Volume 10, Issue 10, October 2024,
Publish Date: 27-10-2024
Doi https://doi.org/10.55640/ijmsdh-10-10-15

International Journal of Medical Science and Dental Health (Open Access)

THE IN VITRO INVESTIGATION OF THE EFFECTS OF DIFFERENT CONTAINING TOOTHPASTES ON GINGIVAL MESENCHYMAL STEM CELLS

DAMLA KAHVECILER TOY[1], FIGEN EREN[2], ZEYNEP TUNCA[3], SABRIYE SENEM KILIÇ[3], ELIF SUEL[3], TUNÇ AKKOÇ[3], BETÜL KARGÜL[1,4]

[1]Department of Pediatric Dentistry, Faculty of Dentistry, Marmara University, Institute of Health Sciences, Istanbul.
[2]Department of Pediatric Dentistry, Faculty of Dentistry, Marmara University, Istanbul, Turkey.
[3]Department of Immunology, Faculty of Medicine, Marmara University, Istanbul, Turkey.
[4]Visiting Professor, Queen Mary University of London, United Kingdom.

ABSTRACT

Background: Toothpaste is the most commonly used personal care products for maintaining oral hygiene. Its composition varies based on its intended purpose, with surfactants being one key ingredient, notable for their cleaning and foaming properties. In addition, fluoride, which is considered the most effective agent in preventing caries, is frequently included in toothpaste formulas. During brushing, gingival tissue is exposed to these components. Gingiva is a tissue from which gingival mesenchymal stem cells with healing and regeneration potential can be isolated. The objective of this study was to evaluate the effects of these components on the viability of gingival mesenchymal stem cells in real-time. **Objectives:** This study aimed to evaluate, in real-time, the cytotoxic effects that may occur on gingival epithelial stem cells exposed to toothpastes with varying surfactant contents and fluoride. **Methods:** Tissue samples for the isolation of gingival mesenchymal stem cells (GMSCs) were obtained during the extraction of impacted third molars. Solutions were prepared from ROCS Mg toothpaste containing sodium lauryl sulfate (SLS), Cocoamidopropyl betaine (CAPB), and ROCS Sensitive containing sodium lauryl sarcosinate, SLS, and Colgate toothpaste with1450 ppm fluoride. Dulbecco's modified eagle medium was used as the negative control group. Cell viability was evaluated in real time with the xCelligence device. Two-way ANOVA was used to evaluate the effects of concentration and toothpaste type, and Tukey Post hoc tests were used for pairwise comparisons. Statistical significance was determined as 0.05 in the study. **Results:** Colgate toothpaste containing SLS and 1450 ppm fluoride exhibited a statistically significantly lower cell viability compared to the other tested toothpastes ($p<0.05$). This was followed by ROCS Mg toothpaste, which did not contain fluoride but included SLS. ROCS Sensitive toothpaste, containing CAPB and sodium lauryl sarcosinate, showed the highest cell viability. As the concentration of toothpastes increased, a decrease in cell viability was observed. **Conclusions:** As a result of the findings, it was seen that toothpastes containing SLS and

fluoride had a more negative effect on the viability of GMSC cells than toothpastes containing different surfactants.

KEYWORDS: Stem Cells, Tootpaste, SLS, xCelligence, Cell Viability.

INTRODUCTION

Dental caries is a major public health problem that has implications for both oral and general health. Oral and dental health has an important place in quality of life [1]. Toothpastes are the most effective chemotherapeutic and chemical personal care products that provide oral hygiene Toothpastes are made up of many different ingredients, each with a different function. [2]. Although the contents vary depending on the manufacturer, a standard toothpaste contains 20%-40% humectants, 20%-40% abrasives, 20%-40% water, 5% therapeutic agents, 1%-2% detergents, 2% binders, 2% flavors, and 1% colorant agents and preservatives [3]. With the therapeutic substances it contains, it has an important effect in preventing caries and gingivitis and removing plaque and stains. The chemical form of toothpaste may vary depending on its concentration and manufacturing purpose [4]. Toothpastes are in constant contact with the oral cavity, and some studies have proven that toothpaste ingredients remain in saliva for a long time after brushing [5]. It has been stated that some substances in toothpaste may have negative effects on the oral cavity [6], [7].

Sulfactants are components with antibacterial properties that provide foaming of the toothpaste during brushing and removal of plaque residues [8]. The most commonly used surfactant in toothpastes is Sodium Lauryl Sulfate (SLS), which has anionic properties. In addition, Sodium Lauryl Sarcosinate from the group of sarcosinates with anionic properties and Cocoamidopropyl betaine (CAPB), which has amphoteric properties and has fewer foaming properties than SLS, are other surfactants used [9]. SLS dilutes or breaks down the mucosal barrier, making it more permeable, damaging the protein and lipid structures of mucosal cells. It can cause irritation to the mucosa. It has been observed that the frequency of aphthous ulcers increases in the use of SLS-containing pastes. [10], [11]. CAPB has been reported to cause less mucosal irritation than toothpastes containing SLS [12]. Another sulfactant, Sodium Lauryl Sarcosinate, is similarly less irritating than SLS and is therefore more suitable for individuals with sensitive gums [13]. These surfactants are also used in combination.

