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Comparative assessment of biocompatibility of various fluoride agents in cell culture

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Abstract

Objective This study aimed to evaluate the biocompatibility of various fluoride agents using L929 fibroblast and human gingival fibroblast (HGF) cell cultures.

Materials and methods Five different fluoride agents were included in the study; 1.23% Acidulated Phosphate Fluoride (APF) gel (Sultan Health Care, Engle Wood, USA), 2% Sodium Fluoride (NaF) gel (Sultan Health Care, Engle Wood, USA), 1% Titanium Tetrafluoride (TiF_4) solution (Sigma-Aldrich® Chemie GmbH, Steinheim, Germany), 38% Silver Diamine Fluoride (SDF) solution (Saforide®-J. Morita; Toyo Seiyaku Kasei Ltd/Osaka-Japan), and 5% NaF varnish (Duraphat®, Colgate Oral Pharmaceuticals, New York, NY, USA). Cytotoxicity was evaluated using the real-time xCELLigence system, while apoptotic activity was determined through Annexin V/PI and TUNEL assays. Genotoxicity was assessed using the micronucleus test. The expression levels of the pro-apoptotic marker BAX and the anti-apoptotic marker BCL-2 were quantified using real-time PCR. All experiments were performed in triplicate and independently repeated to ensure reproducibility, with data analyzed using non-linear regression for IC_{50} determination, one-way ANOVA with Tukey's post hoc test for group comparisons, and chi-square or Fisher's Exact tests for categorical variables (SPSS 25.0); statistical significance was set at $p < 0.05$.

Results NaF gel exhibited the lowest cytotoxicity in L929 fibroblast cell line, while APF gel demonstrated the least cytotoxicity in HGF cells. Both APF and NaF gels significantly induced apoptosis in L929 cells, with no notable apoptotic effects observed in HGF cells treated with these agents. Conversely, TiF_4 solution and NaF varnish consistently induced apoptosis across both cell lines, whereas SDF solution did not elicit significant apoptotic activity. In terms of genotoxicity, TiF_4 solution showed significant genotoxic effects, while SDF solution demonstrated the lowest genotoxic potential. The *Bax/Bcl-2* ratios for all fluoride agents tested in the present study were found to be greater than 1, indicating that these agents induce apoptosis through the mitochondrial pathway, with a predominance of pro-apoptotic signals.

Conclusions Silver Diamine Fluoride, which gained substantial attention especially during the COVID-19 pandemic, has emerged as a highly effective and minimally invasive option for arresting caries and preventing early enamel lesions. With its rapid action and favorable safety profile, SDF solution is increasingly recognized as a superior alternative to commonly used topical fluoride agents, particularly in clinical settings where traditional dental procedures may carry heightened risks or present logistical challenges.

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Clinical relevance This study provides critical insights into the biocompatibility profiles of commonly used fluoride agents, offering evidence-based guidance for their clinical application. Among the tested agents, SDF stands out for its minimal cytotoxicity and genotoxicity, coupled with its proven efficacy in caries arrest and enamel lesion prevention. By highlighting the distinct biocompatibility and apoptotic profiles of various fluoride agents, the findings support informed decision-making in selecting appropriate fluoride therapies to optimize patient safety and treatment outcomes.

Keywords Biocompatibility, Siver Diamine fluoride, Sodium fluoride, Pediatric dentistry

Introduction

Dental caries continues to be one of the most prevalent chronic diseases worldwide, driven by a multifactorial interplay between microbial, dietary, and host-related factors [1, 2]. The disease primarily develops due to the activity of cariogenic microorganisms, frequent consumption of fermentable carbohydrates, the inherent susceptibility of dental tissues, and prolonged exposure to these risk factors [3, 4]. Beyond its biological determinants, the prevalence and severity of dental caries are influenced by broader social and environmental factors, including socioeconomic status, access to dental care, and oral health education. Inequities in access to preventive measures such as fluoride application, dietary counseling, and routine dental check-ups exacerbate the burden of caries, particularly in low-income communities [1–5].

