values were determined in the toothpaste without detergent content, followed by the toothpaste containing CAPB.

Clinical intraoral side effects such as mucosal sensitivity, epithelial desquamation and recurrent aphthous ulcerations in vitro studies point to the possible problems of these ingredients used in adult toothpaste [27, 33, 34]. Studies examining the effects of these components in children's toothpastes on intraoral tissues are very few. When looking at the contents of children's toothpaste, it is seen that many paste contents contain SLS as a type of

---

**Fig. 6** Flow cytometry graph related to the effect of Perlodent toothpaste solutions on gingival epithelial cells (x: Annexin V FITC, y: PIPE). A: Perlodent 0.4%, B Perlodent 20%, C Perlodent 50%, D Perlodent 80%
detergent. However, considering the side effects of SLS, the different degree of keratinization and morphology of the gingival of children suggests that these side effects may occur more in children.

Therefore, in this study, the effects of 5 different children's toothpaste with different detergent content on cells were investigated. There are different evaluation methods to investigate the effects on cells, to determine the toxic effects of the materials to be used or to investigate their biocompatibility. These tests can be classified as clinical use tests, in vivo animal experiments and in vitro cell culture tests. Among these alternative methods, cell culture tests are frequently used in cytotoxicity studies due to their ability to mimic the physiological conditions of living tissues. In addition, cell culture studies have many advantages such as rapid application, repeatability, standardization, low cost, easy control of the experimental environment during the experiment and not being affected by different individual factors [35, 36]. Since there are some ethical and legal problems in other test methods, in vitro cell culture tests constitute the starting point of such studies in biocompatibility and cytotoxicity studies. In this study, in vitro cell culture tests were preferred to determine the effects of toothpastes on cells.

The cell type used in cell culture studies should be selected in relation to the area of use of the material...

**Fig. 7** Flow cytometry graph related to the effect of Colgate toothpaste solutions on gingival epithelial cells (x: Annexin V FITC, y: PIPE). A Colgate 0.4%, B Colgate 20%, C Colgate 50%, D Colgate 80%
## Table 2: Analysis results of the main effects of toothpaste brand and concentration factors and interaction effect on viable, early apoptotic, late apoptotic and necrotic cell rates of gingival epithelial cells