Fluoride is an important component in preventing tooth decay. It also provides antibacterial effects by affecting the cell membrane structure of bacteria [14]. Regular brushing with toothpaste containing fluoride increases the fluoride concentration in the oral cavity [15]. Accordingly, the fluoride concentration in the gingival epithelial cells also increases. Studies have shown that fluoride induces gingival apoptosis depending on the dose and time [16].

Stem cells are cells that have the ability to regenerate, multiply and differentiate according to need. With their differentiation feature, they have undertaken important tasks in the construction and repair events in the organism [17], [18]. Stem cells can be successfully used for the repair of damage to the dental, the gingival or the craniofacial region [19]. Zhang et al (2009) identified human gingival tissue as a potential source of stem cells for tissue regeneration and therapy, Gingival Mesenchymal stem cells (GMSC) are promising in terms of both accessibility and quantity [20].

The gingiva is exposed to toothpastes and the potentially toxic ingredients mentioned above due to daily dental cleaning.

The aim of our study was to evaluate the dose and time-dependent effects of different toothpaste concentrations on gingival mesenchymal stem cells (GMSC), which are found in gingival tissues, play a crucial role in tissue regeneration and healing, and can be easily isolated under in vitro conditions.

MATERIALS AND METHODS

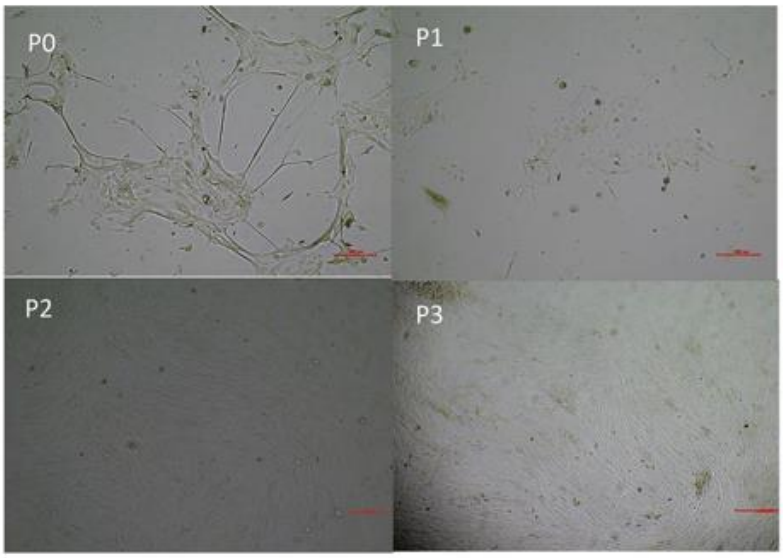
The study was approved by the ethics committee of Marmara University, Faculty of Dentistry (2022-108), according to Helsinki Declaration guidelines.

Cell Isolation

Gingival tissue samples were taken together with extractions from 5 systemically healthy patients with third molars with bone and/or mucosal retention. The tissue samples were dissected and transferred to a falcon tube and exposed to collagenase enzyme for 45 min at 37°C. In order to inactivate the collagenase enzyme, they were incubated in Dulbecco's Modified Eagle medium (DMEM) and centrifuged. When the cells reached an average of 70-80% confluence, the adherent cells were dissociated by adding 0.25% trypsin-EDTA solution. Characterization of GMSCs was performed in the third passage stage (Figure-1A).

The adipogenic, chondrogenic and osteogenic differentiation capacities of G-MSCs brought to passage 3 were characterised. The procedures were briefly as follows: 10x105 G-MSCs in 6-well plates were cultured in DMEM containing 10% FBS. After 24 hours of incubation, cells showing 60-70% confluency were replaced with differentiation medium. Adipogenic differentiated cells were stained with Oil Red on day 14 of differentiation, osteogenic differentiated cells with Alizarin Red and chondrogenic differentiated cells with Alcian Blue on day 21 of differentiation (Figure-1B).