The application of topical fluoride remains a fundamental strategy in preventive dentistry, playing an essential role in the control and mitigation of dental caries [6]. Extensive research has validated fluoride's effectiveness in remineralizing enamel, preventing demineralization, and inhibiting bacterial activity within dental plaque [7]. These attributes make fluoride an essential component of contemporary dental care, particularly for populations at increased risk of caries, such as children, the elderly, and special needs individuals [6, 7]. These agents, including sodium fluoride (NaF), acidulated phosphate fluoride (APF), titanium tetrafluoride (TiF_4), and silver diamine fluoride (SDF), are renowned for their ability to enhance enamel remineralization and inhibit demineralization. A variety of fluoride formulations are available to meet specific clinical or preventative needs [6–9].

Neutral sodium fluoride was the first fluoride compound introduced as a topical fluoride agent in dental practice and is available in solutions, gels, foams, and varnishes. Subsequently, acidulated phosphate fluoride was developed and is similarly offered in solution, gel, and foam formulations [6, 7]. Titanium tetrafluoride, commonly used at concentrations of 1% and 4%, has been reported to form a glaze-like surface layer on enamel, enhancing its protective properties [8]. Fluoride varnishes, introduced during the 1960s and 1970s, were specifically designed for professional use, prolonging the contact time between fluoride and enamel to improve efficacy [9].

More recently, silver diamine fluoride has emerged as a highly effective agent for caries prevention, cavity disinfection, and dentin desensitization. Its mechanism of action involves inhibiting bacterial growth and biofilm formation, as demonstrated in numerous bacterial and biofilm models. SDF is available in concentrations of 12%, 30%, and 38%, with the 38% concentration being most commonly used due to its superior efficacy [10, 11].

During the COVID-19 pandemic, SDF gained considerable attention as a non-invasive, time-efficient, and aerosol-free alternative to traditional clinical procedures. Its use aligns with infection control measures by minimizing aerosol generation and reducing the risk of viral transmission. The ability of SDF to arrest caries without extensive instrumentation has made it particularly beneficial in pediatric and high-risk populations. Notably, SDF solution, along with APF and NaF gels, has achieved significant clinical acceptance due to its proven efficacy in preventing dental caries [12, 13].

While fluoride agents are the cornerstone of preventive dental care, their safety profiles particularly their cytotoxic and genotoxic potentials warrant careful evaluation. Numerous in vitro studies have demonstrated that topical fluoride agents can induce cytotoxic effects on oral fibroblasts and epithelial cells, potentially leading to cell cycle arrest, oxidative stress, apoptosis, or necrosis depending on the concentration and exposure duration. These effects raise important concerns regarding the safe use of fluoride products, especially in vulnerable populations such as children and individuals with mucosal sensitivities [14, 15]. The increasing clinical application of high-concentration agents such as silver diamine fluoride (SDF) and titanium tetrafluoride (TiF4) further necessitates a comprehensive understanding of their biological effects at the cellular level [14]. The biocompatibility of various fluoride agents was systematically evaluated using cell culture assays to examine their cytotoxicity, apoptosis, genotoxicity, and gene expression [16]. Therefore, this study aimed to systematically assess the cytotoxic, apoptotic, and genotoxic properties of five commonly used fluoride agents using L929 murine fibroblasts and primary human gingival fibroblast (HGF) cultures. The use of advanced technologies including real-time cell analysis

(xCELLigence), flow cytometric apoptosis assays, and RT-PCR gene expression profiling allowed for a robust and multifaceted evaluation of fluoride biocompatibility.

