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Effects of different curcumin concentrations on human periodontal ligament fibroblast adhesion and proliferation on periodontally involved root surfaces: In-vitro study

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ABSTRACT

Introduction: Periodontopathic endotoxins infiltrate root surface and prevent cellular adhesion to tooth surfaces. Naturally occurring curcumin has anti-inflammatory, antioxidant and antibacterial qualities that promote fast wound healing by increasing fibroblast cell proliferation and migration in concentration-dependent manner.
Objectives: This study was conducted to evaluate the effect of 0.12 %, 1 % and 2 % curcumin concentrations on PDL cell adhesion, viability and proliferation to periodontally affected root surfaces.
Materials and methods: 20 periodontally affected teeth sectioned into root samples were included. PDL fibroblasts were collected from freshly extracted teeth, cultured and expanded. PDL fibroblast (1×10^5 cells/ml) was seeded on curcumin coated root samples in different concentrations. Study samples were divided into 4 groups: G1 (0.12 % of curcumin paste), G2 (1 % curcumin paste), G3 (2 % curcumin paste) and G4 (control/unconditioned group). All samples were investigated by SEM and MTT assay.
Results: G3 showed highest viability and cell proliferation compared to other groups where well defined multilayered adherent cells covering entire surface with totally flat polyhedral bodies with long cytoplasmic extensions and little or no bacterial colonization.
Conclusion: Curcumin 2 % provides optimal stimulation of cellular attachment, viability, proliferation and antibacterial effects over periodontitis affected root surfaces.
Clinical relevance: Determination of optimal curcumin concentration in this study revealed 2 % concentration produced highest levels of PDL cellular attachment, viability, proliferation and antibacterial action over root surfaces afflicted by periodontitis. Therefore, the use of that optimal curcumin concentration as adjunctive to non-surgical periodontal therapy may modify the periodontal pocket ecology to improve the healing of periodontal tissues.

1. Introduction

Periodontal disease is a serious infection and inflammation involving the supportive apparatus surrounding tooth known as the periodontium, which is periodontal ligament, gingival tissue, cementum and alveolar bone. It is considered as one of the most concerning global oral health burdens affecting almost 10–15 % of world's population and 89.8 % of Egyptian population.[1,2] Apical migration of epithelial attachment, connective tissue deterioration, alveolar bone loss and ultimately tooth loss are common sequalae of periodontitis if left untreated.[3] The principal

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cells in periodontal ligament (PDL) are the PDL fibroblasts which are responsible for the synthesis of collagen bundles in the PDL in addition to renewal and replacement of old and damaged collagen fibrils.[4,5] PDL fibroblasts through its proliferation and differentiation can remodel tissues and repopulate wounds, thus contributing periodontal tissue homeostasis.[6,7] These PDL cells must be viable, in good health and able to adhere to the root surfaces of teeth for periodontal therapy to be effective and for regeneration to occur predictably.[8] However, their cellular attachment is inhibited as suggested by Lucas et al. by the persistent periodontopathic endotoxins that penetrate the root surfaces of teeth leading to irreversible loss of resident PDL fibroblasts.[9] Bacterial endotoxin absorbed to dental surfaces inhibited cell attachment to dental surfaces. Moreover, elimination of bacterial endotoxins had improved human gingival fibroblasts cellular attachment.[10]

For centuries, people have utilized herbal extracts to treat illnesses. Many inflammatory processes can be managed by using herbal formulations that can regulate the generation of pro-inflammatory mediators, thereby managing related inflammatory processes.[11] Curcumin has strong antibacterial activity which suppresses the growth of diverse types of gram-positive and gram-negative bacteria.[12,13] Curcumin is a natural extract with antioxidant and anti-inflammatory properties.[14] In addition, curcumin is an excellent natural option for promoting fast wound healing by increasing fibroblast cell proliferation and migration in concentration-dependent manner.[15]

Konain et al., treated skin fibroblasts with different concentrations of curcumin (0.1, 1, and 10 μg/m) and concluded that there were no significant differences in the proliferative response to that different concentrations.[16] On other hand, curcumin concentration greater than 36.8 μg/mL was reported to have a toxic effect on cultured cells.[17] Furthermore, curcumin paste reported to enhance cell-cell adhesion and cell-matrix adhesion.[18] To the best of our knowledge, no studies have been conducted to examine the effect of different curcumin concentrations on the attachment of human PDL fibroblasts to periodontally affected root surfaces.

Therefore, the current study was carried out to evaluate the proliferation, viability and attachment of PDL fibroblasts to affected root surfaces modified by different concentrations of curcumin as a primary outcome. Secondary outcome was the effect of different curcumin concentration on bacterial colonization over periodontally affected root surfaces.