A



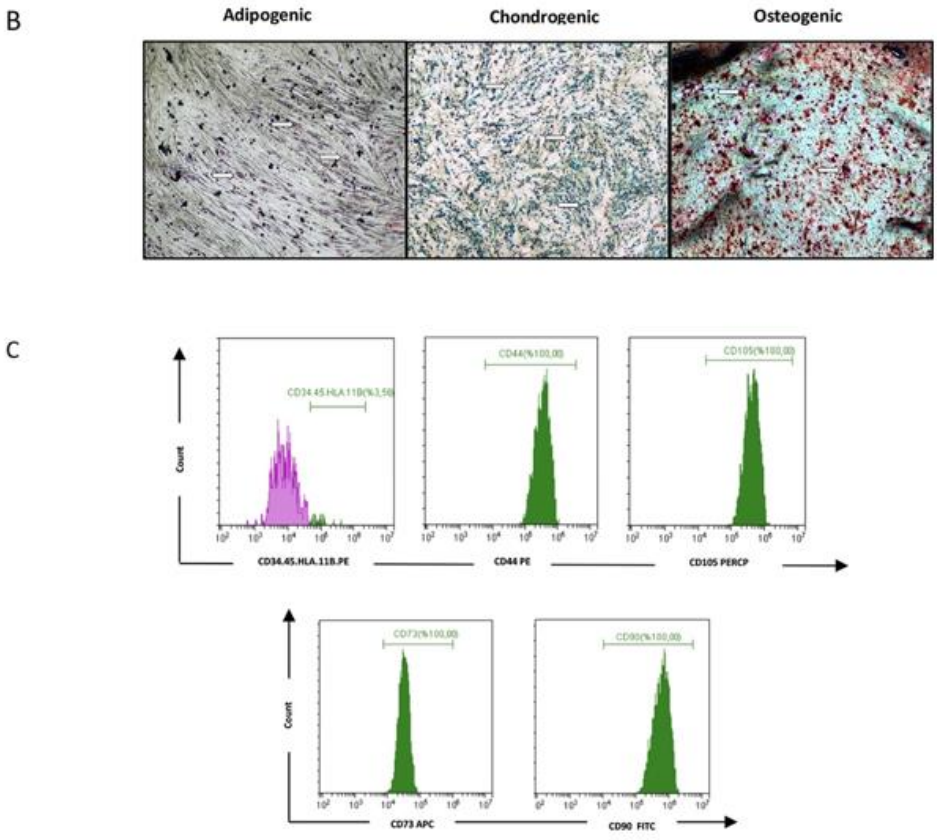


Figure-1: Characterization of Gingival Mesenchymal Stem Cells (G-MSCs) A) Morphological Appearance of G-MSCs at Different Passages (x4 Magnification). P0: G-MSCs at 0th passage, P1: G-MSCs at 1st passage, P2: G-MSCs at 2nd passage, P3: G-MSCs at 3rd passage, Bar: 100µm B) Differentiation Potential of G-MSCs: Differentiation of G-MSCs into adipocytes was confirmed by Oil Red O staining (x4), into chondrocytes by Alcian Blue staining (x4) and into osteoblasts by Alizarin Red staining, (x4), Bar: 80µm C) Representative flow cytometry analysis of mesenchymal stem cell negative and positive surface markers on G-MSCs.

The positive and negative surface markers of passage 3 G-MSCs were characterised by flow cytometry. The procedures are briefly described as follows: 1x105 G-MSCs were suspended in DPBS (washing solution) containing 1% FBS. For positive surface markers, 10 µL antibody against CD90 FITC, CD73 APC and CD105, PerCP.Cy5.5 (BD, 51-9007663) was added; for negative surface markers, 10 µL antibody against CD45, CD11b, CD19 and HLA-DR (BD, 51-9007661) was added and cells were incubated in the dark for 15 minutes at room temperature. After incubation, the cells were centrifuged at 1500 rpm for 5 minutes. The supernatant was discarded. The stained cells were resuspended in the staining solution and analysed using a Beckman Coulter Cytoflex flow cytometer and Cytoexpert software (Figure-1C).

Preparation of Toothpaste Solutions

The toothpastes we used in the study were Colgate +6 (SLS+F Tp.), ROCS Active Mg (SLS Tp.) and ROCS sensitive (CAPB+Sodium Lauryl Sarcosinate Tp.). The contents of these toothpastes are given in Table 1. Appropriate concentrations for determining cell viability values were determined according to ISO-10993-5, 2009 standards [21]. Solutions were prepared at 4 different ratios (0.4%, 20%, 50% and 80%) for each toothpaste. A sufficient amount of toothpaste was weighed on a precision scale to obtain a

solution at 80% concentration, and dilution was performed in sterile conical tubes in serum-free culture medium. Dilution was performed with DMEM to obtain a solution at 0.4%, 20%, and 50% concentrations over the 80% concentration. It was centrifuged at 4200 rpm for 10 min and filtered for the resulting particulate structure [22]. DMEM solution was determined as the negative control group.

Table 1 *(Composition of evaluated toothpaste.)*

Material	Composition	Manufacturer
ROCS Active Mg	Aqua, Silica, Glycerin, Xylitol (6 %), Magnesium Chloride, **Sodium Lauryl Sulfate (SLS)**, Xanthan Gum, Aroma, Sodium Glycerophosphate, Calcium Glycerophosphate, Methylparaben, Sjdium Saccharine, Stevioside, Sodium Silicate, Propylparaben,Limonene	ROCS, DRC Group, Russia
ROCS Sensitive	Aqua, Silica, Glycerin, Xylitol, Hydroxyapatite, Xanthan Gum, Aroma, Calcium Glycerophosphate, **Cocamidopropyl Betaine (CAPB), Sodium Lauryl Sarcosinate,** Hydroxyacetophenone, Sodium Benzoate, Sodium Saccharine, Magnesium Chloride, Sodium Methylparaben, Sodium Propylparaben, O-cymen-5-ol, CI 74160, Limonene.	ROCS, DRC Group, Russia
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 77891, CI 42,090	Colgate, Palmolive Company,Belgium
Complate DMEM	10% FBS (Fetal bovine serum), DMEM (Dulbecco's Modifed Eagles Medium), Supplemented with 1% penicillin/streptomycin	Gibco, Grand Island, USA

Measurements on Cell Cultures

The xCelligence system (ACEA Biosciences, San Diego, CA, USA) was used to evaluate the real-time changes in cell viability values depending on the solution type and concentration.