Materials and methods

Study design

This in vitro study investigated the biocompatibility of five clinically relevant topical fluoride agents:

1. 1.23% Acidulated Phosphate Fluoride (APF) gel (Sultan Health Care, USA).
2. 2% Sodium Fluoride (NaF) gel (Sultan Health Care, USA).
3. 1% Titanium Tetrafluoride (TiF_4) solution (Sigma-Aldrich®, Germany).
4. 38% Silver Diamine Fluoride (SDF) solution (Saforide® - J. Morita, Japan).
5. 5% Sodium Fluoride varnish (Duraphat®, Colgate, USA).

Biocompatibility was evaluated through cytotoxicity (xCELLigence system), apoptosis (Annexin V/PI and TUNEL assays), genotoxicity (micronucleus test), and gene expression analysis (BAX/BCL-2 via RT-PCR) using L929 murine fibroblasts and human gingival fibroblasts (HGFs). All procedures followed Good Laboratory Practice (GLP) principles and conformed with ISO 10993-5:2009 guidelines. HGF cells were isolated from healthy gingival tissues obtained, following approval by the Ege University Ethics Committee (Ref. 12 – 7/33). Written informed consent was obtained from all participating children and their parents.

Preparation of fluoridated agents

Fluoridated compounds were prepared in sterile phosphate-buffered saline (PBS) to final concentrations of 0.1, 0.5, 1, and 2 mM. Solutions were freshly prepared prior to each experiment, filtered using 0.22 µm syringe filters, and protected from light during handling and incubation. All solutions were freshly prepared prior to each experiment and stored in amber tubes to prevent light-induced degradation, following manufacturer instructions and ISO 10993-12:2021 standards on sample preparation.

Cell culture

L929 fibroblasts (ATCC® CCL-1™) and primary human gingival fibroblasts (HGFs) were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and maintained at 37 °C in a humidified incubator with 5% CO_2. HGF cultures were obtained from gingival biopsies of healthy donors following ethical approval and informed consent. Cells were seeded at a density of 1×10^4 cells/well in 96-well plates and allowed to adhere for 24 h prior to treatment.

Preparation of fluoride agents

Fluoride agents were diluted to working concentrations using sterile phosphate-buffered saline (PBS) immediately prior to application. For varnish and gel formulations, homogenization was achieved via vortexing. All materials were filtered through 0.22 µm syringe filters where applicable.

Cytotoxicity assay

Real-time cell viability was monitored using the xCELLigence RTCA DP system (Agilent, USA). Cell impedance was recorded every 15 min post-treatment for up to 72 h. IC_{50} values (concentration causing 50% viability reduction) were calculated using RTCA software 2.0, based on sigmoidal dose–response curves fitted to the four-parameter logistic (4PL) model, in line with ISO 10993-5:2009 guidelines.

Apoptosis analysis

- **Annexin V/PI Staining**: Apoptosis was evaluated using the Annexin V-FITC Apoptosis Detection Kit (BD Biosciences, Cat. No. 556547), following the manufacturer's protocol. Cells were harvested after 24, 48, and 72 h of exposure and analyzed using a BD FACSCalibur flow cytometer. Data were categorized into viable, early apoptotic, late apoptotic, and necrotic cell populations using quadrant analysis.
- **TUNEL Assay**: DNA fragmentation was assessed via the In Situ Cell Death Detection Kit (TMR red; Roche Diagnostics, Cat. No. 12156792910). Cells were fixed in 4% paraformaldehyde, permeabilized, and stained according to the manufacturer's instructions. Fluorescence was visualized using a Leica DMi8 microscope. The TUNEL-positive rate was expressed as the percentage of apoptotic cells.

Genotoxicity (Micronucleus Test)

Micronucleus formation was evaluated using the cytokinesis-block micronucleus assay, in accordance with OECD Guideline 487 and ISO 10993-3:2014. After 48 h of fluoride exposure, cells were treated with cytochalasin B (3 µg/mL) to arrest cytokinesis. Fixed cells were stained with Giemsa, and micronuclei were scored in 1,000 binucleated cells per sample under light microscopy at 1000× magnification. Genotoxicity was expressed as the frequency of micronucleated cells.

