

Original Research

Effects of Fluor Protector S extracts on gingival fibroblast metabolic activity in vitro



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ARTICLE INFO

Article history:

Received 7 July 2026

Accepted 27 August 2026

Available online 6 October 2026

Keywords:

Cell survival

Fibroblasts

Fluorides, topical

Materials testing

Metabolism

ABSTRACT

Objectives: This study aimed to evaluate the effect of Fluor Protector S[®] extracts on the relative metabolic activity of immortalized human gingival fibroblasts in vitro.

Methods: Human fibroblasts were cultured under standard conditions until approximately 80% confluence. The varnish was applied to hydroxyapatite disks, and extracts were obtained according to ISO 10993-12. Extract dilutions of 100%, 75%, 50%, and 25%, with an untreated control, were tested in three independent experimental runs. Metabolic activity was evaluated at 24 and 72 hours using the resazurin method and expressed relative to the within-run control. Technical replicate wells were averaged within each run and condition. Statistical analysis included a two-way ANOVA without replication (run as block, dilution as treatment) and one-sample t-tests against the normalized control ($\alpha = 0.05$). Results were interpreted as exploratory given the small number of runs.

Results: At 24 hours, metabolic activity was significantly lower in the 100%, 75%, and 50% groups than in the control, with the lowest values for the least diluted extracts. The 25% extract did not differ significantly from the control and showed significantly higher metabolic activity than the 100% and 75% extracts. At 72 hours, activity remained significantly lower than the control in the 100% and 75% groups, whereas the 50% and 25% groups did not differ significantly from the normalized control.

Conclusions: Fluor Protector S[®] extracts reduced relative metabolic activity of human gingival fibroblasts, particularly at lower dilutions. Findings should be interpreted cautiously, and further studies using complementary methods and models are needed. (Rev Port Estomatol Med Dent Cir Maxilofac. 2026;67(x):1-9)

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<http://doi.org/10.24873/j.rpemd.2026.09.1587>

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Introduction

Fluoride varnishes are topical agents widely used in dental practice to prevent dental caries due to their ability to enhance enamel remineralization and reduce the cariogenic activity of dental biofilm. Their effectiveness is associated with prolonged adhesion to surfaces, allowing sustained fluoride release over time.¹⁻¹⁰ Frequent exposure to low concentrations of topical fluoride has been associated with caries reduction by increasing fluoride availability in saliva and promoting the formation of fluorapatite.^{1-3,6,11-13} Fluorapatite is strongly resistant to acid dissolution and demineralization.¹⁴ Clinical studies, including randomized controlled trials, have reported caries reduction rates ranging from 15% to over 60%, depending on fluoride concentration, frequency of application, and patient risk profile.³ Additionally, fluoride may inhibit bacterial metabolism and reduce acid production.^{1,3,5,11-15} In vitro and in situ studies showed that fluoride varnishes release the most fluoride during the first 24-48 hours after application, although lower, sustained fluoride levels can be detected for days to weeks, suggesting a prolonged protective effect.¹⁶

Despite clinical benefits, fluoride varnishes may exert cytotoxic effects on oral soft-tissue cells. Thus, evaluating their biological effects is important given their widespread use.^{8,9} In vitro studies using human gingival fibroblasts (hGFs) have shown that relative metabolic activity varies according to concentration and exposure time, with higher concentrations reducing activity more than the lower ones.^{7,11,17,18} Relative metabolic activity assays using hGFs are widely used to evaluate dental materials' biological effects, given these cells' sensitivity to harmful agents, ease of culture, and role in soft-tissue repair.¹⁹ Therefore, their response to dental materials is clinically relevant, particularly in situations where fluoride varnishes may contact soft tissues.

This study aimed to evaluate the effect of Fluor Protector S[®] extracts on the relative metabolic activity of immortalized hGFs in vitro. The null hypothesis tested was that exposure to different extract dilutions would not affect fibroblast relative metabolic activity.

