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# E-cigarette aerosol exposure drives extracellular matrix gene dysregulation and collagen remodeling in gingival fibroblasts: An *in vitro* study

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ABSTRACT

*Background:* E-cigarettes are promoted as less harmful alternatives to traditional tobacco products; however, their effects on periodontal tissues and orthodontic outcomes remain unexplored. This study aimed to investigate the impact of e-cigarette aerosol extract (ECAE) on human gingival fibroblasts (hGFs), focusing on cell viability, extracellular matrix (ECM) integrity, gene expression, and nicotine–protein interactions.

*Methods:* Primary hGFs were exposed to ECAE (5–100%) prepared using a standardized puffing protocol. Cell viability was assessed at 24 and 72 h using the MTT assay. ECM-related gene expression with COL1A1, MMP1, MMP9, TIMP1 was quantified by qPCR. Collagen matrix organization and cellular morphology were evaluated using Masson's Trichrome staining and phase-contrast microscopy. Molecular docking was performed to assess nicotine interactions with ECM-associated proteins in 2D and 3D structures.

*Results:* ECAE exposure resulted in a concentration- and time-dependent reduction in fibroblast viability, with significant cytotoxicity observed at concentrations ≥25%. qPCR showed downregulation of COL1A1 and TIMP1, and upregulation of MMP1 and MMP9, indicating a matrix-degradative gene profile. Masson's Trichrome staining revealed reduced collagen deposition and fibrotic band-like structures. Microscopy demonstrated morphological alterations, including cell shrinkage and vacuolation. Docking analysis indicated nicotine binding affinity to COL1A1 with −4.45 kcal/mol and TIMP1 with −5.08 kcal/mol, with stronger affinities for MMP1 with −5.42 kcal/mol and MMP9 (−6.55 kcal/mol), suggesting potential functional interference.

*Conclusion:* ECAE exposure disrupts gingival fibroblast viability and ECM homeostasis through altered gene expression and potential nicotine–protein interactions. These findings suggest potential molecular mechanisms by which e-cigarette aerosols may influence periodontal connective tissue integrity and warrant further investigation using *in vivo* and clinical models.

## 1. Introduction

The increasing popularity of electronic cigarettes as substitutes for traditional tobacco products brought about substantial debate on their safety and potential long-term effects on human health. In contrast to traditional combustible cigarettes, e-cigarettes deliver nicotine via aerosolized vapors, avoiding combustion and consequently minimizing many of the harmful by-products found in tobacco smoke. Despite the suggested benefits of harm reduction, growing evidence shows that e-cigarette aerosols comprise a complex array of substances, including nicotine, aldehydes, carbonyl compounds, volatile organic chemicals, heavy metals, and reactive oxygen species (ROS). Numerous constituents exhibit cytotoxic and pro-inflammatory properties, which raises concerns regarding their effects on oral and systemic tissues.[1,2]

The periodontal tissues in the oral cavity act as an important site for exposure to inhaled aerosols. The predominant gingival connective tissue, human gingival fibroblasts (hGFs), plays a crucial role in maintaining periodontal homeostasis. They regulate extracellular matrix

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(ECM) production, collagen remodeling, wound repair, and inflammatory signaling.[3] The interference with these mechanisms may impair ECM stability and collagen homeostasis, thereby contributing to altered tissue remodeling at the cellular level [4–6].

Research investigations have shown that exposure to e-cigarette aerosols impairs oral cell viability, increases oxidative stress, and impacts the expression of genes related to ECM regulation.[7,8] In particular, disruptions in the balance between genes responsible for collagen synthesis, including COL1A1, and matrix-degrading enzymes such as MMP1 and MMP9, along with a reduction in activity of natural inhibitors like TIMP1, generate an atmosphere favorable to collagen degradation and connective tissue destruction.[4,9] To explore the possible molecular basis of these alterations, molecular docking analysis was performed to predict the interactions between nicotine and key ECM-associated proteins (COL1A1, TIMP1, MMP1, and MMP9). This computational approach was included to provide supportive insight into potential nicotine–protein associations that may relate to the observed cellular and molecular responses. The molecular alterations observed in this study resemble early inflammatory and extracellular matrix–related changes described in periodontal tissues exposed to tobacco-related products, including upregulated pro-inflammatory cytokines and matrix-degrading pathways implicated in attachment loss and bone resorption. These mechanisms contribute to dysregulated tissue remodeling processes in periodontal disease.[10]

Although there is a growing awareness, the specific impact of e-cigarette aerosols on the function of gingival fibroblasts and the integrity of the collagen matrix remains largely unexplored. The increasing prevalence of e-cigarette uses among adults and young adults, a population particularly susceptible to early periodontal issues, makes the investigation of these cellular and molecular changes both urgent and significant. The present research was designed to evaluate the effects of e-cigarette aerosol extract on the viability of human gingival fibroblasts, the expression of ECM-related genes, and collagen deposition patterns. In addition, molecular docking analysis was employed as a complementary approach to predict the potential interactions between nicotine and key ECM-associated proteins, thereby integrating experimental and computational findings to enhance understanding of how vaping may contribute to periodontal tissue remodeling and degradation.

## 2. Materials and methods

### 2.1. Molecular docking

Molecular docking analysis was employed to evaluate the interactions between nicotine, a major constituent of e-cigarette aerosols, and selected extracellular matrix–related protein receptors implicated in the process of collagen synthesis and matrix degradation. The three-dimensional structures of collagen type I alpha 1 chain (COL1A1; PDB ID: 5K31), tissue inhibitor of metalloproteinases-1 (TIMP1; PDB ID: 3MA2), matrix metalloproteinase-1 (MMP1; PDB ID: 3SHI), and matrix metalloproteinase-9 (MMP9; PDB ID: 1L6J) were retrieved from the Protein Data Bank of the Research Collaboratory for Structural Bioinformatics (https://www.rcsb.org/). The three-dimensional conformer of nicotine was retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Protein structures were preprocessed using PyMOL software by removing water molecules and extracting relevant domains where applicable. The ligand structure was converted from SDF to MOL2 format using Open Babel software. Molecular docking analysis was done using MGLTools version 1.5.7. Polar hydrogens and Kollman partial charges were added to the protein structures, which were then converted to PDBQT format. The ligand was converted to PDBQT format before docking. Docking simulations were run using the Lamarckian Genetic Algorithm implemented in AutoDock, and grid maps were generated using AutoGrid. The predicted binding poses, binding affinities, and protein–ligand interactions were visualized and analyzed using BIOVIA Discovery Studio Visualizer,[11] with both 2D and 3D interaction profiles evaluated.