Gene expression (RT-PCR)

Total RNA was extracted using TRIzol™ Reagent (Invitrogen, Cat. No. 15596026) and quantified

Table 1 Comparison of IC50 values for fluoride agents in L929 fibroblast and human gingival fibroblast (HGF) cell lines

Fluoride Agent	L929 IC50 (µg/ml)	L929 IC50 (mM)	HGF IC50 (µg/ml)	HGF IC50 (mM)
APF gel	88.5 ± 4.1^{a}	4.66 ± 0.22	223 ± 6.8^{a}	11.74 ± 0.35
NaF gel	182 ± 5.7^{b}	9.58 ± 0.30	128 ± 4.3^{b}	6.74 ± 0.21
TiF_4solution	13.7 ± 1.2^{c}	0.72 ± 0.08	8.21 ± 1.1^{c}	0.43 ± 0.05
SDF solution	19.1 ± 2.3^{c}	1.0 ± 0.12	48.4 ± 3.6^{d}	2.55 ± 0.19
NaF varnish	2.81 ± 0.9^{d}	0.15 ± 0.03	8.8 ± 0.9^{c}	0.46 ± 0.06

Data are presented as mean ± standard deviation (SD). Superscript letters within each column denote statistically significant differences among the fluoride agents, as determined by one-way ANOVA followed by Tukey's post hoc test (***p* < 0.05**). Fluoride agents that share the same superscript letter are not significantly different from each other, whereas those with different superscripts exhibit statistically significant differences

spectrophotometrically (Figure S1). First-strand cDNA synthesis was performed using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Cat. No. K1622). Quantitative real-time PCR (qPCR) was conducted using SYBR Green PCR Master Mix (Roche, Cat. No. 04913914001) on a LightCycler 96 instrument (Roche). Gene expression levels of BAX and BCL-2 were normalized to GAPDH. The ΔΔCt method was applied to calculate relative fold changes. All assays were performed in accordance with the MIQE guidelines.

Statistical analyses

All statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). A two-tailed p-value less than 0.05 was considered indicative of statistical significance.

All experimental procedures were conducted in triplicate and independently repeated three times to ensure reproducibility and statistical robustness. Quantitative data are presented as the arithmetic mean ± standard deviation (SD). The half-maximal inhibitory concentration (IC_{50}) values for each fluoride agent were estimated using non-linear regression models fitted to dose–response curves, applying a sigmoidal dose-response (variable slope) function in accordance with the four-parameter logistic (4PL) model.

For comparisons of continuous variables (e.g., cell viability, gene expression, apoptotic indices), one-way analysis of variance (ANOVA) was employed, followed by Tukey's Honestly Significant Difference (HSD) post hoc test to correct for multiple comparisons and determine pairwise group differences. Prior to ANOVA, data normality and homogeneity of variances were assessed using the Shapiro-Wilk test and Levene's test, respectively.

For categorical variables (e.g., presence or absence of micronuclei, apoptotic stages), associations were analyzed using the chi-square (χ^2) test. When expected cell counts in contingency tables were < 5, Fisher's Exact Test was applied to ensure statistical validity.

Table 2 Annexin V assay results: percentage of apoptotic cells in L929 and HGF cell lines

		L929 (Early + Late Apoptosis, %)	HGF (Early + Late Apoptosis, %)
1	APF Gel	35.2 ± 2.1^{b}	18.3 ± 1.9^{b}
2	NaF Gel	38.7 ± 2.4^{b}	20.5 ± 2.2^{b}
3	TiF_4 Solution	62.1 ± 3.6^{a}	55.6 ± 2.8^{a}
4	SDF Solution	12.5 ± 1.7^{c}	10.2 ± 1.4^{c}
5	NaF Varnish	59.8 ± 3.1^{a}	53.3 ± 2.9^{a}