Materials and Methods

Immortalized hGFs (HGF-hTERT; Applied Biological Materials Inc., Richmond, BC, Canada) were cultured in Dulbecco's Modified Eagle's Medium (DMEM, high glucose; Sigma-Aldrich, St. Louis, MO, USA; RNB0075), supplemented with 10% fetal bovine serum (Biowest, Nuaille, France; Batch S00T5) and 1% penicillin/streptomycin (G255; Applied Biological Materials Inc.), at 37 °C, 97% humidity, and 5% CO₂, until reaching approximately 80% confluence (seven days). Cells were enzymatically detached with trypsin-EDTA (Lonza, Verviers, Belgium; L0930-100) for 5–15 min, and viable cells were identified by Trypan Blue (Amresco, Solon, OH, USA, 0485C027) staining and counted using a Neubauer chamber. The cell suspension was adjusted to a density of 1×10^4 cells in 100 μ L of culture medium per well, seeded in 96-well plates, and incubated for 24 h before exposure to the experimental conditions (Figure 1).

Fluor Protector S[®] varnish (Ivoclar Vivadent, Schaan, Liechtenstein, ZL00GC) was applied with a microbrush to one surface of ten hydroxyapatite disks (Clarkson Chromatography, South Williamsport, PA, USA) (8-mm diameter and 1.8-mm thickness) (Figure 2). The disk surface was fully coated with a single drop, which was left in place for 3 min. The volume or mass of varnish applied per disk was not quantitatively measured.

For extract preparation, each varnish-coated disk was individually immersed in 100 μ L of complete DMEM in a separate well. Three independent extract preparations were performed, each using ten freshly coated hydroxyapatite disks. The entire

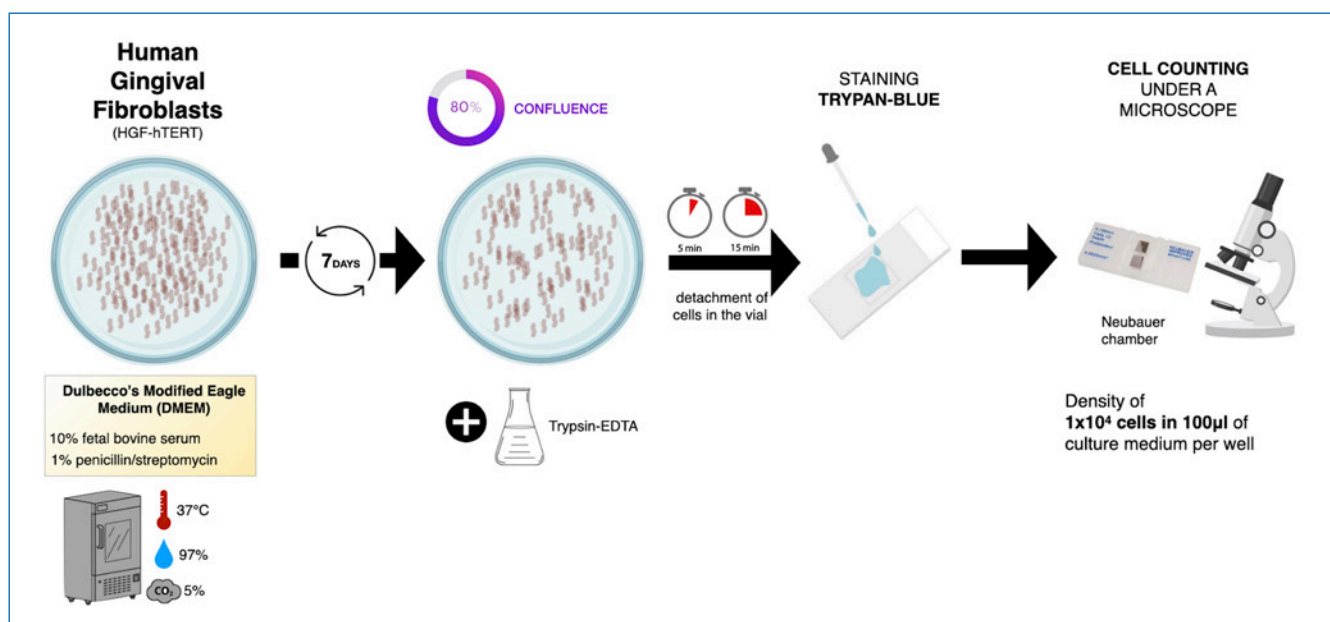
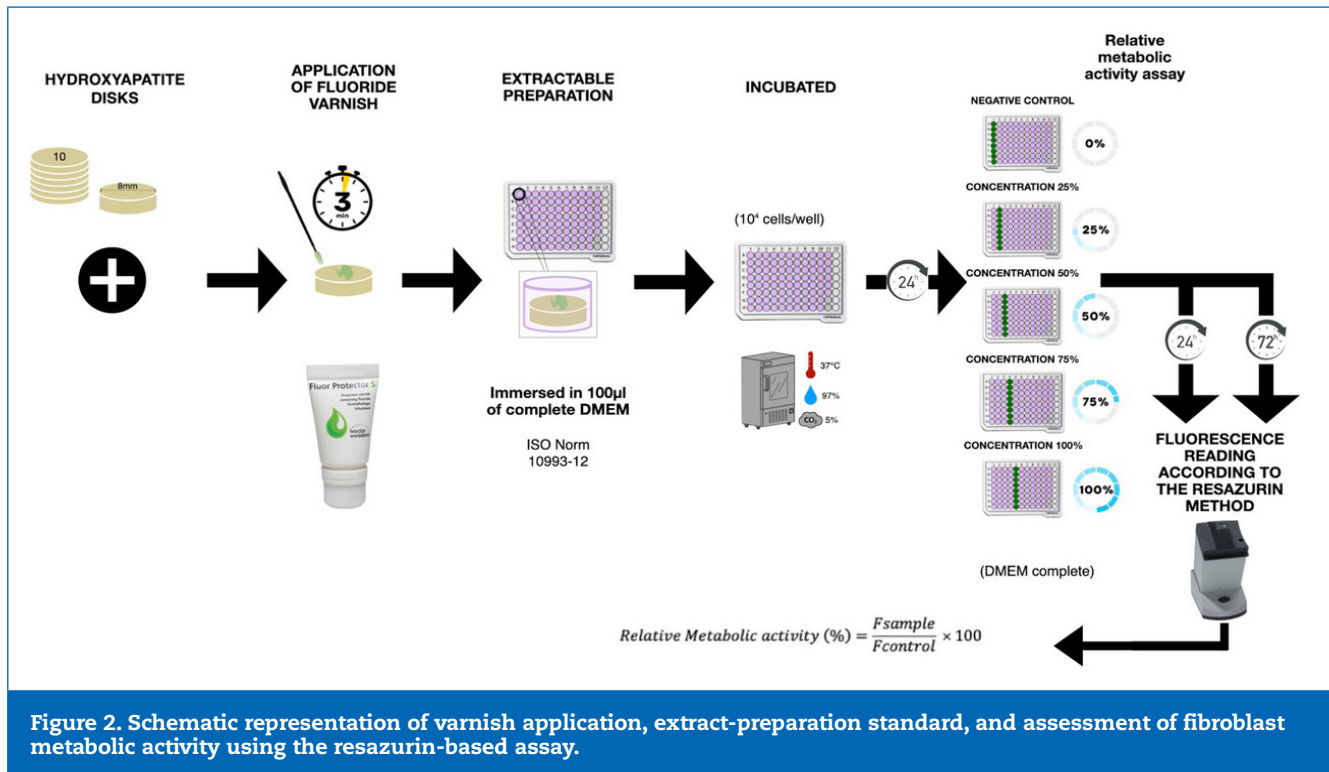


