The in vitro impact of toothpaste extracts on cell viability


Toothpastes contain three main components: detergents, abrasives, and fluoride. Detergents, particularly sodium lauryl sulfate, have been proposed as components that enable toothpastes to produce cytotoxic effects in vitro. However, not all toothpastes contain sodium lauryl sulfate, and almost no studies have found an association between detergents and the in vitro cytotoxicity of toothpastes. The present study examined the in vitro cytotoxicity of nine commercially available toothpastes containing four different detergents. Toothpastes were diluted in serum-free medium, centrifuged, and filter sterilized. The half-lethal concentration of the toothpaste-conditioned medium (TCM) was calculated based on the formation of formazan by gingival fibroblasts, oral squamous cell carcinoma HSC-2 cells, and L929 cells. Cell proliferation was analyzed, and live-dead staining was performed, after exposure of cells to conditioned medium prepared with 1% toothpaste (1% TCM). It was found that toothpastes containing sodium lauryl sulfate and amine fluoride strongly inhibited cell viability with the half-lethal concentration being obtained with conditioned medium prepared with approximately 1% toothpaste (1% TCM). Toothpastes containing cocamidopropyl betaine and Steareth-20 showed higher half-lethal concentration values, with the half-lethal concentration being obtained with conditioned medium prepared with 10% (10% TCM) and 70% (70% TCM) toothpaste, respectively. Proliferation and live-dead data were consistent with the cell-viability analyses. These results demonstrate that the type of detergent in toothpastes can be associated with changes in in vitro cell toxicity.

Toothpaste, also called a dentifrice, together with a toothbrush is routinely used to maintain oral hygiene. The two basic functions are removal of plaque from the teeth and prevention of caries. Removal of plaque is mainly achieved through the use of abrasive insoluble particles, such as silica, aluminum hydroxide, and calcium carbonate, which are dispersed in the soluble fraction of the toothpaste. Caries prevention is supported by the addition of fluoride and other molecules. Further ingredients for additional functions, such as anti-plaque, anti-gingivitis, anti-malodor, anti-tartar, whitening, and erosion prevention, can be added (1). More basic ingredients include sugar alcohols to bind water, antibacterial components, remineralizers, colors, and flavors. Finally, detergents are added, supporting foaming and even distribution of the toothpaste, but with concerns related to safety issues.

Sodium lauryl sulfate (SLS) is a detergent that can change the barrier properties of human oral mucosa in vitro (2) and in vivo (3), and increases gingival blood flow (4). Sodium lauryl sulfate-containing toothpastes are associated with a more frequent occurrence of aphthous ulcers (5), and SLS-free toothpastes can decrease the duration of ulcers and reduce pain (6). In vitro, SLS decreases the viability of oral mucosa cells, such as TERT-1 keratinocytes (7), human oral epithelial cells, and fibroblasts (8). Thus, SLS may be a critical factor in determining the overall in vitro toxicity of toothpastes. However, it has yet to be shown if toothpastes containing SLS reduce cell viability in vitro.

Cocamidopropyl betaine (CAPB) is another detergent that is commonly present in toothpaste. Cocamidopropyl betaine-containing toothpastes are considered less irritating and relieve symptoms of dry mouth (9, 10). Cocamidopropyl betaine has been increasingly used in cosmetics and personal hygiene products (11), but safety concerns have been raised by the Cosmetic Ingredient Review Expert Panel (12). For example, patients with atopic dermatitis and lupus erythematosus have contact hypersensitivity to CAPB (13, 14). Also, the in vitro data are conflicting. Cocamidopropyl betaine supported survival of human keratinocytes exposed to SLS (15), whereas others found CAPB to be even more toxic than SLS (16). In TERT-1 keratinocytes, CAPB and SLS had the same toxicity (7). Toothpastes containing the amine fluoride (AF) Olaflur [N’-octadecyltrimethylendiamine-N,N,N’,tris(2-ethanol)-dihydrofluoride], which acts as a detergent, were also examined regarding their impact on cells (17, 18). Data on other detergents and their associated properties in oral health are critical for understanding the role of detergents in toothpaste formulations.
detergents in toothpastes, such as polyoxyethylene 20 stearyl ether (Steareth-20) are lacking.