| Variable  | Source                        | Type III Sum of Squares | df  | Mean Square  | F     | p     | Partial η² |
|-----------|-------------------------------|-------------------------|-----|--------------|-------|-------|-----------|
|           | Viable                        |                         |     |              |       |       |           |
|           | Intercept                     |                         |     |              |       |       |           |
|           | Hypothesis                    | 546,751.350             | 1   | 546,751.350  | 46.157| 0.000*| 0.865    |
|           | Error                         | 85,443.269              | 7.213| 11,845.392   |       |       |           |
|           | Material                      |                         |     |              |       |       |           |
|           | Hypothesis                    | 35,711.772              | 5   | 7142.354     | 12.781| 0.000*| 0.810    |
|           | Error                         | 8382.503                | 15  | 558.834      |       |       |           |
|           | Concentration                 |                         |     |              |       |       |           |
|           | Hypothesis                    | 15,786.213              | 3   | 5262.071     | 9.416 | 0.001*| 0.653    |
|           | Error                         | 8382.503                | 15  | 558.834      |       |       |           |
|           | Material * Concentration      |                         |     |              |       |       |           |
|           | Hypothesis                    | 8382.503                | 15  | 558.834      | 135.463| 0.000*| 0.955    |
|           | Error                         | 396,033                 | 96  | 4.125        |       |       |           |
|           | Early Apoptotic               |                         |     |              |       |       |           |
|           | Intercept                     |                         |     |              |       |       |           |
|           | Hypothesis                    | 10,231.641              | 1   | 10,231.641   | 6.100 | 0.055 | 0.543    |
|           | Error                         | 8597.625                | 5.126| 1677.416     |       |       |           |
|           | Material                      |                         |     |              |       |       |           |
|           | Hypothesis                    | 4563.250                | 5   | 912.650      | 3.063 | 0.042*| 0.505    |
|           | Error                         | 4469.342                | 15  | 297.956b     |       |       |           |
|           | Concentration                 |                         |     |              |       |       |           |
|           | Hypothesis                    | 3188.166                | 3   | 1062.722     | 3.567 | 0.040*| 0.416    |
|           | Error                         | 4469.342                | 15  | 297.956b     |       |       |           |
|           | Material * Concentration      |                         |     |              |       |       |           |
|           | Hypothesis                    | 4469.342                | 15  | 297.956b     | 103.589| 0.000*| 0.942    |
|           | Error                         | 276,128                 | 96  | 2.876c       |       |       |           |
|           | Late Apoptotic                |                         |     |              |       |       |           |
|           | Intercept                     |                         |     |              |       |       |           |
|           | Hypothesis                    | 6488.581                | 1   | 6488.581     | 6.699 | 0.049*| 0.572    |
|           | Error                         | 4860.174                | 5.018| 968.643a     |       |       |           |
|           | Material                      |                         |     |              |       |       |           |
|           | Hypothesis                    | 2517.559                | 5   | 503.512      | 2.966 | 0.047*| 0.497    |
|           | Error                         | 2546.763                | 15  | 169.784b     |       |       |           |
|           | Concentration                 |                         |     |              |       |       |           |
|           | Hypothesis                    | 1904.747                | 3   | 634.916      | 3.740 | 0.035*| 0.428    |
|           | Error                         | 2546.763                | 15  | 169.784b     |       |       |           |
|           | Material * Concentration      |                         |     |              |       |       |           |
|           | Hypothesis                    | 2546.763                | 15  | 169.784b     | 65.969| 0.000*| 0.912    |
|           | Error                         | 247,074                 | 96  | 2.574c       |       |       |           |
|           | Necrotic                      |                         |     |              |       |       |           |
|           | Intercept                     |                         |     |              |       |       |           |
|           | Hypothesis                    | 30,236.478              | 1   | 30,236.478   | 18.022| 0.005*| 0.738    |
|           | Error                         | 10,730.351              | 6.396| 1677.749a    |       |       |           |
|           | Material                      |                         |     |              |       |       |           |
|           | Hypothesis                    | 7006.300                | 5   | 1401.260     | 14.286| 0.000*| 0.826    |
|           | Error                         | 1471.272                | 15  | 98.085b      |       |       |           |
|           | Concentration                 |                         |     |              |       |       |           |
|           | Hypothesis                    | 1123.721                | 3   | 374.574      | 3.819 | 0.032*| 0.433    |
|           | Error                         | 1471.272                | 15  | 98.085b      |       |       |           |
|           | Material * Concentration      |                         |     |              |       |       |           |
|           | Hypothesis                    | 1471.272                | 15  | 98.085       | 31.576| 0.000*| 0.831    |
|           | Error                         | 298,202                 | 96  | 3.106c       |       |       |           |
whose cytotoxic effects are investigated. Primary cell cultures or continuous cell lines are used in studies as a biological system in biocompatibility tests. It is stated that continuous cell lines such as L929, 3T3, HSC-2, MRC-5 can be used in the cytotoxicity assessment tests of materials used in dentistry, since they can be obtained more easily than primary cell cultures and have rapid reproduction potential. However, since primary cell cultures are more sensitive than continuous cell lines, they are biological systems that best reflect the original physiological state, despite the difficulties that arise during the production phase and the long time to produce [30, 35, 37–41]. For this reason, it was preferred to create a primary cell culture in this study, considering the creation of experimental conditions closer to in vivo conditions. Gingival epithelial cells were used as a biological system that best reflect the original physiological system in biocompatibility tests. It is stated that primary cell culture in this study, considering the creation of experimental conditions closer to in vivo conditions.