Data are presented as the combined percentage of early and late apoptotic cells (mean ± SD) following 24-hour exposure to each fluoride agent. Apoptosis was assessed via flow cytometry using Annexin V/PI staining. Superscript letters indicate statistically significant differences within each column, based on one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Groups sharing the same letter are not significantly different

Results

The IC50 values, determined for each fluoride agent, are summarized in Table 1. The NaF gel exhibited the lowest cytotoxic effect in the L929 fibroblast cell line (NaF gel < APF gel < SDF solution < TiF_4 solution < NaF varnish ($p = 0.033$), respectively) while the APF gel demonstrated the least cytotoxicity in the human gingival fibroblast (HGF) cell line (APF gel < NaF gel < SDF solution < NaF varnish < TiF_4 solution ($p = 0.017$), respectively) (Figures S2, S3, S4, S5, S6).

Regarding apoptosis, the Annexin V assay revealed that both APF and NaF gels significantly induced apoptosis in L929 cells. However, no significant apoptotic effect was observed in HGF cells treated with either APF or NaF gels. In contrast, both TiF_4 solution and NaF varnish consistently induced apoptosis in both cell lines. Notably, SDF solution did not induce any apoptotic response in either L929 or HGF cell lines. These findings suggest distinct apoptotic profiles associated with different fluoride agents, with TiF_4 solution and NaF varnish exhibiting consistent apoptotic activity, while SDF solution demonstrated no significant apoptotic effect in the tested cell lines (Table 2).

The TUNEL assay results further corroborated the apoptosis data, indicating that both TiF_4 solution and NaF varnish significantly induced apoptosis in the L929 cell line. Based on all the tests conducted, TiF_4 solution and NaF varnish demonstrated greater cytotoxic and apoptotic effects compared to APF, NaF gels, and SDF solution in both cell lines (Table 3).

The gene expression levels of the pro-apoptotic marker *Bax* and the anti-apoptotic marker *Bcl-2* were assessed in HGF treated with IC50 concentrations of the fluoride agents. The apoptotic gene expression profiles in HGF exposed to IC50 concentrations of the various fluoride agents were compared with the control group, which was not treated with fluoride agents. To evaluate apoptosis, the *Bax/Bcl-2* ratio; a critical metric reflecting

Table 3 TUNEL assay results: percentage of apoptotic cells in L929 and HGF cell lines

		L929 TUNEL(%)	HGF TUNEL(%)
1	APF Gel	30.5 ± 2.3^{b}	15.7 ± 1.8^{b}
2	NaF Gel	32.8 ± 2.7^{b}	18.1 ± 1.9^{b}
3	TiF_4 Solution	60.4 ± 3.5^{a}	50.2 ± 2.9^{a}
4	SDF Solution	11.3 ± 1.5^{c}	9.8 ± 1.3^{c}
5	NaF Varnish	57.9 ± 3.1^{a}	48.5 ± 2.6^{a}

Data are expressed as the percentage of TUNEL-positive cells (mean ± standard deviation, SD) following 24-hour exposure to each fluoride agent. Apoptotic DNA fragmentation was assessed using the TUNEL assay and fluorescence microscopy. Superscript letters denote statistically significant differences within each column as determined by one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Groups sharing the same letter are not significantly different

Table 4 Bax/Bcl-2 ratios induced by fluoride agents

		Bax/Bcl-2 Ration (Mean ± SD)
1	APF Gel	4.19 ± 0.37^{a}
2	NaF Varnish	2.45 ± 0.22^{b}
3	NaF Gel	5.69 ± 0.45^{c}
4	SDF Solution	5.90 ± 0.41^{c}
5	TiF_4 Solution	5.14 ± 0.39^{c}

Values are expressed as mean ± standard deviation (SD). Superscript letters indicate statistically significant differences between groups as determined by one-way ANOVA with Tukey's post hoc test (***p* < 0.05**). Fluoride agents sharing the same superscript letter are not significantly different from each other

mitochondrial permeability alterations and apoptotic signaling, was calculated for each fluoride agent. The *Bax/Bcl-2* ratios are presented in Table 4. Regarding the cytotoxic and genotoxic effects, although APF, NaF gels, and SDF solution exhibited relatively lower cytotoxic and genotoxic effects compared to other fluoride agents, the *Bax/Bcl-2* ratios for all fluoride agents evaluated in the present study were found to be greater than 1, indicating that these agents induce apoptosis at the gene expression level.