To the best of our knowledge, in vitro studies comparing toothpastes with different detergents, such as SLS, amine fluoride, CAPB, and Steareth-20, have not been performed. Thus, the relationship of detergents in toothpaste to cell toxicity in vitro is not yet understood. The present study investigated the impact of the soluble compounds of commercially available toothpastes on the viability of oral fibroblasts and epithelial cells using in vitro cell-culture systems.

Material and methods

Cell culture

Gingival fibroblasts and epithelial cells, which may come into contact with toothpaste, and the L929 cell line, which is frequently used for cytotoxicity testing, were used for viability testing. Further testing, based on these data, was performed on human gingival fibroblasts prepared from tissue grafts of three independent donors after wisdom tooth extraction (Kantonale Ethikkommission, Bern, Switzerland). The oral squamous cell carcinoma cell line, HSC-2, was kindly provided by Dr Rausch-Fan from the Medical University of Vienna (Vienna, Austria). Murine L929 fibrosarcoma cells were kindly provided by Dr Erik Hedbom, School of Medical Dentistry, University of Bern (Bern, Switzerland). Cells were cultured in a humidified atmosphere at 37°C in growth medium consisting of Dulbecco's modified Eagle's minimum essential medium (DMEM; Invitrogen), 10% fetal bovine serum (FBS; Invitrogen), and antibiotics (Invitrogen). For cell viability testing and proliferation assays, cells were seeded onto microtiter plates (Greiner Bio-One, Frickenhausen, Germany). For live-dead cell staining, cells were washed with PBS and incubated with a cell-permeable green fluorescent dye to stain live cells. Dead cells were stained with propidium iodide, a red fluorescent dye, which in viable cells is actively pumped out of the cytoplasm. Stained cells were visualized by fluorescence microscopy.

Toothpaste conditioned medium

The following toothpastes were purchased: Colgate Total (Colgate-Palmolive, New York, NY, USA), Crest Cavity Protection (Procter & Gamble, Cincinnati, OH, USA), Curaprox Enzycal (Curaden International, Kriens, Switzerland), Elgydium Anti-plaque (Pierre Fabre Oral Care, Allschwil, Switzerland), Elmex Kariesschutz (GABA International, Colgate-Palmolive), Emoform actiflur Protect (Dentaid, Switzerland), Elgydium Anti-plaque (Pierre Fabre Oral Care, Allschwil, Switzerland), Elmex Kariesschutz (GABA International, Colgate-Palmolive), Emoform actiflur Protect (Dentaid, Switzerland). Cells were cultured in a humidified atmosphere at 37°C in growth medium consisting of Dulbecco's modified Eagle's minimum essential medium (DMEM; Invitrogen, Carlsbad, CA, USA), 10% fetal bovine serum (FBS; Invitrogen), and antibiotics (Invitrogen). For cell viability testing and proliferation assays, cells were seeded onto microtiter plates (Greiner Bio-One, Frickenhausen, Germany). For live-dead cell staining, cells were seeded onto chamber slides (Thermo Scientific Nunc, Waltham, MA, USA). Cells were seeded in growth medium at a density of 30,000 cells cm⁻² and were stimulated the next day.

MTT assay and calculation of LC50

Gingival fibroblasts, oral squamous carcinoma cells (HSC-2), and murine fibrosarcoma cells (L929) were exposed for 2 min to TCM containing various concentrations of toothpaste, washed with PBS, and suspended in serum-free medium. Then, 0.5 mg ml⁻¹ of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma-Aldrich St Louis, MO, USA] was added to each well and incubated for 2 h at 37°C. Cell viability was measured by the capacity of the cells, via NAD(P)H-dependent oxidoreductases, to convert MTT into formazan crystals that dissolve in dimethyl sulfoxide. Viability was shown by a more intense color reaction. The optical density of each well was measured using a microplate reader (EL 808; Biotek Instruments, Winooski, VT, USA) and was normalized to that of wells containing untreated cells. The LC50 was calculated by exponential regression analysis using the formula $y = m \times e^{-e}$, where $y$ is the TCM concentration, $m$ is the slope of the regression line, $e$ is exponential, $b$ is the intersection with the x-axis, and $x$ at 50 is LC50.