Figure 1. Schematic of the experimental workflow for cell culture.



extraction procedure was repeated for each experimental run. Based on the coated circular surface of each disk, the ratio of surface area to extraction medium volume was approximately 5.03 cm²/mL. The disks were incubated in complete DMEM for 24 h at 37 °C to obtain extracts, using ISO 10993-12 as the methodological reference.²⁰

After extraction, the medium obtained from the ten individually extracted disks was pooled to produce a single stock extract for each experimental run. The undiluted extract was designated as the 100% extract. The 75%, 50%, and 25% extract dilutions were prepared with complete DMEM at extract-to-medium ratios of 3:1, 1:1, and 1:3, respectively. A negative control containing complete DMEM without extract was included. The medium of the previously seeded hGF cultures was replaced with 100 µL of the corresponding experimental solution.

Relative metabolic activity was assessed at 24 and 72 h using a resazurin-based assay (CellTiter-Blue® reagent; Promega, Madison, WI, USA), according to the manufacturer's instructions and using ISO 10993-5 as the methodological reference for in vitro cytotoxicity assessment.²¹ A working solution containing CellTiter-Blue® reagent and complete medium at a 1:9 reagent-to-medium ratio was prepared by mixing 300 µL of reagent with 2.7 mL of medium, and 100 µL of this mixture was used per well. After 4 h of incubation with the reagent at 37 °C, fluorescence was measured using a Victor Nivo™ Multimode Plate Reader (PerkinElmer®, Waltham, MA, USA) at excitation and emission wavelengths of 560 and 590 nm, respectively. The linear response range of the assay was not formally verified under the present experimental conditions. However, inspection of the raw fluorescence data revealed no evidence of signal saturation, such as clustering of values near the upper detection limit of the plate reader.