### 2.2. Cell culture and experimental design

Primary human gingival fibroblasts (hGFs) were isolated from healthy gingival tissue samples obtained from adult donors aged 18–25 years undergoing routine dental procedures at Saveetha Dental College and Hospitals, Chennai. The study protocol was reviewed and approved by the Institutional Ethics Committee (IHEC/SDC/FACULTY/22/PHARM/574), and written informed consent was obtained from all participants prior to tissue collection. Briefly, gingival tissues were rinsed in sterile phosphate-buffered saline (PBS) containing antibiotics, finely minced, and subjected to enzymatic digestion using a solution of collagenase type I and dispase for 2–3 h at 37 °C to release fibroblastic cells. The cell suspension was centrifuged, and the pellet was resuspended in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cells were seeded into culture flasks and incubated at 37 °C in a humidified atmosphere containing 5% $CO_2$. Upon reaching 70–80% confluence, cells were passaged using trypsin–EDTA, and subsequent passages (P3–P6) were used for *in vitro* experimental assays, including cell viability, special staining, and gene expression analyses.

### 2.3. Preparation of E-cigarette aerosol extract (ECAE) and sample preparation

E-cigarette aerosol extract (ECAE) was prepared using a commercially available electronic cigarette device equipped with a refillable tank system. Aerosol was generated from a nicotine solution (100 mg/mL stock concentration) formulated in a propylene glycol/vegetable glycerin (PG/VG) base without added flavoring agents. The device was operated under standardized puffing conditions, and the generated aerosol was continuously drawn through a sterile collection system into serum-free DMEM. The collected solution was designated as 100% ECAE, representing the highest exposure concentration used in this study for cytotoxicity and cellular response assays. The extract was filtered through a 0.22 μm sterile membrane to remove particulate matter and used immediately for experiments. Working concentrations of ECAE (50%, 25%, 10%, and 5%) were freshly prepared by serial dilution of the 100% ECAE with complete culture medium. Human gingival fibroblasts were exposed to these concentrations for the MTT assay and subsequent analyses, while cells maintained in culture medium alone served as the untreated control.

#### 2.3.1. Cell viability assay

Human gingival fibroblasts ($5 \times 10^3$ cells/well) were seeded into 96-well plates and allowed to adhere overnight. Cells were then exposed to varying concentrations of e-cigarette aerosol extract (5%, 10%, 25%, 50%, and 100%) and untreated control medium for 24 h and 72 h. Following treatment, the medium was replaced with MTT solution (0.5 mg/mL in phenol-red-free DMEM) and incubated at 37 °C for 3 h to allow mitochondrial dehydrogenases in viable cells to convert MTT into purple formazan crystals. The supernatant was carefully removed, and the crystals were solubilized in 100 μL of dimethyl sulfoxide (DMSO) with gentle shaking for 10 min. Absorbance was measured at 570 nm using a spectrophotometer (BioTek, Winooski, VT, USA) and blank wells

**Table 1**
Primer sequences used in the real-time PCR experiment.

| Gene | Forward primer (5′ → 3′) | Reverse primer (5′ → 3′) |
|---|---|---|
| **MMP9** | GCCACTACTGTGCCTTTGAGTC | CCCTCAGAGAATCGCCAGTACT |
| **MMP1** | ATGAAGCAGCCCAGATGTGGAG | TGGTCCACATCTGCTCTTGGCA |
| **TIMP1** | GGAGAGTGTCTGCGGATACTTC | GCAGGTAGTGATGTGCAAGAGTC |
| **COL1A1** | GTGCTAAAGGTGCCAATGGT | ACCAGGTTCACCGCTGTTAC |
| **GAPDH** | GTCTCCTCTGACTTCAACAGCG | ACCACCCTGTTGCTGTAGCCAA |

**Table 2**
List of amino acids residue and chemical interaction of Nicotine and ECM markers.

| PDB ID | CID | AMINO ACID RESIDUES | BINDING AFFINITY |
|---|---|---|---|
| COL1A1 (5K31) | **NICOTINE** Compound CID: 89594 | TRP51, LYS52, VAL194, ASP195, THR193 | −4.45 |
| TIMP1 (3MA2) | **NICOTINE** Compound CID: 89594 | ALA258, ILE256, TYR 261, GLN 262, HIS 239, VAL 236 | −5.08 |
| MMP1 (3SHI) | **NICOTINE** Compound CID: 89594 | Leu147, Pro146, Arg202, Asp200 | −5.42 |
| MMP9 (1L6J) | **NICOTINE** Compound CID: 89594 | Met422, His401, Tyr420, Glu416 | −6.55 |

containing medium without cells. Cell viability was expressed as a percentage relative to the untreated control, and each condition was tested in triplicate for technical and biological replicates.

### 2.3.2. *Phase-contrast microscopy (morphology)*

After the ECAE exposures at 24 h and 72 h, the cells were imaged under phase contrast (20 × objectives). Morphological endpoints included cell spread area, spindle morphology, cytoplasmic vacuolation, and detachment. For quantitative morphometry, cell area and circularity (ImageJ) were computed from ≥100 cells/condition.