Discussion

The biocompatibility of topical fluoride agents is a critical factor influencing their widespread use in preventive and minimally invasive dentistry. While these agents are widely recognized for their efficacy in reducing demineralization and enhancing remineralization, their potential cytotoxic and genotoxic effects warrant careful consideration, particularly when used in pediatric or medically compromised populations. Numerous studies have highlighted the adverse cellular responses induced by high concentrations of fluoride, including mitochondrial dysfunction, apoptosis, and chromosomal instability in oral and non-oral cell lines [14, 15].

Our findings indicate that SDF exhibited the lowest cytotoxic and apoptotic impact across both cell lines, aligning with previous in vitro study highlighting its relative safety on oral fibroblasts [16]. The high biocompatibility of SDF supports its expanded use not only for arresting cavitated lesions but also in preventive protocols, especially where patient cooperation is limited.

In the present study, we demonstrated that among the five fluoride agents evaluated, SDF solution consistently exhibited the most favorable biocompatibility profile, showing minimal cytotoxic and genotoxic effects in both L929 and HGF cell lines. This finding aligns with reports by Lopez-Garcia et al. who noted the low cytotoxicity of SDF on human gingival fibroblasts and its relative safety in vitro [16]. Although fluoride agents are not intended for prolonged mucosal contact, transient exposure during clinical application may elicit biological responses in gingival fibroblasts, thereby justifying their inclusion as a relevant in vitro model for biocompatibility assessment.

Similarly, Mei et al. [10] and Duangthip et al. [12] reported its clinical success in arresting caries while posing minimal risk to soft tissue. Our observation that SDF induced negligible apoptotic activity and maintained cell viability suggests its potential as a safer topical agentA, especially in non-invasive protocols and high-risk patient populations.

Conversely, TiF_4 and NaF varnish displayed significant cytotoxic and apoptotic activity, corroborated by the elevated Bax/Bcl-2 ratios in both cell lines. These results are consistent with previous in vitro study that has identified TiF_4 as a highly reactive compound capable of disrupting cell membranes and triggering oxidative stress responses [16]. Although TiF_4 has shown beneficial effects in increasing enamel resistance through glaze-like layer formation, its biocompatibility concerns may limit its direct application to soft tissues or mucosa [8].

APF and NaF gels demonstrated moderate cytotoxicity, but were well tolerated in HGF cells. These results are in agreement with Tsai et al., who found that 1.23% APF gel induced apoptosis in rabbit oral mucosa but noted that its effects were concentration- and exposure-dependent [15]. Our findings reinforce the idea that both APF and NaF gels are relatively safe when used appropriately and remain clinically effective for fluoride delivery in pediatric dentistry.

The elevated Bax/Bcl-2 ratios observed in all treatment groups suggest that even agents with relatively low cytotoxicity (e.g., SDF, APF) can induce apoptosis at the molecular level through mitochondrial pathways. However, the magnitude of these effects must be interpreted in context. For instance, although all fluoride agents demonstrated Bax/Bcl-2 ratios > 1, SDF induced the lowest magnitude of gene expression change, indicating a lower potential to trigger apoptotic cascades. These findings are supported by Bogdal et al. (2013), who proposed that Bax/Bcl-2 expression ratios are sensitive indicators of fluoride-induced stress, even in sub-cytotoxic conditions [17].

From a clinical perspective, our data underscore the importance of selecting fluoride agents not only based on efficacy but also on cellular safety. Given the low apoptotic and genotoxic potential of SDF, coupled with its well-documented antimicrobial and caries-arresting properties, it emerges as a preferred option for non-invasive caries management, particularly in populations with reduced cooperation or where aerosol-generating procedures must be minimized [10–12].