Following the 24-h exposure period, the exposure medium was removed and replaced with 100 µL of the CellTiter-Blue working solution. After 4 h of incubation, fluorescence was measured. The assay solution was then removed, and the original wells containing the adherent cells were then replenished with 100 µL of fresh complete DMEM, without further exposure to the extracts, and maintained in culture for an additional 48 h before reassessment at 72 h using the same resazurin-based procedure. Therefore, the 72-h assessment reflects an initial 24-h exposure to the extracts followed by a 48-h extract-free period rather than continuous 72-h exposure.

Relative metabolic activity was calculated as follows:

$$\text{Relative metabolic activity (\%)} = \frac{F_{\text{sample}}}{F_{\text{control}}} \times 100$$

where F_{sample} is the background-corrected fluorescence of extract-exposed wells and F_{control} is the mean background-corrected fluorescence of the corresponding untreated control wells. Background fluorescence was determined using cell-free blank wells containing 100 µL of complete DMEM and CellTiter-Blue and was subtracted from all fluorescence measurements before normalization.

Three independent experimental runs were performed using the same cell passage. For each run, a freshly prepared extract was independently generated using a new set of varnish-coated hydroxyapatite disks. Four technical replicate wells were planned per condition within each run; Table 1 reports the number of valid wells and reasons for exclusion. No formal sample-size calculation was performed because the study was designed as an exploratory in vitro investigation using three independent experimental runs.

Table 1. Sample exclusions by time point, experimental group, and run.

Time point	Run	Group	Initial n	Excluded samples	Reason for exclusion	Final n*
24h	1	Control	4	0		4
	2	Control	4	0		4
	3	Control	4	0		4
	1	100%	4	0		4
	2	100%	4	0		4
	3	100%	4	1	Contamination	3
	1	75%	4	0		4
	2	75%	4	1	Pipetting error	3
	3	75%	4	0		4
	1	50%	4	1	Technical fault	3
	2	50%	4	0		4
	3	50%	4	0		4
	1	25%	4	1	Contamination	3
	2	25%	4	0		4
	3	25%	4	0		4
	1	Control	4	0		4
	2	Control	4	0		4
	3	Control	4	0		4
72h	1	100%	4	0		4
	2	100%	4	0		4
	3	100%	3	0		3
	1	75%	4	0		4
	2	75%	3	0		3
	3	75%	4	0		4
	1	50%	3	0		3
	2	50%	4	0		4
	3	50%	4	0		4
	1	25%	3	0		3
	2	25%	4	0		4
	3	25%	4	0		4

Initial n, initial number of samples prepared; Excluded samples, number of samples excluded during or after experimental runs; * Final n, final sample size (calculated as Initial n minus excluded samples).

Statistical analyses were performed using IBM SPSS Statistics, version 30.0 (IBM Corp., Armonk, NY, USA). Technical replicate wells were averaged within each run and experimental condition to obtain one run-level value per condition. The experimental run (n = 3), rather than the individual technical well, was considered the independent statistical unit. Technical exclusions were applied before calculation of the within-run control mean and subsequent normalization. Data were

normalized separately within each experimental run and time point to the mean fluorescence of the corresponding untreated control, defined as 100%.

Descriptive statistics were calculated from the three run-level values for each extract dilution and assessment time. At each time point, a two-way blocked ANOVA without replication was performed, with experimental run included as a blocking factor and extract dilution as the treatment factor. Because only one aggregated observation was available for each run and dilution combination, the run-by-dilution interaction could not be estimated separately from residual error. Accordingly, the blocked ANOVA assumes an additive run-and-treatment structure.

Pairwise comparisons of estimated marginal means between extract dilution groups were performed using the pooled error term from the blocked ANOVA model, without adjustment for multiple comparisons, given the exploratory nature of the analyses.