5-Bromo-2'-deoxyuridine incorporation assay

Gingival fibroblasts were incubated with 1% TCM for 2 min and washed with PBS. Cells were then incubated with serum-free medium before addition of 5-bromo-2'-deoxyuridine (BrdU) to the cells for 2 h. DNA synthesis was determined using the Cell Proliferation ELISA, BrdU (colorimetric) kit from Roche (Basel, Switzerland). Incorporation of BrdU was determined following the procedure of the manufacturer and was normalized to that of untreated cells.

Live-dead cell staining

Gingival fibroblasts were incubated with 1% TCM for 2 min. Following the instructions of the manufacturer of the Live-Dead cell staining kit (Enzo Life Sciences, Lausen, Switzerland), cells were washed with PBS and incubated with a cell-permeable green fluorescent dye to stain live cells. Dead cells were stained with propidium iodide, a red fluorescent dye, which in viable cells is actively pumped out of the cytoplasm. Stained cells were visualized by fluorescence microscopy.

Statistical analysis

The LC50 data were reported as median, 25th–75th percentile, and minimum–maximum of four independent experiments, each performed in duplicate. Differences between the LC50 values obtained for cells treated with the TCM prepared from each toothpaste were tested using a non-parametric Kruskal-Wallis test followed by a post-hoc Mann-Whitney U-test with Bonferroni correction for multiple comparisons (ssrs version 19.0; SPSS, Chicago, IL, USA). The global Alpha-error was set to 5%. Data obtained using the MTT assay and the BrdU incorporation assay are given as mean ± SD.

Results

Toothpastes containing SLS and AF reduced formazan formation

The viability of gingival fibroblasts stimulated for 2 min with TCM prepared with different concentrations of...
toothpaste, measured by the capacity of the cells to convert MTT into formazan crystals, is shown in Fig. 1. Based on these data, we calculated that in all toothpastes containing SLS [i.e. Colgate Total (CLT), Crest Cavity Protection (CRT), Elgydium Anti-plaque (EYA), Odol-med3 Original (OMO), and Oral-B Pro-expert (ORB)] the LC50 was below 5% for gingival fibroblasts, HSC2, and L929 cells. Also, the toothpaste containing AF [Elgydium Anti-plaque (EYA)] exhibited an LC50 of <5% for all types of cells investigated (Table 2).

In parallel, we investigated the potential cytotoxic effects of toothpastes containing CAPB [Emoform actiflur Protect (EMF) and Sensodyne Fluoride (SDF)] and Steareth-20 [Curaprox EnzyCal (CPE)] (Fig. 1). The LC50 values for EMF and SDF were <8% and 24%, respectively, in all cell types (Table 2). With the Steareth-20-containing toothpaste, the LC50 was 75% for gingival fibroblasts. Interestingly, the median LC50 for HSC-2 and L929 remained approximately 100% (Table 2). Together these observations suggest that toothpastes containing SLS and AF, but not CAPB and Steareth-20, substantially reduce the viability of oral fibroblasts (Fig. 2) and epithelial cells in vitro (Table 2).

### Cell proliferation decreased with toothpastes containing SLS

The toxic effect of toothpastes containing SLS almost completely prevented incorporation of BrdU into DNA. Interestingly, toothpaste containing AF did not produce results consistent with the MTT assay because the amount of BrdU incorporated into the DNA of fibroblasts in the presence of such toothpaste was almost the same as in the untreated control. Gingival fibroblasts exposed to 1% TCM from toothpastes containing SLS almost completely prevented incorporation of BrdU into DNA. Interestingly, toothpaste containing AF did not produce results consistent with the MTT assay because the amount of BrdU incorporated into the DNA of fibroblasts in the presence of such toothpaste was almost the same as in the untreated control. Gingival fibroblasts exposed to 1% TCM from toothpastes containing SLS and AF, but not CAPB and Steareth-20, substantially reduce the viability of oral fibroblasts (Fig. 2) and epithelial cells in vitro (Table 2).