### 2.3.3. *Masson's trichrome staining*

For assessment of collagen deposition and fibrotic remodeling, gingival fibroblasts were cultured on sterile coverslips and exposed to e-cigarette aerosol extract for 72 h. Cells were fixed in 4% para-formaldehyde, post-fixed in Bouin's solution, and subjected to standard

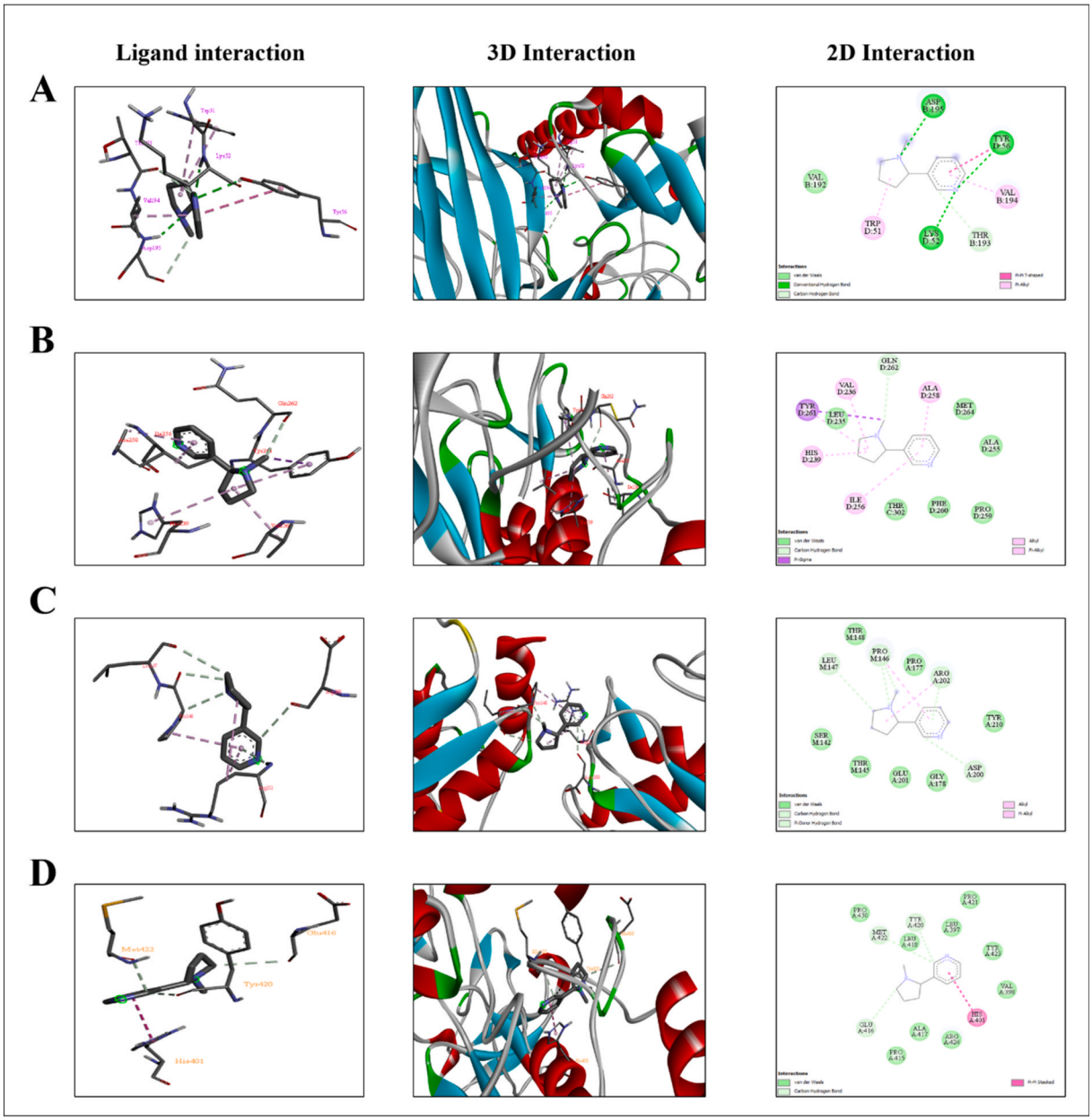


**Fig. 1.** Molecular docking analysis illustrating the interactions between nicotine and selected target proteins involved in collagen synthesis and matrix degradation. (A) Interaction of nicotine with COL1A1; (B) interaction with TIMP1; (C) interaction with MMP1; and (D) interaction with MMP9. Each section displays ligand–protein interactions in both 2D and 3D structural representations, highlighting key amino acid residues and binding affinities.

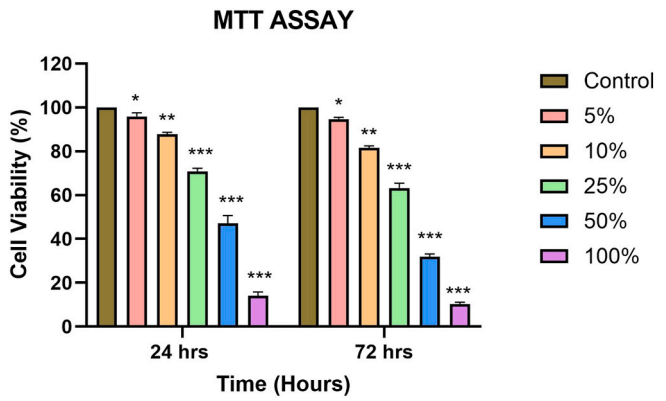


**Fig. 2.** MTT assay data demonstrate a significant concentration- and time-dependent reduction in cell viability of human gingival fibroblasts following E-cigarette aerosol extract (ECAE) exposure at 24 and 72 h. Cell viability is expressed as a percentage relative to the untreated control. Data are presented as mean ± SD from three independent experiments; *p < 0.05, **p < 0.01, and ***p < 0.001 vs control.

Masson's Trichrome staining, including sequential treatment with Weigert's iron hematoxylin, Biebrich scarlet–acid fuchsin, phosphomolybdic–phosphotungstic acid, and aniline blue, followed by a brief acetic acid rinse. Coverslips were dehydrated, cleared in xylene, and mounted in resin medium. Collagen fibers stained blue, cytoplasm red, and nuclei dark brown-black. Stained preparations were visualized under a light microscope at 20 × magnification, and their images were taken. Collagen intensity and distribution patterns, including fibrotic band-like structures, were qualitatively documented and, where applicable, quantified using ImageJ software.

#### 2.3.4. Quantitative real-time PCR (qPCR) gene expression

Total RNA was extracted from treated and control gingival fibroblasts using TRIzol reagent following the manufacturer's protocol, and RNA purity was verified by A260/A280 ratios. One microgram of RNA was reverse transcribed into cDNA using a commercial reverse transcription kit, and quantitative PCR was performed with SYBR Green chemistry on a real-time thermal cycler. Target genes included COL1A1, MMP1, MMP9, and TIMP1, normalized to GAPDH as the reference and their primer sequence listed in Table 1. Primer pairs were validated for specificity and efficiency, and reactions were run in duplicate under the following cycling conditions: 95 °C for 2 min, followed by 40 cycles of 95 °C for 10 s and 60 °C for 30 s, with melt-curve analysis confirming single amplicons. Data were analyzed by the $2^{-\Delta\Delta Ct}$ method, with untreated control cells serving as the calibrator, and fold-change expression was reported as mean ± SD.