Each extract dilution was additionally compared with the normalized reference value of 100% using a two-sided one-sample t-test based on the three independent run-level observations. Given the small number of independent experimental runs, all inferential analyses were considered exploratory. Statistical significance was set at $\alpha = 0.05$. Residual normality of the blocked ANOVA models was assessed using the Shapiro-Wilk test (24 h: $W = 0.920$, $p = 0.285$; 72 h: $W = 0.982$, $p = 0.989$), which provided no evidence against normality. However, the limited number of independent experimental units substantially restricts the power of this diagnostic. Since the blocked design and small number of independent observations also limited the formal assessment of homogeneity of variance, residual dispersion and individual run-level observations were examined descriptively (Figure 3).

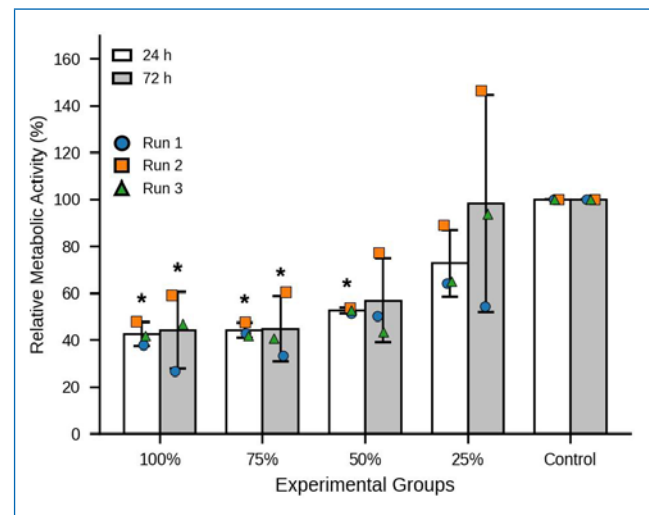


Figure 3. Relative metabolic activity after 24 and 72 hours. Bars represent mean $\pm$ standard deviation (n = 3 independent experimental runs) expressed as a percentage of the corresponding within-run untreated control. Individual points represent the value of each experimental run (circle = Run 1; square = Run 2; triangle = Run 3). * indicates a statistically significant difference from the control ($p < 0.05$, one-sample t-test).

Table 2. Two-way ANOVA for relative metabolic activity across groups and experimental runs.

Time point	Source of variation	Sum of squares	df	Mean square	F	p-value
Relative metabolic activity (24 h)	Group	1733.609	3	577.870	16.734	0.003
	Run (block)*	268.715	2	134.358	—	—
	Error	207.198	6	34.533	—	—
	Total	2209.523	11	—	—	—
Relative metabolic activity (72 h)	Group	5834.469	3	1944.823	6.802	0.023
	Run (block)*	4137.315	2	2068.658	—	—
	Error	1715.518	6	285.920	—	—
	Total	11687.303	11	—	—	—

* Experimental run was treated as a blocking factor to control for inter-assay variability.

Because the same cell cultures were assessed at 24 and 72 h, the two assessment times were not statistically independent. However, no inferential comparison between time points was performed. Analyses were conducted separately at each assessment time and no conclusions regarding a time effect or interaction between time and dilution were drawn.

Results

The results showed a significant overall effect of extract dilution on relative metabolic activity at 24 hours ($F(3,6) = 16.734$, $p = 0.003$) (Table 2). The 100%, 75%, and 50% groups showed a significant reduction in relative metabolic activity, expressed as a percentage of the corresponding within-run untreated control (Table 3, Figure 3). The 100% group showed the lowest relative metabolic activity (mean $42.43\% \pm 5.08\%$; $p = 0.003$ vs. control) (Table 4), followed by the 75% group ($44.10\% \pm 3.18\%$; $p = 0.001$) and the 50% group ($52.50\% \pm 1.35\%$; $p < 0.001$). The 25% group showed the highest relative metabolic activity among the treated groups ($72.67\% \pm 14.15\%$) and did not differ significantly from the control ($p = 0.079$) (Table 4). Pairwise comparisons showed that the 25% group differed significantly from all other extract dilutions ($p \leq 0.006$), whereas the 100%,

Table 3. Descriptive statistics of cell relative metabolic activity (%) at 24 and 72 hours in the control and in the 100%, 75%, 50%, and 25% groups. Values are expressed as mean, standard deviation, minimum, and maximum.