| Variable     | Brands                  | Concentration |
|--------------|-------------------------|---------------|
|              | 0.40%  | 20%   | 50%   | 80%   |
| Viable       | Colgate 6+           | 75.74 ± 3.18a | 47.56 ± 3.49a | 30.77 ± 4.26a | 13.04 ± 2.98a |
|              | Splat Juicy         | 90.14 ± 0.95d | 85.1 ± 1.77b  | 84.82 ± 1.6b  | 83.19 ± 1.88b  |
|              | Sensodyne Pronamel 6+| 84.66 ± 1.58c | 78.8 ± 1.16c  | 67.47 ± 1.68c | 57.63 ± 0.83c  |
|              | Nenedent Kids       | 80.23 ± 0.93b | 64.21 ± 0.91d | 55.17 ± 1.2d  | 39.15 ± 0.91d  |
|              | Perlodent Junior 6+  | 78.66 ± 1.84ab| 64.75 ± 0.91d | 45.21 ± 1.81e | 30.43 ± 4.05e  |
|              | CDMEM                | 90.82 ± 1.04d | 9.82 ± 1.04e  | 90.82 ± 1.04f | 90.82 ± 1.04f  |
|              |                       | 0.000*        | 0.000*         | 0.000*         | 0.000*         |
| Early apoptotic| Colgate 6+       | 3.38 ± 2.01   | 9.75 ± 3.55cd | 33.83 ± 2.81d | 28.11 ± 3.07b  |
|              | Splat Juicy         | 3.12 ± 1.48   | 2.52 ± 1.14a  | 2.29 ± 0.78a  | 1.97 ± 0.4a    |
|              | Sensodyne Pronamel 6+| 1.85 ± 0.53   | 4.52 ± 0.67ab | 12.09 ± 1.11c | 9.73 ± 0.57c   |
|              | Nenedent Kids       | 4.24 ± 0.98   | 10.55 ± 1.79d | 8.69 ± 1.36b  | 38.08 ± 1.27d  |
|              | Perlodent Junior 6+  | 2.83 ± 0.67   | 6.39 ± 1.14bc | 10.9 ± 0.99bc | 17.82 ± 3.44e  |
|              | CDMEM                | 2.24 ± 1.26   | 2.24 ± 1.26a  | 2.24 ± 1.26a  | 2.24 ± 1.26a   |
|              |                       | 0.290         | 0.000*         | 0.000*         | 0.000*         |
| Late apoptotic| Colgate 6+       | 5.73 ± 1.66c  | 11.23 ± 3.2c  | 10.29 ± 2.55b | 37.19 ± 2.68e  |
|              | Splat Juicy         | 1.93 ± 0.32ab | 3.51 ± 0.65a  | 3.87 ± 0.90a  | 3.46 ± 0.97ab  |
|              | Sensodyne Pronamel 6+| 0.70 ± 0.37a  | 1.5 ± 0.37a   | 5.25 ± 0.64a  | 11.96 ± 1.02c  |
|              | Nenedent Kids       | 0.89 ± 0.26a  | 7.65 ± 1b     | 12.31 ± 0.53b | 7.65 ± 0.6bc   |
|              | Perlodent Junior 6+  | 6.13 ± 1.01c  | 3.66 ± 0.64a  | 10.9 ± 1.19b  | 18.78 ± 4.84d  |
|              | CDMEM                | 2.97 ± 0.86b  | 2.97 ± 0.86a  | 2.97 ± 0.86a  | 2.97 ± 0.86a   |
|              |                       | 0.000*        | 0.000*         | 0.000*         | 0.000*         |
| Necrotic     | Colgate 6+           | 15.16 ± 2.4c  | 31.46 ± 4e    | 25.11 ± 2.91d | 21.67 ± 2.84c  |
|              | Splat Juicy         | 4.79 ± 0.54a  | 8.86 ± 0.87b  | 9.03 ± 1.03b  | 11.39 ± 1.53b  |
|              | Sensodyne Pronamel 6+| 12.79 ± 0.96bc| 15.18 ± 1.09c | 15.19 ± 1.28c | 20.69 ± 0.91c  |
|              | Nenedent Kids       | 14.65 ± 0.61bc| 17.6 ± 1.1c   | 23.83 ± 0.7d  | 15.15 ± 0.92b  |
|              | Perlodent Junior 6+  | 12.35 ± 0.68b | 25.21 ± 1.3d  | 31.99 ± 1.2e  | 32.97 ± 3.99d  |
|              | CDMEM                | 3.98 ± 1.09a  | 3.98 ± 1.09a  | 3.98 ± 1.09a  | 3.98 ± 1.09a   |
|              |                       | 0.000*        | 0.000*         | 0.000*         | 0.000*         |

Each F tests the simple effects of material within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means

a-b-c-d-e-f: There is no difference between the groups with the same

Univariate Test; Tukey post-hoc Test

*Significant p-value at 0.05 level
in this study, since the majority of oral tissues that toothpastes come into contact with during tooth brushing are gingival tissues.