The xCelligence system features specially designed microtiter plates containing nested gold microelectrodes. Using these plates, it measures cell viability non-invasively and in real time. The electrical impedance value of the gold microelectrodes is expressed as the cell index (CI). When there

are no viable cells, this value is close to zero [23]. In a 16-well xCelligence plate, 2x104 cells were cultured in DMEM at 37°C and 0.5% CO2. At the end of 24 hours, 50 µl of supernatant was removed and replaced with the same amount of toothpaste solution. No addition was made to the control group. The viability indices of the cells were monitored for 72 hours and the experiments were repeated three times.

Statistical Analysis

IBM statistics SPSS 23 program was used for statistical evaluation of the findings. Two Way Anova and Post hoc Bonferroni test were applied to evaluate concentration and time-dependent data. Significance value was determined as $p<0.05$.

Results

Real-time values of toothpastes at 0.4%, 20%, 50% and 80% concentrations using Xcelligence was shown in Figure 2. Time-dependent cell index values of GMSCs exposed to different toothpastes and concentrations are shown in Table 2. The toothpaste with the highest cell index at 24 and 72 hours at 0.4% concentration is CAPB+Sodium Lauryl Sarcosinate Tp. At this concentration, an increase in cell index was observed in all toothpastes and the control group over time. This increase was statistically significant in all toothpastes except SLS+ F Tp. ($p<0.05$)

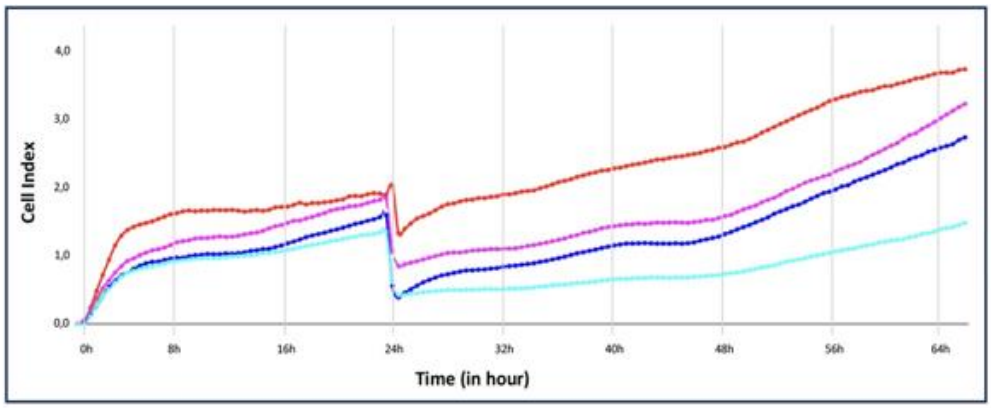
At 20% concentration, SLS Tp. had the highest cell index value at the end of 24 hours, and CAPB+ Sodium Lauryl Sarcosinate Tp. at the end of 72 hours. At this concentration, a statistically significant decrease in cell index was observed in CAPB+Sodium Lauryl Sarcosinate Tp. and SLS+F Tp. at the end of 72 hours ($p<0.05$). A statistically significant increase was observed in CAPB+Sodium Lauryl Sarcosinate Tp. and SLS Tp. at the end of 72 hours ($p<0.05$)

Table 2 *(Comparison of cell index between groups with two-way repeated ANOVA test and change over time for all p<0,05.)*

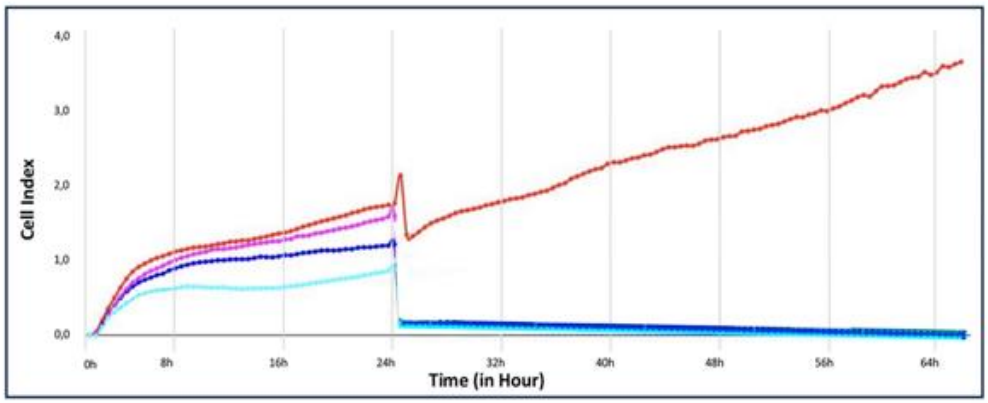
Concentration	Contents	24 Hours Mean±SD	72 Hours Mean±SD	p-value
%0,4	SLS Tp.	1,14± 0,43	2,11± 0,43	0,005*
	CAPB+Sodyum Lauryl Sarkosinate	1,29 ± 0,48	2,31± 0,61	0,001*
	SLS+F Tp.	0,97± 0,35	1,08± 0,28	0,60
	Control	1,56±0,51	3,05± 0,44	0,006*
%20	SLS Tp.	1,66±0,88	0,05±0,01	0,001*
	CAPB+Sodium Lauryl Sarcosinate Tp.	1,63±1,21	2±0,3	0,002*
	SLS+F Tp.	1,35±1,21	-0,02±0,09	0,002*