Time point	Group	n	Mean	SD	Minimum	Maximum
24 h	Control	3	100.00	0.00	100.00	100.00
	100%	3	42.43	5.08	37.70	47.80
	75%	3	44.10	3.18	41.70	47.70
	50%	3	52.50	1.35	51.10	53.80
	25%	3	72.67	14.15	64.10	89.00
72 h	Control	3	100.00	0.00	100.00	100.00
	100%	3	44.10	16.42	26.50	59.00
	75%	3	44.77	14.02	33.30	60.40
	50%	3	56.90	17.92	43.30	77.20
	25%	3	98.13	46.25	54.20	146.40

75%, and 50% groups did not differ significantly from each other ($p \geq 0.081$) (Table 5).

At 72 hours, the overall effect of extract dilution remained significant, although weaker than at 24 hours ($F(3,6) = 6.802$,

Table 4. Relative metabolic activity of extract dilution group compared with the untreated control (one-sample t-test, test value = 100%).

Time point	Group	Mean (SD)	n	SE	95% CI	t (vs. 100)	p-value
24 h	100%	42.43 (5.08)	3	2.93	29.81 to 55.05	-19.63	0.003
	75%	44.10 (3.18)	3	1.83	36.21 to 51.99	-30.50	0.001
	50%	52.50 (1.35)	3	0.78	49.14 to 55.86	-60.82	< 0.001
	25%	72.67 (14.15)	3	8.17	37.51 to 107.82	-3.35	0.079
72 h	100%	44.10 (16.42)	3	9.48	3.32 to 84.88	-5.90	0.028
	75%	44.77 (14.02)	3	8.10	9.93 to 79.60	-6.81	0.021
	50%	56.90 (17.92)	3	10.34	12.40 to 101.40	-4.17	0.053
	25%	98.13 (46.25)	3	26.70	-16.76 to 213.03	-0.07	0.950

Note: The control is the fixed normalization reference (mean = 100%, SD = 0 by construction); it was used as the test value in this table and was excluded from the ANOVA (Table 2) and the pairwise comparisons (Table 5).

Table 5. Pairwise comparisons of relative metabolic activity among extract dilution groups (unadjusted pairwise comparisons based on the pooled error term from the blocked ANOVA model).

Time point	Group comparison	Mean difference	Std. error	95% CI	p-value
Relative metabolic activity (24 h)	100% vs 75%	-1.667	4.798	-13.407 to 10.073	0.740
	100% vs 50%	-10.067	4.798	-21.807 to 1.673	0.081
	100% vs 25%	-30.233	4.798	-41.973 to -18.493	< 0.001
	75% vs 50%	-8.400	4.798	-20.140 to 3.340	0.131
	75% vs 25%	-28.567	4.798	-40.307 to -16.827	0.001
	50% vs 25%	-20.167	4.798	-31.907 to -8.427	0.006
Relative metabolic activity (72 h)	100% vs 75%	-0.667	13.806	-34.449 to 33.115	0.963
	100% vs 50%	-12.800	13.806	-46.582 to 20.982	0.390
	100% vs 25%	-54.033	13.806	-87.815 to -20.251	0.008
	75% vs 50%	-12.133	13.806	-45.915 to 21.649	0.413
	75% vs 25%	-53.367	13.806	-87.149 to -19.585	0.008
	50% vs 25%	-41.233	13.806	-75.015 to -7.451	0.024

$p = 0.023$) (Table 2). Relative metabolic activity in the 100% and 75% groups remained significantly lower than that of the normalized control ($44.10\% \pm 16.42\%$, $p = 0.028$ and $44.77\% \pm 14.02\%$, $p = 0.021$, respectively) (Table 4). The 50% group also showed lower mean relative metabolic activity, although the difference from the normalized control was not statistically significant ($p = 0.053$). The 25% group showed the highest relative metabolic activity among the treated groups ($98.13\% \pm 46.25\%$), with substantial between-run variability, and did not differ significantly from the control ($p = 0.950$) (Table 3, Table 4, Figure 3). Pairwise comparisons showed that the 25% group differed significantly from the 100%, 75%, and 50% groups ($p \leq 0.024$), whereas these three groups did not differ significantly from each other ($p \geq 0.390$) (Table 5).