In studies for the characterization of gingival epithelial cells, the method of determining epithelial cells specific CK13 and Vimentin genes by PCR, analysis of phenotypic properties of cells by transmission electron microscopy, determination of a specific epithelial marker cytokeratin by immunofluorescence method, staining of cells with Papanicoula staining method and analysis under light microscopy, methods were used [42]. In this study, the cells, which are easier and quicker to apply than other methods and are also more cost-effective, were stained with hematoxylin and eosin dyes after fixation with alcohol on the slide, and the cells were analyzed under light microscopy, and the presence of epithelial cells was determined.

Many in vitro tests such as MTT, trypan blue exclusion test, micronucleus are used to determine cell viability [27, 28, 43–48]. Flow cytometry analysis is frequently recommended in terms of providing more reliable, faster and more sensitive results than other methods in evaluating cell viability and cytotoxicity [29]. In addition to determining cell viability, information about different properties of cells such as immunophenotypic properties, enzyme activities, and specific markers of the cell can be obtained with this method [49, 50]. In addition, the separation of apoptotic and necrotic cells with this method is important in terms of different biological responses of these two types of death [49, 51]. In this study, since gingival cells are labeled with Annexin V and propidium iodide dyes, since they give faster, more sensitive and reliable results compared to alternative methods used in cell culture studies, it was ensured that live, early apoptotic, late apoptotic and necrotic cells were determined by flow cytometry analysis.

In the literature, changes caused by SLS, which is frequently used in toothpaste, on the oral mucosa have been reported. In addition, in a few studies examining the effects of SLS on cells, it has been stated that they have a negative effect on cell viability [27, 28, 52–57]. In this study, SLS, sodium lauryl sarcosinate, sodium C14-16 olefin sulfonate, CAPB containing toothpastes which are reported to be more biocompatible than SLS, toothpaste without detergent and CDMEM were selected as experimental groups. While determining the concentrations of
In this study, when the viability rates of different detergent-containing children's toothpaste solutions on human gingival epithelial cells were evaluated, it was seen that the lowest proportion of viable cells was in toothpaste solutions containing SLS. After the control group, the highest vitality values were detected in toothpaste without detergent content, followed by toothpaste containing CAPB. The effects of this study on cell viability Cvikl et al's findings in studies examining the effects of adult toothpastes and children's toothpaste on cells [27, 28]. Moore et al. also found that cell viability rates in SLS and betaine containing toothpastes were lower than the control group. These findings are also similar to the findings in our study.

In the literature, the effects of toothpastes on cells have been examined only in terms of living cell proportions [27, 28]. In this study, early apoptotic, late apoptotic and necrotic cell ratios were evaluated as well as the live cell ratios. In the comparisons between the groups, the Colgate group generally shows the highest value in terms of early apoptotic, late apoptotic and necrotic cell ratios, while Splat and the control group generally have similar values in terms of cell death type rates. Considering that SLS increases cellular permeability by causing denaturation of cellular proteins in this study, we think that the