	Control	1,73±0,62	3,55±0,61	0,001*
%50	SLS cont.	2,96±1,45	-0,24±0,03	0,001*
	CAPB+Sodium Lauryl Sarcosinate	2,64±1,15	0,44±0,52	0,001*
	SLS+F Tp.	2,63±1,33	-0,32±0,03	0,000*
	Control	2,56±1,12	2,85±0,41	0,21
%80	SLS Tp.	2,72±1,35	-0,4 ± 0,04	0,000*
	CAPB+Sodium Lauryl Sarcosinate	2,75±1,19	-0,02± 0,12	0,000*
	SLS+ F Tp.	2,58±1,16	-0,4 ± 0,05	0,001*
	Control	3,21±1,19	2,77±0,50	0,009

A



B



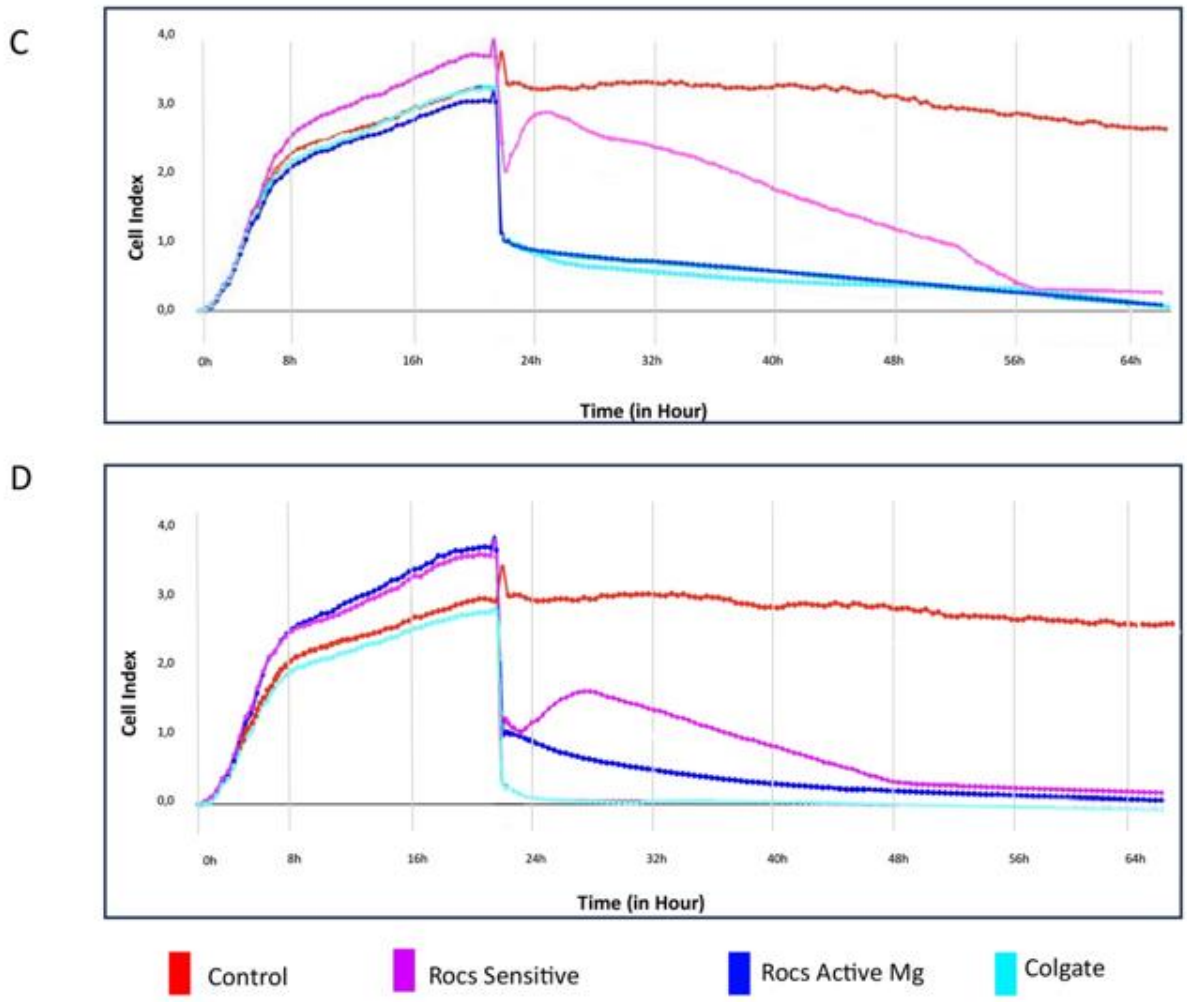


Figure 2: Real-time Monitoring of Cell Viability Using Xcelligence A) Time-dependent change of cell index (ΔCI) of Toothpastes at 0.4 % concentration B) Time-dependent change of cell index (ΔCI) of remineralising agents at 20 % concentration C) Time-dependent change of cell index (ΔCI) of remineralising agents at 50 % concentration D) Time-dependent change of cell index (ΔCI) of remineralising agents at 80 % concentration.

Table 3 *(P values of different toothpastes at fixed concentrations at 24 and 72 hours compared to each other and p values of each toothpaste at different concentrations at 24 and 72 hours.)*

		24 Hours p value	**72 Hours p value**
p values for toothpaste	%0,4	0,010*	0,000*
	%20	0,004*	0,000*
	%50	0,003*	0,000*
	%80	0,018*	0,000*

	SLS	0,000*	0,000*
p values for concentration	CAPB+Sodium Lauryl Sarcosinate	0,003*	0,000*
	SLS+F	0,000*	0,000*
	Control	0,000*	0,000*

At 50% concentration, the toothpaste with the highest cell index at the end of 24 hours was SLS Tp., and at the end of 72 hours, the toothpaste with the highest cell index was CAPB+Sodium Lauryl Sarcosinate Tp. At this concentration, at the end of 72 hours, the cell index decreased statistically significantly in all toothpastes except the control group ($p<0.05$). Similarly, cell index values decreased over time in all toothpastes except the control group at 80% concentrations. This decrease was statistically significant ($p<0.05$) The p values of different toothpastes at fixed concentrations at 24 and 72 hours and the p values of each toothpaste at different concentrations at 24 and 72 hours are given in Table 3. Accordingly, the cell index value of all toothpastes with 0.4% concentration at 24 hours was found to be significant compared to each other. ($p<0.05$) Similarly, it was found to be statistically significant at 20%, 50% and 80% concentrations. ($p<0.05$) The cell index values of CAPB+Sodium Lauryl Sarcosinate Tp. at 0.4%, 20%, 50% and 80% concentrations at 24 and 72 hours were found to be statistically significant. ($p<0.05$) The 0.4%, 20%, 50% and 80% values of SLS Tp. and SLS+ F Tp. at 24 and 72 hours were found to be statistically significant. ($p<0.05$) The effects of concentration and toothpaste on cell index changes was shown in Table 4.