### Table 1

<table>
<thead>
<tr>
<th>Toothpaste (Manufacturer)</th>
<th>Detergents</th>
<th>Composition</th>
<th>Expiry date</th>
<th>Country of purchase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colgate Total (CLT)</td>
<td>Sodium lauryl sulfate</td>
<td>Aqua, aroma, carrageenan, cellulose gum, titanium dioxide, glycerin, hydrated silica, propylene glycol, sodium saccharin, sodium fluoride (1,400 p.p.m.), sodium hydroxide, sorbitol, triclosan, maleic anhydride copolymer</td>
<td>11-2015</td>
<td>Austria</td>
</tr>
<tr>
<td>Crest Cavity Protection (CRT)</td>
<td>Sodium lauryl sulfate</td>
<td>Aqua, aroma, cellulose gum, Brilliant Blue FCF/Bluel, titanium dioxide, hydrated silica, polycrylic acid, sodium saccharin, sodium fluoride (1,100 p.p.m.), sodium phosphate, sorbitol, trisodium phosphate</td>
<td>08-2015</td>
<td>USA</td>
</tr>
<tr>
<td>Curaprox EnzyCal (CPE)</td>
<td>Steareth-20</td>
<td>Amyloglucosidase, aque, aroma, carrageenan, citric acid, titanium dioxide, glucose oxidase, glycerin, hydrated silica, lactoperoxidase, lactose, mentha piperita oil, potassium thioxyanate, sodium benzoate, sodium saccharin, sodium chloride, sodium fluoride (950 p.p.m.), sodium hydrogen phosphate, sorbitol</td>
<td>12-2016</td>
<td>Switzerland</td>
</tr>
<tr>
<td>Elmex Kariesschutz (EMF)</td>
<td>Sodium lauryl sulfate</td>
<td>Aqua, aroma, benzyl alcohol, cellulose gum, chlorhexidine digluconate, Patent Blue V, quinoline yellow, hydrated silica, limonene, nico methanol hydrofluoride (1,250 p.p.m.), sodium saccharin, sodium methylparaben, sorbitol</td>
<td>07-2015</td>
<td>Switzerland</td>
</tr>
<tr>
<td>GABA (Schweiz)</td>
<td>Amine fluoride (1,400 p.p.m.)</td>
<td>Aqua, aroma, titanium dioxide, hydrated silica, hydrocholic acid, hydroxyethylcellulose, limonene, sodium saccharin, sorbitol/glycerin</td>
<td>12-2015</td>
<td>Austria</td>
</tr>
<tr>
<td>Emoform actiflur Protect (Dr Wild &amp; Co., Muttenz, Switzerland)</td>
<td>Cocamidopropyl betaine</td>
<td>Aqua, aroma, cellulose gum, titanium dioxide, glycerin, limonene, polyethylene glycol-8, hydrogenated castor oil, rebaudioside A, silica, sodium fluoride (1,400 p.p.m.), xylitol</td>
<td>08-2015</td>
<td>Switzerland</td>
</tr>
<tr>
<td>Odol-med3 Original (GlaxoSmithKline, Brentford, UK)</td>
<td>Sodium lauryl sulfate</td>
<td>Aqua, aroma, carrageenan, thiouindigo colors, copper phthalocyanine, titanium dioxide, glycerin, hydrated silica, limonene, polyethylene glycol, sodium saccharin, sodium fluoride and stannous fluoride (1,450 p.p.m.), sorbitol, xanthan gum</td>
<td>12-2015</td>
<td>Austria</td>
</tr>
<tr>
<td>Oral-B Pro-expert (GlaxoSmithKline)</td>
<td>Sodium lauryl sulfate</td>
<td>Aqua, aroma, chondrus crispus, copper phthalocyanine green, titanium dioxide, glycerin, hydrated silica, limonene, polyethylene glycol, propylene glycol, silica, sodium saccharin, sodium fluoride (350 p.p.m.), sodium gluconate, sodium hexametaphosphate, stannous fluoride (1,100 p.p.m.), trisodium phosphate, xanthan gum, zinc lactate</td>
<td>12-2014</td>
<td>Austria</td>
</tr>
<tr>
<td>Sensodyne Fluoride (GlaxoSmithKline)</td>
<td>Cocamidopropyl betaine</td>
<td>Aqua, aroma, titanium dioxide, glycerin, hydrated silica, limonene, potassium nitrate, sodium saccharin, sodium fluoride (1,450 p.p.m.), sorbitol, sucralose, xanthan gum</td>
<td>08-2015</td>
<td>USA</td>
</tr>
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Live–dead cell staining of cells treated with toothpastes