| Variable | Concentration | p  |
|----------|----------------|----|
|          | 0.40%          | 20% | 50% | 80% |
| Viable   |                |     |     |     |
| Colgate 6+ | 75.74 ± 3.18A | 47.56 ± 3.49B | 30.77 ± 4.26C | 13.04 ± 2.98D | 0.00* |
| Splat Juicy | 90.14 ± 0.95A | 85.1 ± 1.77B | 84.82 ± 1.68B | 83.19 ± 1.88B | 0.00* |
| Sensodyne Pronamel 6+ | 84.66 ± 1.58A | 78.8 ± 1.16B | 67.47 ± 1.68C | 57.63 ± 0.83D | 0.00* |
| Nenedent Kids | 80.23 ± 0.93A | 64.21 ± 0.91B | 55.17 ± 1.2C | 39.71 ± 0.91D | 0.00* |
| Perlodent Junior 6+ | 78.66 ± 1.84A | 64.75 ± 0.91B | 45.21 ± 1.81C | 30.43 ± 4.05D | 0.00* |
| Early apoptotic |                |     |     |     |
| Colgate 6+ | 3.38 ± 2.01A | 9.75 ± 3.55B | 33.83 ± 2.81C | 28.11 ± 3.07D | 0.00* |
| Splat Juicy | 3.12 ± 1.48 | 2.52 ± 1.14 | 2.29 ± 0.78 | 1.97 ± 0.4 | 0.745 |
| Sensodyne Pronamel 6+ | 1.85 ± 0.53A | 4.52 ± 0.67B | 12.09 ± 1.11C | 9.73 ± 0.57C | 0.00* |
| Nenedent Kids | 4.24 ± 0.98A | 10.55 ± 1.79B | 8.69 ± 1.36B | 38.08 ± 1.27C | 0.00* |
| Perlodent Junior 6+ | 2.83 ± 0.67A | 6.39 ± 1.14B | 10.9 ± 0.99C | 17.82 ± 3.44D | 0.00* |
| Late apoptotic |                |     |     |     |
| Colgate 6+ | 5.73 ± 1.66A | 11.23 ± 3.2B | 10.29 ± 2.55AB | 37.19 ± 2.68C | 0.00* |
| Splat Juicy | 1.93 ± 0.32 | 3.51 ± 0.65 | 3.87 ± 0.96 | 3.46 ± 0.97 | 0.238 |
| Sensodyne Pronamel 6+ | 0.70 ± 0.37A | 1.5 ± 0.37A | 5.25 ± 0.64B | 11.96 ± 1.02C | 0.00* |
| Nenedent Kids | 0.89 ± 0.26A | 7.65 ± 1B | 12.31 ± 0.53C | 7.65 ± 0.6B | 0.00* |
| Perlodent Junior 6+ | 6.13 ± 1.01A | 3.66 ± 0.64A | 10.9 ± 1.19B | 18.78 ± 4.84C | 0.00* |
| Necrotic |                |     |     |     |
| Colgate 6+ | 15.16 ± 2.4A | 31.46 ± 4B | 25.11 ± 2.91C | 21.67 ± 2.84C | 0.00* |
| Splat Juicy | 4.79 ± 0.54A | 8.86 ± 0.87B | 9.03 ± 1.03B | 11.39 ± 1.53C | 0.00* |
| Sensodyne Pronamel 6+ | 12.79 ± 0.96A | 15.18 ± 1.09B | 15.19 ± 1.28B | 20.69 ± 0.91C | 0.00* |
| Nenedent Kids | 14.65 ± 0.61A | 17.6 ± 1.1B | 23.83 ± 0.7C | 15.15 ± 0.92A | 0.00* |
| Perlodent Junior 6+ | 12.35 ± 0.68A | 25.21 ± 1.3B | 31.99 ± 1.2C | 32.97 ± 3.99C | 0.00* |

Each F tests the simple effects of concentration within each level combination of the other effects shown. These tests are based on the linearly independent pairwise comparisons among the estimated marginal means

A-B-C-D: There is no difference between the groups with the same

Univariate Test, Tukey post-hoc Test

*Significant p value at 0.05 level
opening of the pores between cells may cause the release of apoptosis-inducing proteins into the cytosol and ultimately stimulate apoptosis/necrosis mechanisms. It has been reported that stimulation of apoptosis and necrosis mechanisms in gingival epithelial cells may prevent periodontal wound healing and prolong the healing period [61, 62]. In this study, it is thought that the increase in the ratio of apoptotic and necrotic cells of SLS-containing toothpaste may delay the healing time of periodontal diseases and oral aphthous ulcers and adversely affect wound healing.

This study has some limitations due to the absence of saliva, the protective and immunological properties of tissue barriers. In addition, this study suggests that other ingredients in toothpaste may also have toxic effects, since detergent ingredients cannot be supplied in pure form. However, in order to eliminate this limitation, toothpastes used in similar age groups and having similar contents formed the study groups in our study.

Abbreviations
GEC: Gingival epithelial cells; SLS: Sodium lauryl sulfate; CDMEM: Complete dulbecco’s modified eagles medium; CABP: Cocamidopropyl betaine; DPBS: Dulbecco's phosphate buffered saline.

Acknowledgements
The authors would like to extend their sincere thanks to anyone who contributed to this study.

Authors’ contributions
FS and SB designed the study. SB generated the data. YD and TA analysed the data. SB wrote the paper. All authors reviewed the manuscript.

Funding
This study was supported by the Research Fund of Istanbul University, Project No: 25696.

Availability of data and materials
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.
Declarations

Ethics approval and consent to participate
The study was approved by the ethics committee of Istanbul University, Faculty of Dentistry (1/70/2017) following Helsinki Declaration guidelines. Informed consent was obtained from all subjects and/or their legal guardian(s).

Consent for publication
NA.

Competing interests
The authors do not have any conflict of interest.