### 2.4. Statistical analysis

All data were tested for normality using the Shapiro–Wilk test and for homogeneity of variances using Levene's test. Group comparisons were conducted using one-way ANOVA followed by Tukey's post hoc test, with significance set at $\alpha = 0.05$. When assumptions of normality or equal variance were violated, non-parametric alternatives were applied using the Kruskal–Wallis test with Dunn's post hoc correction. For qPCR data, statistical analyses were performed on ΔCt values, while fold changes are presented in the figures for clarity. All graphs display mean ± standard deviation (SD) with individual data points shown. Statistical analyses were performed using GraphPad Prism software, version 10.4.2 (GraphPad Software, San Diego, CA, USA).

## 3. Results

### 3.1. Molecular docking of nicotine with collagen synthesis protein and matrix-degrading enzymes

The molecular interactions between nicotine and selected protein receptors were validated through docking studies, which revealed favorable binding affinities and specific amino acid interactions, as detailed in Table 2. Notably, nicotine exhibited a binding affinity of −4.45 kcal/mol with COL1A1, forming hydrogen bonds with LYS52, VAL194, ASP195, and THR193, and a π-alkyl interaction with TRP51. For TIMP1, nicotine demonstrated a stronger binding affinity of −5.08 kcal/mol, forming a hydrogen bond with GLN262 and π-alkyl interactions with HIS239, VAL236, ALA258, and ILE256. Furthermore, docking affinity with matrix-degrading enzymes revealed even stronger interactions. Nicotine bound to MMP1 with a binding affinity of −5.42 kcal/mol, involving hydrogen bonds with LEU147, PRO146, ARG202, and ASP200. The highest binding affinity was observed with MMP9, at −6.55 kcal/mol, where nicotine formed hydrogen bonds with MET422, TYR420, and GLU416. These molecular interactions are visually represented in (Fig. 1), highlighting the key binding residues and the nature of ligand-receptor interactions.

### 3.2. Cytotoxicity effects of ECAE on human gingival fibroblasts

#### 3.2.1. MTT assay

The MTT assay revealed an evident dose- and time-dependent cytotoxic impact of ECAE on human gingival fibroblasts. After 24 h, cells treated with lower concentrations of ECAE (5% and 10%) exhibited a slight reduction in viability compared to untreated controls, while significant reductions were observed at concentrations of 25% ECAE. Following a 72-h exposure, the decrease in viability became more evident, with the 25% ECAE group reducing viability to nearly 65–70% of control values. In contrast, the 50% and 100% ECAE groups exhibited significant suppression of metabolic activity, resulting in viability dropping below 40% and 25% of control levels, respectively. The results demonstrate a cumulative cytotoxic effect resulting from extended exposure to ECAE, with a notable decline in fibroblast viability observed at elevated concentrations and prolonged durations, as shown in Fig. 2. The observed dose-dependent reduction of fibroblast viability in this study indicates impaired periodontal healing and attachment among e-cigarette users. Previous clinical studies have associated vaping with increased gingival inflammation, altered immune responses, and higher susceptibility to periodontal breakdown.[12,13] Our results provide direct *in vitro* evidence supporting these clinical correlations, emphasizing that chemical exposure from aerosols alone can compromise fibroblast survival, even in the absence of microbial challenge.

#### 3.2.2. Morphological evaluation through phase-contrast microscopy

The distinct morphological alterations were observed in gingival fibroblasts following exposure to ECAE. In the control, fibroblasts exhibited the characteristic elongated, spindle-shaped morphology, indicative of healthy and metabolically active cells. In contrast, fibroblasts treated with higher concentrations of ECAE, including 50% and 100%, demonstrated concentration-dependent morphological changes, including cytoplasmic shrinkage, cellular rounding, vacuole formation, and loss of the distinctive spindle-like structure, as depicted in Fig. 3a & b. These morphological disruptions were consistent with the reduced cell viability observed in the Masson's Trichrome staining assay, supporting a concentration-dependent cytotoxic effect of ECAE. Furthermore, in groups exhibiting dense, fibrotic band-like collagen staining, fibroblasts were observed to be contracted and clustered, suggesting impaired cell–matrix interactions and altered fibroblast function.