Discussion

The results of the present study showed reduced relative metabolic activity in hGFs following exposure to Fluor Protector S[®] extracts under the tested in vitro conditions. At 24 hours, the 100%, 75%, and 50% groups showed significantly lower relative metabolic activity than the control, with greater reductions observed in the less diluted extracts. The 25% group did not differ significantly from the control ($p = 0.079$), although the reduction was directionally consistent with that observed for the other dilutions. Previous in vitro studies have similarly reported reduced relative metabolic activity of gingival fibroblasts and human gingival cells following exposure to fluoride-containing dental materials.^{18,19} Although these findings are broadly consistent with the present results, differences in material composition, extract dilution, exposure conditions, and experimental models should be considered when comparing studies. A recent review of fluoride varnish formulations currently recommended in dental prophylaxis highlights this heterogeneity across products.²²

At 72 hours, relative metabolic activity remained statistically lower than the control in the less diluted 100% and 75% extract groups, whereas the 25% group did not differ significantly from the control. The 72-hour assessment reflected an initial 24-hour exposure to the extracts followed by a 48-hour extract-free period in the same wells, rather than continuous exposure for 72 hours. Because no continuous 72-hour exposure condition was tested and no direct statistical comparison between time points was performed, these findings should not be interpreted as evidence of recovery, persistence, or a temporal plateau. They should instead be interpreted specifically within the context of the 48-hour extract-free interval. Previous evidence indicates that the biological effects of fluoride compounds on fibroblasts may be influenced by concentration, exposure time, and the experimental model.¹⁷

Considerable between-run variability was observed in raw control fluorescence at 72 hours. Because data were normalized within each run to that run's own control, this variability is not reflected in the normalized control values, which were fixed at 100% by construction, but may contribute to dispersion in the normalized values of the treated groups. This was most evident in the 25% group, which showed the greatest between-run variability ($SD = 46.25$). This pattern suggests the presence of between-run variability in baseline fluorescence rather than solely random well-to-well variation. Normalization does not eliminate this underlying variability, which represents a limitation of the study.

The resazurin-based assay is a sensitive method for assessing cellular metabolic activity. However, resazurin reduction primarily reflects cellular metabolic status and should not be interpreted as a direct measure of irreversible cell damage or cell death. The observed reductions in fluorescence should therefore be interpreted specifically as reduced relative metabolic activity under the tested experimental conditions rather than as definitive evidence of cytotoxicity.²³ In

addition, the linear response range of the resazurin assay was not formally verified under the present experimental conditions, although no evidence of fluorescence signal saturation was observed.

The mechanisms underlying the observed reduction in relative metabolic activity cannot be determined from the present study. Previous experimental evidence suggests that fluoride-related cellular effects may involve mitochondrial dysfunction, oxidative stress, and activation of apoptosis-related pathways, depending on concentration and exposure conditions.^{24,25} However, these mechanisms were not directly investigated here. Moreover, because Fluor Protector S[®] is a multicomponent formulation, the observed biological response cannot be attributed exclusively to fluoride. Previous studies comparing commercial fluoride varnishes, including Fluor Protector S[®], have demonstrated formulation-dependent differences in their biological effects on hGFs.⁸ Therefore, components of the varnish formulation and their extractable products may also contribute to the observed cellular response. Further studies combining metabolic assays with assessments of oxidative stress, mitochondrial function, apoptosis, and membrane integrity are required to clarify the mechanisms underlying these effects.

The clinical relevance of these findings should be interpreted with caution, as the present *in vitro* model does not fully reproduce the dynamic and complex conditions of the oral environment. The use of an immortalized hGF cell line (HGF-hTERT), while providing reproducibility and consistency across experiments, may not fully reflect the physiological variability and biological responses of primary human gingival cells. Furthermore, the experimental conditions did not reproduce factors such as salivary flow, dilution, buffering capacity, protective salivary components, and tissue repair mechanisms that may influence the biological response to fluoride varnishes *in vivo*.

Additional methodological limitations should also be acknowledged. The present study evaluated a single fluoride varnish (Fluor Protector S[®]), which limits comparisons with other commercially available formulations. The varnish applied per disk was not measured quantitatively by volume or mass, which is considered a methodological limitation. The biological assessment was restricted to relative metabolic activity using the resazurin assay and did not include complementary endpoints such as membrane integrity, apoptosis, inflammatory responses, or oxidative stress.

Moreover, all three independent experimental runs used the same cell passage; therefore, biological replication across independent cell passages was not assessed. Because the same cell cultures were assessed at both time points, cultures evaluated at 72 hours had previously been exposed to the resazurin reagent during the 24-hour assessment. Although the assay medium was subsequently removed and replaced with fresh complete DMEM, a potential influence of the first assay exposure on subsequent cellular metabolic behavior cannot be excluded.