We next examined whether the incubation of gingival fibroblasts with 1% TCM changed the ratio of green live cells to red dead cells. Consistent with the viability assays, toothpastes containing SLS and AF caused cells to take up propidium iodide, and thus to appear red. Only a few gingival fibroblasts stained green (a sign of live cells). In contrast, gingival fibroblasts incubated with 1% TCM from toothpastes containing CAPB and Steareth-20 showed mainly green live cells (Fig. 4).

Discussion

The results described herein demonstrate that TCM containing SLS and AF is significantly more cytotoxic...
to fibroblasts and epithelial cells than is TCM containing CAPB and Steareth-20. This evidence is supported by data on formazan formation and live–dead cell staining. Collectively, the findings of this report establish that detergents, key components of toothpaste, are associated with disruption of cell membranes in vitro. Our data are consistent with other in vitro observations that incubation with SLS for 2 min reduces viability of TERT-1 keratinocytes in vitro (7). Our concept, however, to prepare a TCM and use this preparation in vitro, is new. The present study was based on the assumption that toothpastes have a complex composition and that single components cannot necessarily reproduce the effect of toothpaste on cells. We can thus not directly relate our findings to those of others. In addition, our findings are contradictory to those

![Fig. 2. Determination of the half-lethal concentration (LC50) values of gingival fibroblast viability upon exposure to toothpaste-conditioned medium (TCM). Gingival fibroblasts were exposed for 2 min to various concentrations of toothpaste in TCM, cell viability was measured using the MTT assay, and LC50 values were calculated. Global differences in the median LC50 values of each toothpaste were revealed after analysis using the Kruskal-Wallis test ($P < 0.001$). In post-hoc analysis, the level of significance was adjusted to 0.006, after Bonferroni correction. This showed significant differences between the median LC50 values of the following groups, as indicated by different letters. (a) Emoform actiflur Protect (EMF) vs. Curaprox Enzycal (CPE) ($P < 0.002$); EMF vs. Elmex Kariesschutz (EMX), Crest Cavity Protection (CRT), Elgydium Anti-plaque (EYA), and Odol-med3 Original (OMO) (all $P < 0.002$); EMF vs. Colgate Total (CLT) ($P < 0.002$); and Sensodyne Fluoride (SDF) vs. CPE, EMX, CLT, CRT, EYA, OMO, and Oral-B Pro-expert (ORB) (all $P < 0.001$). (b) CPE vs. EMX, CLT, CRT, EYA, OMO, and ORB (all $P < 0.001$). (c) EMX, CRT, EYA, and OMO vs. CLT ($P < 0.001$). (d) CLT vs. ORB ($P < 0.001$). (e) ORB vs. SDF, CPE, and CLT (all $P < 0.001$).](image1)

![Fig. 3. Proliferation of gingival fibroblasts. Following exposure to conditioned medium prepared with 1% toothpaste (1% TCM), the proliferation of gingival fibroblasts was expressed as 5-bromo-2’-deoxyuridine (BrdU) incorporation during DNA synthesis. CLT, Colgate Total; CPE, Curaprox Enzycal; CRT, Crest Cavity Protection; EMF, Emoform actiflur Protect; EMX, Elmex Kariesschutz; EYA, Elgydium Anti-plaque; OMO, Odol-med3 Original; ORB, Oral-B Pro-expert; SDF, Sensodyne Fluoride.](image2)