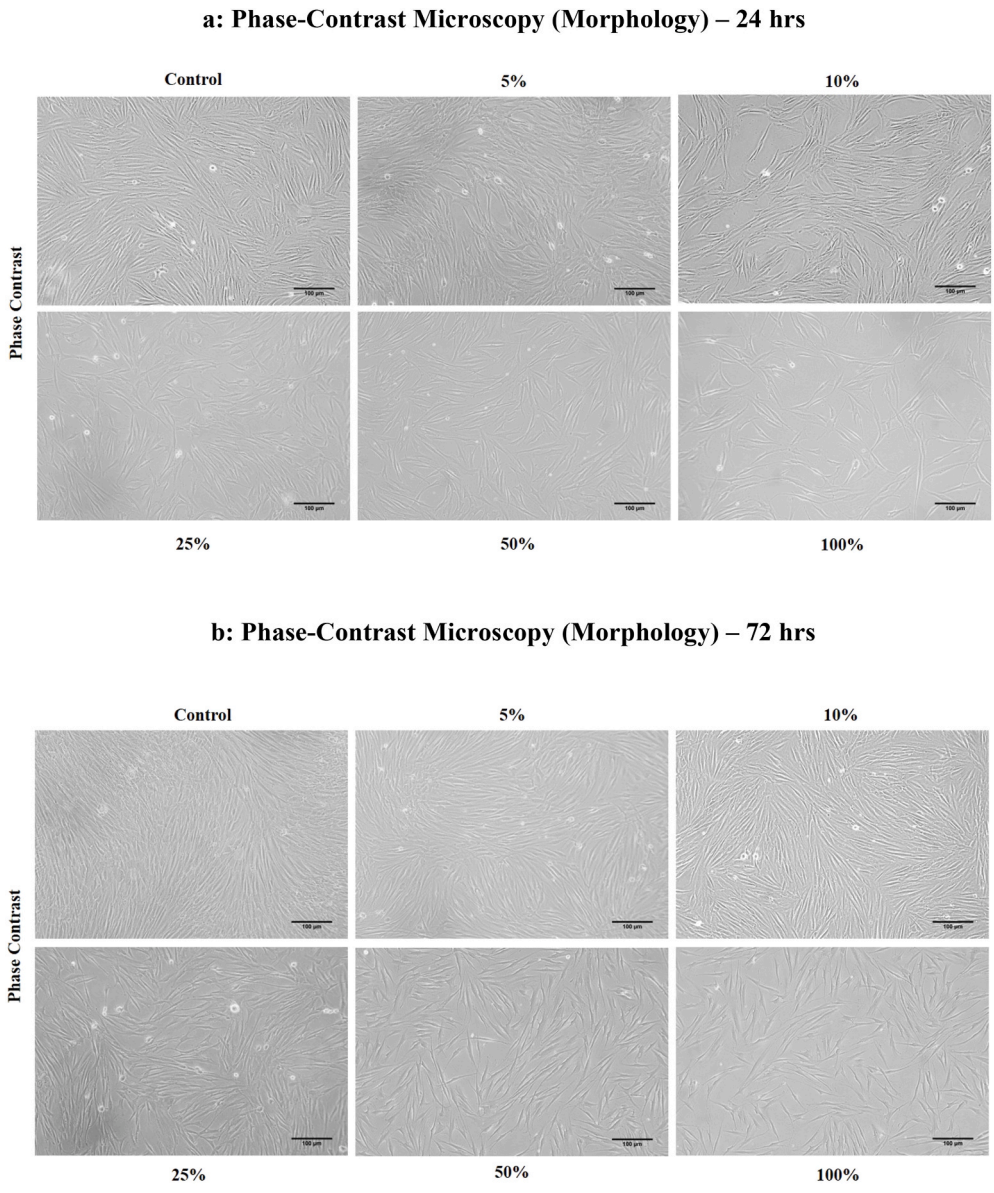


**Fig. 3.** a & 3b: Representative phase-contrast micrographs of human gingival fibroblasts exposed to increasing concentrations of E-cigarette aerosol extract (ECAE) (5–100%) showing dose-dependent alterations in cell morphology compared with control. Lower concentrations (5–10%) maintain spindle-shaped fibroblastic morphology, whereas higher concentrations (25–100%) exhibit reduced cell spreading and altered cellular organization. Scale bar = 100 μm.

### *3.2.3. Assessment of collagen deposition patterns through Masson's trichrome staining*

Masson's trichrome staining (MTS) revealed concentration-dependent alterations in collagen staining patterns and extracellular matrix (ECM) organization in human gingival fibroblasts exposed to ECAE. The untreated control cells exhibited intense, uniform blue staining consistent with well-organized collagen fibers distributed throughout the extracellular and pericellular regions. In contrast, fibroblasts treated with 25% ECAE showed a noticeable reduction in collagen staining intensity accompanied by early disruption of fibrillar organization. At higher exposure concentrations (50% and 100% ECAE), collagen staining was markedly diminished, appearing irregular and fragmented, with a pronounced loss of normal ECM architecture. These alterations were associated with morphological changes, including cytoplasmic shrinkage, vacuolation, and partial cellular detachment. Additionally, disorganized band-like collagen structures were observed at the highest ECAE concentration, suggesting aberrant ECM remodeling rather than definitive changes in collagen synthesis (Fig. 4).

### *3.2.4. Gene expression analysis*

The quantitative real-time PCR analysis revealed significant, concentration-dependent alterations in extracellular matrix–related gene expression in human gingival fibroblasts exposed to ECAE compared with untreated controls (Fig. 5). The expression of COL1A1 was progressively downregulated with increasing ECAE concentrations, with the most pronounced suppression observed at 50% and 100% ECAE. In contrast, MMP1 and MMP9 expression levels were significantly upregulated in a dose-dependent manner, with marked increases at higher concentrations, indicating enhanced matrix-degradative activity. Concurrently, TIMP1 expression was significantly reduced in cells treated with ≥25% ECAE, further shifting the gene expression profile toward matrix degradation. Collectively, these molecular changes

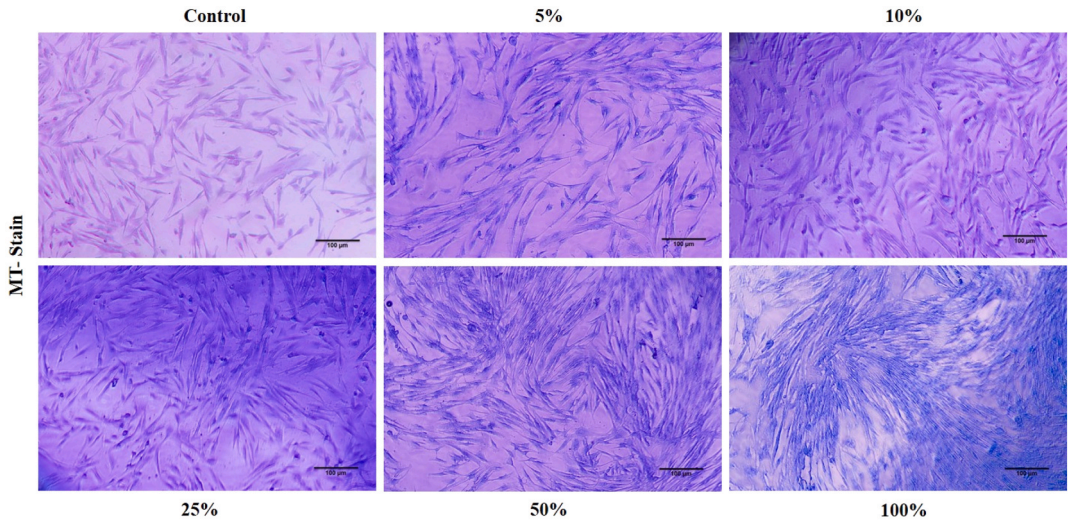


**Fig. 4.** Representative Masson's Trichrome–stained (MTS) images of human gingival fibroblasts showing concentration-dependent alterations in collagen staining and extracellular matrix organization following E-cigarette aerosol extract (ECAE) exposure. Control cells exhibit well-organized collagen, while 5–10% ECAE shows mild disruption. 25–50% ECAE demonstrates progressively altered collagen organization and cell alignment, and 100% ECAE displays marked matrix disarray. Images are representative of three independent experiments. Scale bar = 100 μm.