Table 4 *(Evaluation of the effects of concentration and toothpaste on cell index change in 72-hour period.)*

Material	**%0.4**	**20%**	**50%**	**80%**	**p-value**
SLS	1,52±0,42Aa	1,73± 0,19Aa	0,85±1,49Aa	0,68± 1,45Aa	0,06
CAPB+Sodium Lauryl Sarcosinate	1,64±0,48Aa	1,74± 0,19Aa	1,77±0,96Aa	1,17± 1,16Aa	0,052
SLS+Fluoride	0,90 ± 0,19Ba	0,46± 0,63Ba	0,69±1,37Ba	0,38± 1,38Ba	0,60
Control	2,21±0,62Ca	2,58± 0,75Ca	3,11±0,58Ca	3,27± 0,43Ca	0,51
p-value	0,000*	0,001*	0,001*	0,001*	

*Two-way ANOVA Test, Post hoc Tukey HSD test *p < 0.05*
a-b-c-d: There is no difference between the groups with the same letter in same line
A-B-C: There is no difference between the groups with the same letter in same colomn.

The cell index values of all toothpastes at 0.4% concentration are statistically significant when compared with the control group ($p<0.05$). In pairwise comparisons, there is no statistically significant difference between the cell index values of CAPB+Sodium Lauryl Sarcosinate Tp. and SLS Tp.($p<0.05$). However, there is a statistically significant difference between the cell index values of SLS+Fluoride Tp. and CAPB+Sodium Lauryl Sarcosinate Tp. and SLS Tp.($p<0.05$). A similar situation applies to 20%, 50% and 80% concentrations. When we compare all toothpastes at concentrations of 0.4%, 20%, 50% and 80%, we observe that the cell index values decrease, but this decrease is not statistically significant ($p<0.05$)

DISCUSSION

Toothpastes consist of many components to serve different purposes. Surfactants are antibacterial ingredients that have cleansing, foaming properties and also allow the toothpaste to disperse in the mouth. Toothpastes usually contain surfactant types such as SLS, CAPB, sodium lauryl sarcosinate and sodium methyl cocoyl taurate. The most commonly preferred surfactant in toothpastes is SLS. However, studies have reported that some surfactant types have negative effects on oral tissues [26]. Fluoride in toothpaste is the most widely used agent for the prevention of dental caries, but concerns about fluoride toxicity persist [16]. With tooth brushing, not only the dental tissues but also the gingival tissues are exposed to the toothpaste contents. It has been reported that these contents reach the deep layers of the gingival tissues after brushing. GMSC are cells with regeneration and transformation properties that can be easily isolated from gingival tissues [27]. We aimed to examine the effect of toothpaste exposure on the viability values of these cells, which have an important effect on tissue damage and cell regeneration.