To minimize potential adverse effects, we advocate for the use of protective barriers such as rubber dams, petroleum jelly, or gingival protectors during fluoride application. Delivery methods should be optimized using localized approaches (e.g., cotton pellet application) with strong suction to avoid systemic ingestion. These recommendations align with protocols established by Horst et al. on fluoride safety [11].

One of the principal strengths of this study lies in its comprehensive and comparative approach to evaluating the biocompatibility of multiple clinically relevant fluoride agents. By employing a combination of advanced in vitro techniques including real-time impedance-based cytotoxicity analysis (xCELLigence), flow cytometry-based apoptosis assays (Annexin V/PI), TUNEL assay for DNA fragmentation, and RT-PCR for apoptotic gene expression profiling this study provides a robust, multidimensional assessment of fluoride-induced cellular responses. The dual use of L929 fibroblast and primary human gingival fibroblast (HGF) cell lines further enhances the translational value of the findings, offering insights into both general fibroblast sensitivity and tissue-specific oral cell responses.

Additionally, the inclusion of fluoride agents widely used in current dental practice (e.g., SDF, APF gel, TiF_4) ensures clinical relevance and provides evidence-based guidance for their safe and effective application, particularly in pediatric and high-risk populations.

However, several limitations must be acknowledged. First, although in vitro models offer controlled environments to assess cellular responses, they cannot fully replicate the complexity of in vivo conditions, including factors such as salivary buffering, immune modulation, and tissue regeneration. Second, while the study used IC_{50} concentrations to standardize comparisons, it does not account for the variable exposure durations and bioavailability of fluoride agents in clinical settings. Third, the analysis focused primarily on early-stage apoptotic and genotoxic markers; long-term or chronic effects of repeated fluoride exposure remain unexplored. Lastly, while the RT-PCR data provide molecular insight into apoptosis, additional protein-level confirmation (e.g., Western blotting) could further substantiate the observed gene expression trends.

Despite these limitations, the study offers meaningful contributions to the literature on fluoride biocompatibility and underscores the importance of safety-informed decision-making in preventive dentistry.

In conclusion, the current study contributes valuable comparative data on the biocompatibility of commonly used fluoride agents. Among them, SDF exhibited relatively higher cellular compatibility compared to other agents tested, while maintaining therapeutic efficacy, may represent a viable option for broader use in modern caries management strategies. Comparisons with existing literature confirm the consistency of these findings and highlight the importance of integrating biocompatibility assessments into fluoride agent selection. Future studies, particularly in vivo and clinical trials, are warranted to validate these observations and refine best-practice guidelines.

Abbreviations

APF	Acidulated Phosphate Fluoride
NaF	Sodium Fluoride
TiF_4	Titanium Tetrafluoride
SDF	Silver Diamine Fluoride
PCR	Polymerase Chain Reaction
HGF	Human gingival fibroblast
DMEM	Dulbecco's Modified Eagle Medium

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

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Author contributions

B.A., D.C. and C.G. conceived the ideas; B.A., Ç.K., and D.C. collected the data; B.A., Ç.K., D.C. and C.G. analyzed the data, and B.A., and D.C. led the writing. All authors read and approved the final manuscript.

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Data availability

All data that support the findings of this study have been deposited in the Ege University Faculty of Dentistry Archive.

Declarations

Ethics approval and consent to participate

This study was approved by the Ege University Faculty of Medicine Medical Research Ethics Committee (Reference Number: 12 – 7/33) and was conducted in accordance with the ethical principles of the Declaration of

Helsinki (2013 version). Written informed consent was obtained from all participating children and their parents.

Competing interests
The authors declare no competing interests.

Consent for publication
Written informed consent for publication was obtained from all participants and their legal guardians prior to their inclusion in the study.

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