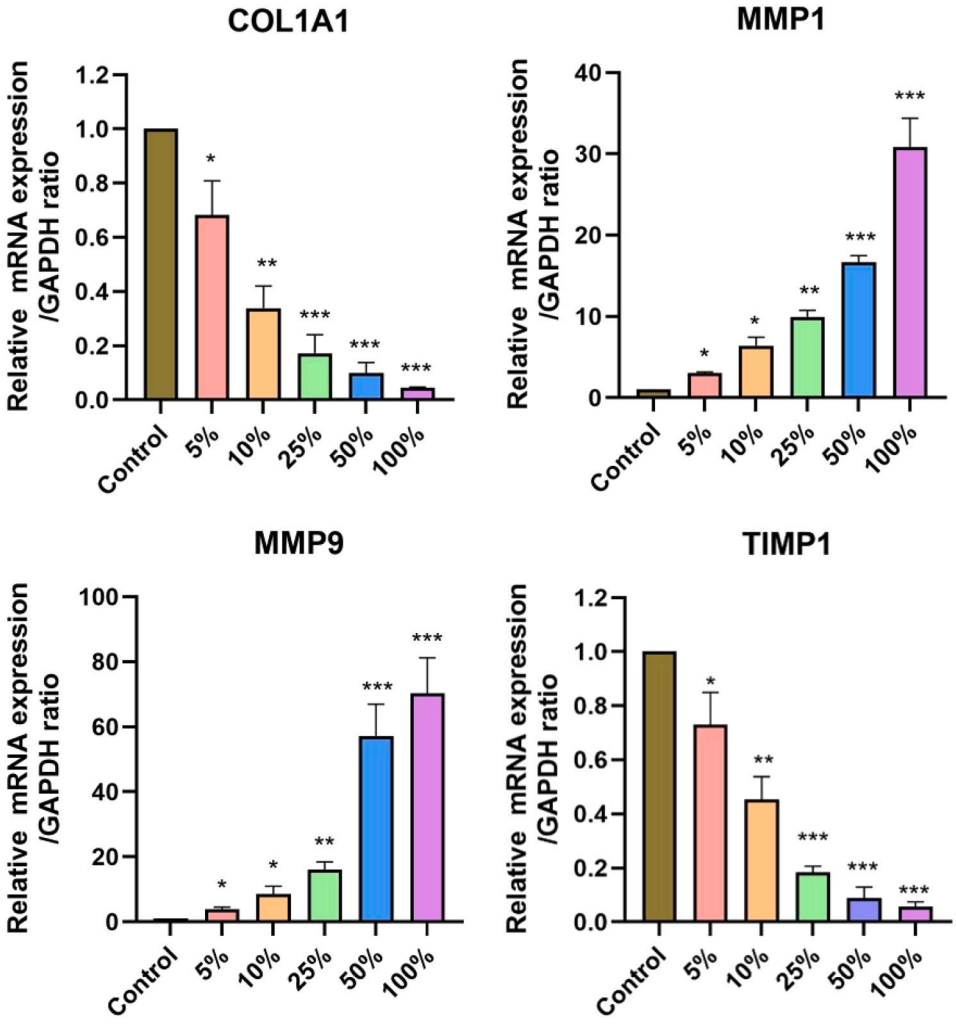


**Fig. 5.** Quantitative PCR analysis showing the effects of ECAE on the expression of collagen synthesis-related genes and matrix-degrading enzymes in gingival fibroblasts. Data are presented as mean ± standard deviation (SD) from experiments performed in triplicate. Statistically significant differences compared to the control group are indicated as $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***).

demonstrate a clear imbalance between collagen synthesis and degradation and are consistent with the observed disruption of organized collagen staining in ECAE-treated fibroblasts.

## 4. Discussion

The present investigations demonstrate that ECAE has cytotoxic effects on human gingival fibroblasts that are dependent on both concentration and exposure time. These findings are aligned with previous studies indicating reduced cell viability, metabolic activity, and proliferation of oral cells following exposure to e-cigarette vapor.[14,15] The significant reduction in MTT assay at higher ECAE concentrations, such as 50% and 100% suggests mitochondrial dysfunction and suppressed metabolic activity, both characteristic features of early-stage apoptosis and necrosis.

The observed cytotoxicity can be scientifically attributed to key constituents of e-cigarette aerosols, such as nicotine, aldehydes, reactive oxygen species (ROS), and trace metals. These components are well-established inducers of oxidative stress, mitochondrial injury, and DNA fragmentation, leading to cellular dysfunction and death.[16] Due to their pivotal role in maintaining ECM homeostasis and facilitating periodontal tissue regeneration, gingival fibroblasts appear especially susceptible to these challenges. The reported decrease in fibroblast viability following ECAE exposure highlights the reduced potential for repair and regeneration in gingival connective tissues.[17]

The Masson's Trichrome staining results demonstrated concentration-dependent alterations in collagen staining patterns and extracellular matrix organization in ECAE-treated gingival fibroblasts. The progressive reduction in well-organized blue-stained collagen fibers with increasing ECAE concentrations indicates disruption of ECM organization and collagen homeostasis. These observations are consistent with the qPCR findings showing downregulation of COL1A1 and suppression of TIMP1, along with upregulation of MMP1 and MMP9, collectively suggesting an imbalance favoring matrix degradation. The presence of disorganized band-like collagen staining at higher ECAE concentrations likely reflects aberrant matrix remodeling associated with cellular stress responses. The phenomenon of fibrotic remodeling aligns with previous research indicating that chronic exposure to nicotine and aldehydes affects fibroblast function and disturbs ECM homeostasis.[18,19]

Importantly, the substantial reduction in TIMP1 alongside the upregulation of MMP1 and MMP9 in gene expression assays indicates an imbalance between matrix metalloproteinases and their natural inhibitors. This imbalance accelerates ECM breakdown, leading to compromised structural integrity of the gingival tissue structure.[20,21] Clinically, these changes may lead to compromised periodontal attachment, delayed wound healing, and increased susceptibility to periodontal disease progression in chronic e-cigarette users.