Author details
1 Department of Pediatric Dentistry, Faculty of Dentistry, Istanbul University-Cerrahpaşa, Istanbul, Turkey. 2 Department of Pediatric Allergy-Immunology, Faculty of Medicine, Marmara University, Istanbul, Turkey. 3 Department of Pediatric Dentistry, Faculty of Dentistry, Altinbas University, Istanbul, Turkey.

Received: 8 September 2021   Accepted: 22 February 2022

Published online: 09 March 2022

References
1. Rosan B, Lamont RJ. Dental plaque formation. Microb Infect. 2000;2:1599–607.
2. Takeshita T, Yasa M, Shibata Y, Furuta M, Saeli Y, Eshima N, et al. Dental plaque development on a hydroxyapatite disk in young adults observed by using a barcoded pyrosequencing approach. Sci Rep. 2015;5:1–9.
3. Walsukul A. Fluoride compounds in dental caries prophylaxis in children and adolescents—review of polish literature. Przegl Epidemiol. 2017;71:603–11.
4. Claydon NC. Current concepts in toothbrushing and interdental cleaning. Periodontol. 2000;2008(48):10–22.
5. Trubey RJ, Moore SC, Chestnut IG. Parents reasons for brushing or not brushing their child's teeth: a qualitative study. Int J Paediatr Dent. 2014;24(2):104–12.
6. Deery C, Heanue M, Deacon S, Robinson PG, Walmsley AD, Worthington J, et al. The effectiveness of manual versus powered toothbrushes for children and adolescents—review of polish literature. Przegl Epidemiol. 2017;71:603–11.
7. Wilder RS, Bray KS. Improving periodontal outcomes: merging clinical and behavioral science. Periodontol. 2000;2016(71):65–81.
8. Welbury R, Duggal M, Hosey TM. Paediatric dentistry. 3rd ed. Oxford: Oxford University Press, 2005.
9. Ozkok CB, Kaaaslan SE, Aytaç F. Saliva proteins and their effects on caries. Turkey Dental Clinics J. 2017;23:56–64.
10. Chambers HF. Miscellaneous antimicrobial agents, disinfectants, antiseptic, and bacteriostatic. In: Katzung BG, editor. Basic & clinical pharmacology. 10th ed. Connecticut: Appleton & Lange; 2007. p. 803–11.
11. Ozyurt M. Aldehydes, peroxide and peracetic acid and other disinfectants that do not contain chlorine donating agents and are recommended as instrument disinfectants, their general use and antimicrobial effectiveness. In: Gunaydin M, Sanic A, Gurler B, editors. 4th national sterilization disinfection congress, congress book. Scientific Medicine Publishing House: Ankara, 2005. p. 180–99.
12. Ananthapadmanabhan KP, Moore DJ, Subramanyan K, Misra M, Meyer F. Cleansing without compromising the risk of cleansers on the skin barrier and the technology of mild cleansing. Dermatol Ther. 2004;17:16–25.
13. Walters KA, Blalik W, Brain KR. The effects of surfactants on penetration across the skin. Int J Cosmet Sci. 1993;15:260–70.
14. Shah SK, Niraula TP, Bhattachar A, Chatterjee SK. A comparative study of cationic and anionic surfactants on the micellar behavior through different composition of methanol-water mixed solvent. Conductometric Method Bibechean. 2012;8:387–45.
15. Forwood JC, James AH, Barnett P, Jackson RJ. Gum health product formulations: what is in them and why? Periodontol. 2000,15:32–9.
16. Petersen FC, Assev S, Scheie AA. Combined effects of NaF and SLS on acid and polyacrylamide formation of biofilm and planktonic cells. Arch Oral Biol. 2006;51:665–71.
17. Burow R, Maeda T, Karney M, Kourai H. Pathogenic bacteria carried by companion animals and their susceptibility to antibacterial agents. Biocontrol Sci. 2006;11:1–9.
18. Law V, Sewo WK. A longitudinal controlled study of factors associated with mutans streptococci infection and caries lesion initiation in children 21 to 72 months old. Pediatr Dent. 2006;28:58–55.
19. Nordstrom A, Myrtikos C, Ramberg P, Birkehed D. Effect on denovo plaque formation of rinsing with toothpaste slurries and water solutions with a high fluoride concentration (5,000 ppm). Eur J Oral Sci. 2009;117:563–7.