The control strategy was limited to untreated cell cultures maintained in complete DMEM. Although cell-free assay blank wells containing complete DMEM and CellTiter-Blue were used for background correction, no cell-free

varnish-extract blank was included. Consequently, the present experimental design cannot completely distinguish potential effects associated with the varnish from those related to the hydroxyapatite extraction substrate, nor can it exclude possible optical or chemical interference from extract components with the resazurin signal. These factors should also be considered limitations of the study. Accordingly, the present findings do not allow differentiation between reduced cellular metabolism and reduced proliferation and should therefore be interpreted specifically as changes in relative metabolic activity rather than as evidence of other cellular effects.

The observed biological response supports further investigation of fluoride varnish exposure effects on oral soft tissues under clinically relevant conditions. Future studies should incorporate independent biological replication, complementary biological assays, additional fluoride varnish formulations, and experimental models that more closely reproduce the dynamic conditions of the oral environment to provide a more comprehensive assessment of the biological response to Fluor Protector S[®].

Conclusions

Under the tested *in vitro* conditions, exposure to Fluor Protector S[®] extracts was associated with reduced relative metabolic activity of hGFs, with greater reductions observed for the less diluted extracts. Since only three independent experimental runs using the same cell passage were performed and metabolic activity was assessed using a single resazurin-based assay, these findings should be interpreted cautiously and cannot be considered evidence of irreversible cell death or direct clinical cytotoxicity. Further studies incorporating independent biological replication, complementary cellular endpoints, and experimental models that more closely reproduce the oral environment are needed to characterize the biological effects of Fluor Protector S[®] more comprehensively.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Marta Martins Coelho: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Resources; Validation; Writing – original draft; Writing – review & editing. **Inês Cardoso-Martins:** Conceptualization; Methodology; Resources; Validation; Visualization; Writing – original draft; Writing – review & editing. **Neusa Silva:** Conceptualization; Methodology; Resources; Supervision; Visualization. **Joana Faria Marques:** Conceptualization; Data curation; Methodology; Resources; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing. **António Mata:** Resources; Validation. **Paula Faria Marques:** Conceptualization; Data curation; Methodology; Resources; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

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Efeito dos extratos de Fluor Protector S na atividade metabólica de fibroblastos gengivais *in vitro*

R E S U M O

Objetivos: Avaliar o efeito de extratos de Fluor Protector S® na atividade metabólica relativa de fibroblastos gengivais humanos imortalizados *in vitro*.

Métodos: Cultivaram-se fibroblastos em condições padrão até 80% de confluência. Aplicou-se o verniz em discos de hidroxiapatite e obtiveram-se os extratos segundo a ISO 10993-12. Avaliaram-se diluições de 100%, 75%, 50% e 25% e um controlo não tratado em três ensaios independentes. A atividade metabólica foi avaliada às 24 e 72 h por resazurina e expressa face ao controlo de cada ensaio. As réplicas técnicas foram agregadas por média em cada ensaio e condição, sendo o ensaio a unidade experimental. A análise usou ANOVA de dois fatores sem repetição (ensaio como bloco, diluição como tratamento) e testes t de uma amostra face ao controlo normalizado ($\alpha = 0,05$). Interpretaram-se os resultados como exploratórios dado o reduzido número de ensaios.

Resultados: Às 24 h, a atividade metabólica foi significativamente inferior nos grupos de 100%, 75% e 50% face ao controlo, com va-

lores mais baixos nos extratos menos diluídos; o grupo de 25% não diferiu do controlo e foi significativamente superior aos grupos de 100% e 75%. Às 72 h, os grupos de 100% e 75% mantiveram-se significativamente inferiores ao controlo, enquanto os grupos de 50% e 25% não diferiram significativamente, com maior variabilidade entre ensaios.

Conclusões: Os extratos de Fluor Protector S® reduziram a atividade metabólica relativa dos fibroblastos, sobretudo nas diluições mais baixas. Os resultados devem ser interpretados com precaução; são necessários estudos com métodos complementares e modelos representativos. (Rev Port Estomatol Med Dent Cir Maxilofac. 2026;67(x):xxx-xxx)

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Palavras-chave:

Sobrevivência celular
Fibroblastos
Fluoretos tópicos
Teste de materiais
Metabolismo