The coordinated downregulation of COL1A1 together with the upregulation of MMP1 and MMP9 and suppression of TIMP1, as demonstrated by the qPCR results, indicates a shift toward matrix degradation and provides a molecular basis for the observed alterations in collagen organization and cellular morphology. Previous studies have reported similar findings, where both cigarette smoke and e-cigarette vapors induced structural disorganization of fibroblast matrices and reduced their regenerative potential.[22,23] These changes result in weakened periodontal connective tissues, delayed healing potential, and an increased risk of periodontal attachment loss, bone resorption, and tooth mobility.[24]

The qPCR results provide strong molecular evidence that e-cigarette aerosols disrupt the balance of ECM homeostasis in gingival fibroblasts. The downregulation of COL1A1 confirms the impaired ability of fibroblasts to synthesize structural collagen, a critical component of the gingival connective tissue. Simultaneously, the strong upregulation of MMP1 and MMP9, key collagenases and gelatinases, implies a degradative phenotype that favors ECM breakdown. This effect is compounded by the observed downregulation of TIMP1, the primary tissue inhibitor of MMPs, thereby releasing a critical regulatory check on proteolysis.[25,26] The combined effect of reduced collagen synthesis and insufficient MMP activity provides experimental insights for the loss of collagen staining and fibrotic disorganization observed in Masson's Trichrome experiments.

These findings align with previous *in vitro* reports demonstrating that cigarette smoke condensates and e-cigarette aerosols alter fibroblast gene expression toward a catabolic profile characterized by decreased collagen synthesis and increased protease activity [27–29]. Furthermore, nicotine, aldehydes, and reactive oxygen species present in e-cigarette vapor have been shown to activate intracellular pathways such as NF-κB and MAPKs, which upregulate pro-inflammatory cytokines and MMPs, thereby accelerating tissue degradation [30–32].

The molecular docking analysis further supported these cellular findings by demonstrating that nicotine can directly bind to ECM-related proteins with strong binding affinities. The interaction with COL1A1 (−4.45 kcal/mol) involved residues essential for fibril stability, which may compromise collagen assembly. Nicotine interactions with TIMP1 (−5.08 kcal/mol) occurred in the inhibitory domain, suggesting functional impairment of its MMP-regulatory role. MMP1 has shown an excellent affinity with nicotine at −5.42 kcal/mol and especially MMP9 (−6.55 kcal/mol) at residues, suggesting a potential increase in enzymatic activity. Together, these docking results indicate that nicotine may compromise ECM integrity not only at the gene expression level but also by directly interfering with structural and regulatory protein functions.

From a clinical perspective, this gene expression pattern resembles molecular characteristics associated with periodontitis, where excessive MMP activity drives connective tissue destruction and alveolar bone resorption. The concurrent inhibition of COL1A1 and TIMP1 suggests that even in the absence of bacterial infection, chronic vaping might stimulate gingival tissues for progressive periodontal attachment loss. The observed changes also provide a molecular basis for the cytotoxicity and collagen disorganization identified in this study, emphasizing a coherent pathway of injury from gene regulation to functional tissue remodeling. This study is limited by the use of an *in vitro* gingival fibroblast model, which does not fully recapitulate the structural and biological complexity of periodontal tissues *in vivo*. In addition, the use of a single e-liquid formulation and a standardized exposure condition may not reflect the diversity of commercially available products or real-world vaping behaviors. Accordingly, further studies using *in vivo* models and clinical investigations are warranted to validate and extend these findings.

## 5. Conclusion

This study demonstrates that exposure to e-cigarette aerosol extract induces cytotoxic effects in gingival fibroblasts, disrupts collagen matrix organization, and alters the expression of key extracellular matrix–related genes. MTT and Masson's Trichrome staining revealed concentration- and time-dependent reductions in cell viability accompanied by marked disorganization of collagen staining patterns. The coordinated downregulation of COL1A1 and TIMP1, together with the upregulation of MMP1 and MMP9, indicates a shift toward matrix degradation and impaired ECM homeostasis. While these findings suggest that e-cigarette aerosols can adversely affect fibroblast-mediated processes essential for periodontal tissue maintenance, the conclusions are limited to an *in vitro* model. Accordingly, further validation using *in vivo* models and clinical studies is required to confirm these effects and to elucidate the long-term implications of e-cigarette exposure on periodontal health.

### Patient's/Guardian's consent

Not Applicable.

## CRediT authorship contribution statement

Padmanathan Ramasamy: Conceptualization, manuscript writing. Karthikeyan Kandaswamy: Methodology, experimental work, data curation, and results arrangement. Navaneethan R: Language editing, clinical insights, critical revision. Raghunandhakumar Subramanian: Study conception, experimental design, supervision, data interpretation, and final approval of the manuscript.

## Data availability statement

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

## Ethical statement

This study was approved by the Institutional Ethical Committee of the Saveetha Dental College and Hospital (IHEC/SDC/FACULTY/22/PHARM/574).

## Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author used Quillbot for grammar checking and to assist in the writing process. Following the use of this tool, the author reviewed and edited the content and takes full responsibility for the content of the manuscript.

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## Declaration of competing interest

The authors declare no conflicts of interest.