20. Moran J, Addy M, Newcombe R. The antibacterial effect of toothpastes on the salivary flora. J Clin Periodontol. 1988;15:193–9.
21. Moran J, Addy M, Newcombe R. Comparison of the effect of toothpastes containing enzymes or antimicrobial compounds with a conventional fluoride toothpaste on the development of plaque and gingivitis. J Clin Periodontol. 1989;16:295–9.
22. Evans A, Leishman SJ, Walsh LJ. Inhibitory effects of children's toothpastes on Streptococcus mutans, Streptococcus sanguinis and lactobacillus acidophilus. Eur Arch Paediatr Dent. 2015;16:219–26.
23. Macdonald JM, Tobin CA, Burkemper NM, Hurley MY. Oral Leukoedema with mucosal desquamation caused by toothpaste containing sodium lauryl sulfate. Case Let. 2015;97:4–5.
24. Neppelberg E, Costea DE, Vintermyr OK, Johannaessen AC. Dual effects of sodium lauryl sulphate on human oral epithelial structure. Experiment Dermatol. 2007;16:574–9.
25. Siegel IA, Gordon HP. Surfactant-induced alterations of permeability of rabbit oral mucosa in vitro. Exp Mol Pathol. 1986;44:132–7.
26. Ahlfors EE, Lyberg T. Contact sensitivity reactions in the oral mucosa. Acta Odontol Scand. 2001;59:248–54.
27. Cilić B, Lussi A, Moritz A, Gruber R. The in vitro impact of toothpaste extracts on cell viability. Eur Oral Sci. 2015;13:179–85.
28. Cilić B, Lussi A, Moritz A, Gruber R. Dentifrices for children differentially affect cell viability in vitro. Clin Oral Investig. 2017;21:453–61.
29. Zhou H, Shen Y, Wang Z, Li L, Zheng Y, Hakkinen L, et al. In vitro cytotoxicity evaluation of a novel root repair material. J Endod. 2013;39:78–83.
30. Russo FB, Pignatari GC, Fernandes IR, Dias JLRM, Beltrão-Braga PCB. Epithelial cells from oral mucosa: how to cultivate them? Cytotechnol. 2016;68:2105–14.
31. Birant S, Duran Y, Gokalp M, Akkoc T, Seymen E. Effects of diffferent-containing children's toothpastes on the viability, osteogenic and chondrogenic differentiation of human dental periodontal ligament stem cells and gingival stem cells in vitro. Tissue Cell. 2017;21:1–2.
32. Lawrence LM, Farquharson A, Brown RS, Vatanka HO. Oral tissue irritants in toothpaste: a case report. J Clin Pediatr Dent. 2013;37:75–8.
33. Herlofson BB, Barkvoll P. Oral mucosal desquamation caused by two toothpaste detergents in an experimental model. Eur J Oral Sci. 1996;104:21–6.
34. Skaare AB, ROLL G, Barkvoll P. The influence of tricosan, zinc or propylene glycol on oral mucosa exposed to sodium lauryl sulphate. Eur J Oral Sci. 1997;105:277–33.
35. Craig RG, Powers JM, Sakauchi RL. Craig's restorative dental materials. 12th ed. St. Louis: Mosby, 2006.
36. Schmalz G. Use of cell cultures for toxicity testing of dental materials—advantages and limitations. J Dent. 1996;22:6–11.
37. Arendt-Hildeslev D, Bleeg H. Characterization of two types of human oral fibroblast with a potential application to cellular toxicity studies; tooth pulp fibroblasts and buccal mucosa fibroblasts. Int Endod J. 1990;23:84–91.
38. Illeperuma RP, Park YJ, Kim JM, Bae JY, Che ZM, Son HK, et al. Immortalized gingival fibroblasts as a cytotoxicity test model for dental materials. J Mater Sci Mater Med. 2012;23:573–62.
39. International Organization for Standardization. Dentistry-Biological evaluation of medical devices. Tests for in vitro cytotoxicity. ISO 10993-5. 2009. https://www.iso.org/standard/36406.html. Accessed 13 Feb 2019.
40. Schmalz G. Concepts in biocompatibility testing of dental restorative materials. Clin Oral Investig. 1997;1:154–62.
41. Tuncer S, Demirci M. Biocompatibility evaluations of dental materials. J Ataturk Univ Dentist. 2011;21:141–9.