![Fig. 4. Live–dead cell staining of gingival fibroblasts. Gingival fibroblasts were exposed to conditioned medium prepared with 1% toothpaste (1% TCM) for 2 min, then stained to determine the proportions of live and dead cells. Viable cells stained green and dead cells stained red. Consistent with the results of the viability assays, following exposure to toothpaste containing sodium lauryl sulfate (SLS) or amine fluoride (AF) mainly red-stained cells were observed, whereas following exposure to toothpastes containing cocamidopropyl betaine (CAPB) and polyoxyethylene 20 stearyl ether (Steareth-20), mainly green-stained cells were observed. CLT, Colgate Total; CPE, Curaprox Enzycal; CRT, Crest Cavity Protection; EMF, Emoform actiflur Protect; EMX, Elmex Kariesschutz; EYA, Elgydium Anti-plaque; OMO, Odol-med3 Original; ORB, Oral-B Pro-expert; SDF, Sensodyne Fluoride.](image3)
showing that CAPB alone has a similar cytotoxic effect compared with SLS in vitro (7, 8). However, others have shown that CAPB even protected cells from the adverse response to SLS in vitro. Thus, the overall situation remains unclear. As our approach is based on extracts in the form of TCM – and not on the detergents alone – and we have included a large spectrum of methods to assess the effects of TCM, we have great support for the conclusion that TCM containing SLS and AF is more harmful to cells in vitro than is TCM containing CAPB and Steareth-20. The underlying mechanisms for the loss of formazan formation remain to be elucidated but are probably associated with disruption of the cell membrane, which can be expected following exposure to detergents.

The clinical relevance of the in vitro data presented has to be interpreted with caution. For example, isolated cells in vitro are not protected by the salivary pellicle layer, and thus higher concentrations in vivo are presumably necessary to produce cytotoxic effects (19). The in vitro situation cannot simulate the immunologic aspects in vivo or the protective barriers at the tissue level. However, both SLS and CAPB have been considered to cause adverse reactions in vivo – particularly in compromised situations where, for example, SLS can change the barrier properties of human oral mucosa in vivo (3). The irritation caused by SLS may lead to increased gingival blood flow (4) and has been linked to recurrent aphthous stomatitis (5). For example, patients with atopic dermatitis and lupus erythematosus have an increased incidence of contact hypersensitivity to CAPB (13, 14), and CAPB-containing toothpastes are considered as less irritating than those containing SLS and relieve symptoms of dry mouth (9, 10). The mechanisms described in the present study may be partially responsible for the complications observed in these compromised oral conditions.

The present study has limitations. Even though the results suggest an association between the content of the detergent and the in vitro cell toxicity, we have no definitive proof that SLS or AF was responsible for the decrease in cell viability in vitro. Moreover, we have based our analysis on specific dilutions of extracts from toothpastes (TCM); however, as the precise composition of the toothpastes is not publicly available, the concentration of the detergents is not defined, particularly with respect to the LC50 values. However, if we consider the concentration of detergents in toothpaste to range from 0.5% to 2.0% (1, 20, 21), we have a final half-lethal concentration of approximately 1% in TCM – this would be 0.01% SLS in the cell culture experiments. This concentration is also considered as cytotoxic for other in vitro models, such as corneal epithelial cells with an IC50 of 0.002% (22), rat submandibular salivary gland acinar cells with an IC50 of approximately 0.005% (23), and cultured keratinocytes with an IC50 of 0.0014% (24). These observations basically support the conclusion that SLS in toothpastes could be responsible for the effects observed in the present study. However, the conclusion that should be derived from the data on CAPB is far from clear.

If we now consider the concentration of CAPB in dentifrices ranging from 0.5 to 2.0% (21), the cells in the present study were exposed to approximately 0.01% CAPB in vitro – without any significant changes in cell viability. This was unexpected because in other studies the LC50 of CAPB for cultured keratinocytes was approximately 0.001% (24). Cocamidopropyl betaine was even considered more toxic than SLS (16). We have no explanation of why toothpastes containing CAPB produce significantly less cytotoxicity compared with those in which SLS is the detergent. Therefore, it would now be important to know the actual concentration of CAPB in toothpaste to calculate the concentration in the TCM. Interestingly, toothpastes containing Steareth-20 showed no signs of toxicity, even at the high concentrations that are reached in vivo. At this stage, the discrepancy in the in vitro data between the pure surfactants, SLS and CAPB, and the respective experiments with the toothpastes containing the two surfactants remains unclear.

Within the limitations of this study, it can be concluded that the type of detergent in toothpastes can be associated with changes in in vitro cell toxicity. Toothpastes containing SLS and AF strongly inhibited cell viability at low concentrations, whereas toothpastes containing CAPB and Steareth-20 showed higher LC50 values.

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Conflicts of interest – The authors declare no conflict of interest.

References

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