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## References

1. Grana R, Benowitz N, Glantz SA. E-cigarettes: a scientific review. *Circulation*. 2014;129(19):1972–1986.
2. Orr MS. Electronic cigarettes in the USA: a summary of available toxicology data and suggestions for the future. *Tob Control*. 2014;23(suppl 2):ii18–ii22.
3. Naruishi K. Biological roles of fibroblasts in periodontal diseases. *Cells*. 2022;11(21):3345.
4. Elgezawi M, Haridy R, Almas K, et al. Matrix metalloproteinases in dental and periodontal tissues and their current inhibitors: developmental, degradational and pathological aspects. *Int J Mol Sci*. 2022;23(16):8929.
5. Feng Y, Li HP. Optimizing collagen-based biomaterials for periodontal regeneration: clinical opportunities and challenges. *Front Bioeng Biotechnol*. 2024;12, 1469733.
6. Deepika BA, Ramamurthy J, Kannan B, Jayaseelan VP, Arumugam P. Overexpression of insulin-like growth factor-2 mRNA-binding protein 1 is associated with periodontal disease. *J Oral Biol Craniofac Res*. 2024;14(5):494–499.
7. Szumilas P, Wilk A, Szumilas K, Karakiewicz B. The effects of e-cigarette aerosol on oral cavity cells and tissues: a narrative review. *Toxics*. 2022;10(2):74.
8. Guo J, Hecht SS. DNA damage in human oral cells induced by use of e-cigarettes. *Drug Test Anal*. 2023;15(10):1189–1197.
9. Rojas MA, Ceccarelli S, Gerini G, et al. Gene expression profiles of oral soft tissue-derived fibroblast from healing wounds: correlation with clinical outcome, autophagy activation and fibrotic markers expression. *J Clin Periodontol*. 2021;48(5):705–720.
10. Rom O, Avezov K, Aizenbud D, Reznick AZ. Cigarette smoking and inflammation revisited. *Respir Physiol Neurobiol*. 2013;187(1):5–10.
11. Kandaswamy K, Panda SP, Subramanian R, et al. Synergistic berberine chloride and curcumin-loaded nanofiber therapies against methicillin-resistant Staphylococcus aureus infection: augmented immune and inflammatory responses in zebrafish wound healing. *Int Immunopharmacol*. 2024;140, 112856.
12. Kazi S, Khader AA, Sheth AM, et al. Effects of vaping on Periodontium-A review. *J Adv Med Dent Sci Res*. 2020;8(2):69–72.
13. Aldalaeen MO, Haddad RH, Alhusamiah BK, Abuejheisheh AJ. The impact of cigarette smoking and vaping use on the development and progression of periodontitis: a systematic review. *Health Sci Rep*. 2025;8(9), e71245.
14. Vermehren MF, Wiesmann N, Deschner J, Brieger J, Al-Nawas B, Kämmerer PW. Comparative analysis of the impact of e-cigarette vapor and cigarette smoke on human gingival fibroblasts. *Toxicol Vitro*. 2020;69, 105005.
15. Rouabhia M, Park HJ, Semlali A, Zakrzewski A, Chmielewski W, Chakir J. E-cigarette vapor induces an apoptotic response in human gingival epithelial cells through the caspase-3 pathway. *J Cell Physiol*. 2017;232(6):1539–1547.
16. Anderson C, Majeste A, Hanus J, Wang S. E-cigarette aerosol exposure induces reactive oxygen species, DNA damage, and cell death in vascular endothelial cells. *Toxicol Sci*. 2016;154(2):332–340.
17. Alanazi H, Park HJ, Chakir J, Semlali A, Rouabhia M. Comparative study of the effects of cigarette smoke and electronic cigarettes on human gingival fibroblast proliferation, migration and apoptosis. *Food Chem Toxicol*. 2018;118:390–398.
18. Takeuchi-Igarashi H, Kubota S, Tachibana T, et al. Matrix remodeling response of human periodontal tissue cells toward fibrosis upon nicotine exposure. *Odontology*. 2016;104(1):35–43.
19. Aarthi SS, Pandiar D, Subramanian R, Krishnan RP. An in-vitro exploration of the antifibrotic activity of naringenin: a potential therapeutic agent for oral submucous fibrosis management. *J Oral Biol Craniofac Res*. 2025;15(4):849–857.
20. Wang T, Liu X, Li J, et al. Mechanisms of mechanical force in periodontal homeostasis: a review. *Front Immunol*. 2024;15, 1438726.
21. Radzki D, Negri A, Kusiak A, Obuchowski M. Matrix metalloproteinases in the Periodontium—vital in tissue turnover and unfortunate in periodontitis. *Int J Mol Sci*. 2024;25(5):2763.
22. Martinez JD, Easwaran M, Ramirez D, Erickson-DiRenzo E. Effects of electronic (e)-cigarette vapor and cigarette smoke in cultured vocal fold fibroblasts. *Laryngoscope*. 2023;133(1):139–146.
23. Alanazi H, Rouabhia M. Effect of e-cigarette aerosol on gingival mucosa structure and proinflammatory cytokine response. *Toxicol Rep*. 2022;9:1624–1631.
24. Salvi G.E., Roccuzzo A., Imber J.C., Stähli A., Klinge B., Lang N.P. Clinical periodontal diagnosis. Periodontol 2000. 2023;00:1-19.
25. Sharma C, Dobson GP, Davenport LM, Morris JL, Letson HL. The role of matrix metalloproteinase-9 and its inhibitor TIMP-1 in burn injury: a systematic review. *Int J Burns Trauma*. 2021;11(4):275.
26. Molière S, Jaulin A, Tomasetto CL, Dali-Youcef N. Roles of matrix metalloproteinases and their natural inhibitors in metabolism: insights into health and disease. *Int J Mol Sci*. 2023;24(13), 10649.
27. Mavrogonatou E, Papadopoulou A, Pratsinis H, Kletsas D. Senescence-associated alterations in the extracellular matrix: deciphering their role in the regulation of cellular function. *Am J Physiol Cell Physiol*. 2023;325(3):C633–C647.
28. Ryder MI, Hyun W, Loomer P, Haqq C. Alteration of gene expression profiles of peripheral mononuclear blood cells by tobacco smoke: implications for periodontal diseases. *Oral Microbiol Immunol*. 2004;19(1):39–49.
29. Sancilio S, Gallorini M, Cataldi A, Sancillo L, Rana RA, di Giacomo V. Modifications in human oral fibroblast ultrastructure, collagen production, and lysosomal compartment in response to electronic cigarette fluids. *J Periodontol*. 2017;88(7):673–680.
30. Yang X, Che W, Zhang L, Zhang H, Chen X. Chronic airway inflammatory diseases and e-cigarette use: a review of health risks and mechanisms. *Eur J Med Res*. 2025;30(1):223.
31. Ma T, Wang X, Li L, Sun B, Zhu Y, Xia T. Electronic cigarette aerosols induce oxidative stress-dependent cell death and NF-κB mediated acute lung inflammation in mice. *Arch Toxicol*. 2021;95(1):195–205.
32. Wang L, Wang Y, Chen J, Liu P, Li M. A review of toxicity mechanism studies of electronic cigarettes on respiratory system. *Int J Mol Sci*. 2022;23(9):5030.