42. Ghapanchi J, Kamali F, Moattari A, Pooshahidi S, Shahin E, Rezaeadeh F, et al. In vitro comparison of cytotoxic and antibacterial effects of 16 commercial tooth pastes. J Int Oral Health. 2015;7:39–43.
43. Fiori J, Teti G, Gotti R, Mazzotti G, Falconi M. Cytotoxic activity of guaiazulene on gingival fibroblasts and influence of light exposure on guaiazulene-induced cell death. Toxicol In Vitro. 2011;25:64–72.
44. Eyuboglu GB, Yesilyurt C, Erturk M. Evaluation of cytotoxicity of dentin desensitizing products. Oper Dentist. 2015;40:503–14.
45. Kalil Bussadori S, Marcilio Santos E, Cardoso Guedes C, Jansiski Motta L, Santos Fernandes RP, Mesquita-Ferrari RA, et al. Cytotoxicity assessment of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste. ConScient Saude. 2010;9:354–9.
46. Fernandes JPS, Mello Moura ACV, Marques MM, Nicoletti MA. Cytotoxicity evaluation of Curcuma zedoaria Roscoe fluid extract used in oral hygiene products. Acta Odontol Scand. 2012;70:610–4.
47. Olgun Erdemir E, Sengun A, Ulker M. Cytotoxicity of mouthrinses on epithelial cells by micronucleus test. Eur J Dent. 2007;1:80–5.
48. Kafev MO, Gokalp Muranlı FD. Flow cytometry and usage areas. J SAÜ Fen Bil. 2016;20:33–8.
49. Coskun G, Ozgur H. Molecular mechanism of apoptosis and necrosis. Arch Med Rev J. 2011;20:145–58.
50. Salzer S, Rosema NAM, Maertin ECJ, Slot DE, Timmer CJ, Dorfer CE, et al. The effectiveness of dentifrices without and with sodium lauryl sulfate on plaque, gingivosis and gingival abrasion—a randomized clinical trial. Clin Oral Invent. 2016;20:443–50.
51. Healy CM, Paterson M, Joyston-Bechal S, Williams DM, Thomhill MH. The effect of a sodium lauryl sulfate-free dentifrice on patients with recurrent oral ulceration. Oral Dis. 1999;5:39–43.
52. Barkvoll P, Rolla G, Svendsen AK. Interaction between chlorhexidine digluconate and sodium lauryl sulfate in vivo. J Clin Periodontol 1989;16:593–5.
53. Allen AL, Hawley CE, Cutright DE, Seibert JS. An investigation of the clinical and histologic effects of selected dentifrices on human palatal mucosa. J Periodontol. 1975;46:102–12.
54. Shihi Y, Choi JH, Ahn HJ, Kwon JS. Effect of sodium lauryl sulfate on recurrent aphthous stomatitis: a randomized controlled clinical trial. Oral Dis. 2012;18:655–60.
55. Moore C, Addy M, Moran J. Toothpaste detergents: a potential source of oral soft tissue damage? Int J Dent Hyg. 2008;6:193–8.
56. Herlufson BB, Brodin P, Aars H. Increased human gingival blood flow induced by sodium lauryl sulfate. J Clin Periodontal. 1996;23:1004–7.
57. Melsen B, Rolla G. Reduced Clinical effect of monofluorophosphate in the presence of sodium lauryl sulphate. Caries Res. 1983;17:549–53.
58. Rantanen I, Jutila K, Nicander I, Tenovuo J, Soderling E. The effects of two sodium lauryl sulphate-containing tooth pastes with and without betaine on human oral mucosa in vivo. Swed Dent J. 2003;27:31–4.
59. Semlali A, Chakir J, Goulet JP, Chmielowski W, Rouabhia M. Whole cigarette smoke promotes human gingival epithelial cell apoptosis and inhibits cell repair processes. J Periodont Res. 2011;46:533–41.
60. Rouabhia M. Interactions between host and oral commensal microorganisms are key events in health and disease status. Can J Infect Dis. 2002;13:47–51.
61. Weindl G, Wagener J, Schaller M. Epithelial cells and innate antifungal defense. J Dent Res. 2010;89:666–75.
62. Bahri R, Saidane-Mostahbi D, Rouabhia M. Candida famata modulates toll-like receptor, beta-defensin, and proinflammatory cytokine expression by normal human epithelial cells. J Cell Physiol. 2010;222:209–18.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:
- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.
Learn more biomedcentral.com/submissions