Many studies have been conducted on the effects of surfactants and fluoride among these ingredients. However, there is no study in the literature evaluating the effect of toothpastes on gingival mesenchymal stem cell viability with the xCelligence device. For this purpose, toothpastes with different surfactant types and additionally toothpaste containing fluoride were preferred in our study. SLS containing Rocs Mg toothpaste, SLS and fluoride containing Colgate +6 toothpaste and Sodium Lauryl Sarcosinate and Cocoamidopropyl Betaine (CABP) containing Rocs Sensitive toothpaste solutions were prepared and aimed to evaluate their effects on GMSC.

There are many studies examining the toxicity of surfactants. Birant et al. reported in their study that toothpastes containing SLS had the lowest vitality values [22] Similarly, Cvikl et al. found the vitality values of toothpastes containing SLS to be low [24]. In our study, the vitality values of toothpastes containing SLS were found to be lower than those of toothpastes without SLS. In the study conducted by Lloret et al., it was stated that the vitality values of toothpastes using Sodium Lauryl Sarcosinate instead of SLS were higher than those containing SLS [25]. Similarly, in our study, the vitality values of toothpastes containing CABP and Sodium Lauryl Sarcosinate instead of SLS were found to be higher.

Tadin et al. have found that toothpastes without SLS and fluoride and toothpastes without SLS but with fluoride have similar cytotoxic effects [7]. In our study, the vitality value of toothpaste containing SLS and fluoride was found to be lower than that of toothpaste containing SLS and not containing fluoride.

There are many traditional methods (flow cytometry, MTT) used to evaluate cell viability. xCelligence monitors the dynamic behavior of cells as continuous data. Classical methods provide information about the current status of cells and cannot monitor behavioral changes. It provides measurements without the need for extracellular intervention and provides biologically appropriate results without the need for the addition of markers or chemicals.

For this reason, we preferred the xCelligence system, a modern, non-invasive method that provides real-time detection of cell viability. Although there are many studies examining the relationship between cell viability and toothpaste, there is no study in the literature evaluating the effect of toothpastes on gingival mesenchymal stem cell viability with the xCelligence system.

One of the limitations of our study is that the exposure of toothpaste ingredients to gingival tissue cannot be fully reflected in vitro. Oral tissues are dynamic, and the level of dilution that will occur with saliva or rinsing cannot be fully reflected. In addition, the toothpastes we used in our study consist of many different components. Although we evaluated the effects of surfactant and fluoride in our study, different components may also have an effect on cell viability.

Based on the results of this study, toothpastes without surfactants should be preferred due to the negative effects on cell viability. New formulations and research are needed to evaluate toothpastes and their contents.

CONCLUSIONS

Our study is the first to evaluate the effect of surfactants in toothpastes on gingival mesenchymal stem cell viability in real time using the xCelligence device. According to the results of the study, although all toothpastes containing surfactants negatively impact GMSC viability, toothpaste containing both SLS and fluoride has the most pronounced negative effect. Further studies are required to investigate this effect.

Acknowledgements

Funding

This study was supported by grants from Marmara University Scientific Research Projects Coordination Unit with TDK-2023-10875 protocol ID. This article is derived from the doctoral thesis titled 'Investigation of the Effects of Different Remineralization Agents on Gingival Epithelial Stem Cells' conducted at the Institute of Health Sciences, Marmara University.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of Interest

The authors have no conflicts of interest relevant to this article

Ethical Approval

This study protocol was submitted to and approved by the local ethical committee (protocol code is 2022-108, date: 10.11.2022). The study was conducted in accordance with the guidelines of the Declaration of Helsinki.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Goldman AS, Yee R, Holmgren CJ, Benzian H. Global affordability of fluoride toothpaste. Globalization and Health. 2008; 4:7. doi:10.1186/1744-8603-4-7.
2. Forward GC, James AH, Barnett P, Jackson RJ. Gum health product formulations: what is in them and why? Periodontol 2000. 2000; 15:32-9.
3. Bulut M, Yıldırım S, Ulukapı I. Diş macunları ve fluor. Turkiye Klinikleri, Diş Hekimliğinde Fluor. 1. baskı. Ankara: Turkiye Klinikleri; 2020. p. 42-7.
4. Petrović B, Kojić S, Milić L, Luzio A, Perić T, Marković E, Stojanović GM. Toothpaste ingestion—evaluating the problem and ensuring safety: systematic review and meta-analysis. Front Public Health. 2023; 11:1279915. doi: 10.3389/fpubh.2023.1279915.
5. Zamataro, C.B.; Tenuta, L.M.; Cury, J.A. Low-fluoride dentifrice and the effect of postbrushing rinsing on fluoride availability insaliva.Eur. Arch. Paediatr. Dent.2008,9, 90–93
6. Kasi SR, Özcan M, Feilzer AJ. Side effects of sodium lauryl sulfate applied in toothpastes: a scoping review. Am J Dent. 2022; 35:84–88.
7. Tadin A, Gavic L, Govic T, et al. In vivo evaluation of fluoride and sodium lauryl sulphate in toothpaste on buccal epithelial cells toxicity. Acta Odontol Scand. 2019; 77:386–393. https://doi.org/10.1080/00016357.2019.1577988.
8. Lindenmuller IH, Lambrecht JT. Oral care. Curr Probl Derma- tol 2011 40: 107–115.
9. Van Loveren C, ed. Toothpastes: an introduction to toothpaste—its purpose, history, and ingredients. Monogr Oral Sci. Basel: Karger; 2013. vol 23, pp. 1-14. doi:10.1159/000350456.
10. Sälzer S, Rosema NAM, Martin ECJ, Slot DE, Timmer CJ, Dörfer CE, van der Weijden GA. The effectiveness of dentifrices without and with sodium lauryl sulfate on plaque, gingivitis and gingival abrasion - A randomized clinical trial. Clin Oral Investig 2016; 20:443-450.
11. Siegel IA, Gordon HP. Surfactant-induced alterations of permeability of rabbit oral mucosa in vitro. Exp Mol Pathol 1986; 44:132-137.
12. Rantanen I, Tenovuo J, Pienihakkinen K, Soderling E. Effects of a betaine-containing toothpaste on subjective symptoms of dry mouth: a randomized clinical trial. J Contemp Dent Pract 2003; 4: 11–23.
13. Fiume MM, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler DC, et al. Amended safety assessment of fatty acyl sarcosines and sarcosinate salts as used in cosmetics. Int J Toxicol. 2021;40(Suppl 2):117S-133S. doi:10.1177/10915818211023881.
14. Arslan İ, Baygın Ö. Cavity disinfection methods used in pediatric dentistry. J Dent Fac Atatürk Uni. 2019; 29:124–132.
15. Souza DC, Maltz M, Hashizume LN. Fluoride retention in saliva and in dental biofilm after different home-use fluoride treatments. Braz Oral Res. 2014; 28:1.
16. Lee JH, Jung JY, Jeong YJ, et al. Involvement of both mitochondrial and death receptor-dependent apoptotic pathways regulated by Bcl-2 family in sodium fluoride-induced apoptosis of the human gingival fibroblasts. Toxicology 2008; 243:340–347.
17. Huang S., Fu R., Shyu W., 2013. Adipose-derived stem cells: Isolation, characterization, and differentiation potential. Cell Transplant, 22, 701- 709.

18. Çerçi E, Erdost H. Kök Hücre. Atatürk Üniversitesi Vet Bil Derg. 2019;14(2):221-8. doi:10.17094/ataunivbd.483253.
19. Abou Neel, E. A.; Chrzanowski, W.; Salih, V. M.; Kim, H. W.; Knowles, J. C. Tissue engineering in dentistry. J. Dent. 2014, 42 (8), 915−928.
20. Shaikh F, Langade N, Khan M, Muglikar S, Ansari NZ. Mesenchymal stem cells and tissue engineering in dentistry. Recent Update on Mesenchymal Stem Cells. IntechOpen; 2023. pp. 1-22. doi:10.5772/intechopen.1002291.
21. ISO 10993-5, 2009. Biological Evaluation of Medical Devices-part 5: Test for in Vitro Cytotoxicity. International organization for standardization, Geneva, Switzerland.
22. Birant S, Duran Y, Gokalp M, Akkoc T, Seymen F. Effects of different detergent-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. 2021; 72:101538. doi: 10.1016/j.tice.2021.101538.
23. Ke N, Wang X, Xu X, Abassi YA. The xCELLigence system for real-time and label-free monitoring of cell viability. Methods Mol Biol. 2011; 740:33-43. doi:10.1007/978-1-61779-108-6_5.
24. Cvikl B, Lussi A, Gruber R. The in vitro impact of toothpaste extracts on cell viability. Eur J Oral Sci. 2015; 123:179-85. doi:10.1111/eos.12177.
25. Pecci-Lloret MP, López-García S, Rodríguez-Lozano FJ, Álvarez-Novoa P, García-Bernal D. In vitro biocompatibility of several children's toothpastes on human gingival fibroblasts. Int J Environ Res Public Health. 2022; 19:2954. doi:10.3390/ijerph19052954.
26. Stovell AG, Newton BM, Lynch RJM. Important considerations in the development of toothpaste formulations for children. Int Dent J. 2013;63(Suppl 2):57-63. doi:10.1111/idj.12083.
27. Kuroda, Y., Kitada, M., Wakao, S., Nishikawa, K., Tanimura, Y., Makinoshima, H., et al., 2010. Unique multipotent cells in adult human mesenchymal cell populations. Proc. Natl. Acad. Sci. U.S.A. 107, 8639–8643.