2. Materials and methods

2.1. Sample selection and procurement

Recruitment for this study was done through screening of patients receiving treatment at the Oral Medicine and Periodontology Department's outpatient clinic of Faculty of Dentistry where patients with hopeless periodontal affected teeth indicated for extraction were selected for the study. Every patient received detailed explanation of the research procedures and signed an informed consent form allowing collection of research related data prior to extraction and the use of their extracted teeth in research work. The current study was approved by Research Ethics Committee at Faculty of Dentistry with approval number (RECO6U/25–2022) and the authors present the current article following the Modified CONSORT checklist for reporting *in-vitro* studies of dental materials.

Sample size calculation was performed using Epicalc program (version 1.02) adopting a power of 80 % and alpha = 0.05 based on mean difference of cell adhesion on diseased cementum discs with different curcumin concentration retrieved from previous research.[19] A suggested minimum number of patients if groups equaled 4 was 16 patients and the final sample size of 20 patients was decided to be used in the current study where only one tooth was contributed by each patient. The teeth included in the study were caries-free, anterior and posterior teeth with grade III mobility diagnosed with stage 4 periodontitis[20] and indicated for extraction as an integral aspect of the future dental therapy of the selected patients. This study excluded patients with systemic disorders that could impact the periodontium, smokers, and pregnant women. Additionally, patients who had undergone dental prophylaxis, scaling and root planing or root surface debridement within the preceding three months were not allowed to participate. In addition, ten freshly extracted third molars from medically-free adolescents were utilized as a source of the PDL fibroblasts. These impacted molars were obtained from Oral Surgery Department of Faculty of Dentistry following patients consent for extraction and use of their extracted teeth for research purposes.

Measuring the clinical probing depth and attachment level served as the basis for the periodontal clinical parameters for tooth collection. A calibrated periodontal probe (Williams Periodontal Probe, Aksim Surgical Ltd, TW3 1 EA, UK) was used to determine probing depth from gingival margin to base of probing depth and attachment level from cementoenamel junction (CEJ) to base of probing depth. At least a 5 mm probing depth and a 5 mm clinical attachment loss were present on diseased tooth surfaces that were part of the investigation. Just before extraction, a pencil mark was drawn at level of gingival margin to indicate future regions of teeth exposed to pocket environment. These extraction procedures were carried out under local anesthesia to prevent disturbing the test areas. Once every tooth was extracted, a fine 0 round bur (Brassler, Savannah, GA, USA) was used to create a groove at the pencil mark. Another groove was positioned at the base of the pocket at the most coronal level of periodontal tissue attachment to the root surface. The test areas were then Ultrasonically cleaned until hard and sound tooth structure becomes evident. This procedure was performed using an ultrasonic scaler (Yimei UD-32, Yimei Dentistry Industry Co. Ltd, Zhengzhou, China) followed by manual root debridement.

2.2. Sample preparation, sterilization, and assignment

According to Gamal et al., a horizontal cut was made at the groove level to remove the root from the crown of each tooth while it was continuously cooled with water. After that, a longitudinal sectioning of the root through the pulp to the test area's apical point was performed.[19] To create a specimen chip, a second horizontal cut was made at the pocket's base to cut the test area from the root body. Finally, those specimens were placed in sterile saline solution (4 °C) as a storage medium to carry out the next experimental steps.[21]

2.3. Human PDL fibroblast isolation and culture

PDL fibroblasts were prepared in the central lab of stem cells and biomaterials applied research (CLSBAR) at the Faculty of Dentistry. The methods described in Mailhot et al. were followed for the culture processes.[22] PDL fibroblasts were collected from 10 impacted third molars for PDL harvesting and expansion. Teeth were extracted and repeatedly cleaned in biopsy medium under precise aseptic conditions. A sterile centrifuge tube containing 10 ml transferring medium was used to transfer the teeth to the lab. Phosphate-buffered saline (Bio-Whittaker Inc., Walkersville, MD, USA) with 100 μg/mL streptomycin and 100μ/mL penicillin was used twice to clean the teeth under the sterile atmosphere of a laminar flow chamber and a sterile scalpel (No.15) was used to scrap teeth roots to collect the periodontal tissues. The tissues were then divided into 1–2 mm^2 pieces and placed in a flask to be incubated in 2 mg/mL collagenase IV, 1 mg/ml dispase II and 0.2 μmol/L Eagle's minimum essential medium (Bio-Whittaker Inc., Walkersville, MD, USA) at 37 °C for 30 min and this procedure was repeated twice using fresh solution and incubated for another 90 min at same temperature. After centrifugation, those tissues were seeded in Eagle's minimal essential medium (EMEM) supplemented with 100 μg/mL streptomycin, 100μ/mL penicillin, 200 μmol/L L-glutamine, 1 % amphotericin and 15 % fetal bovine serum (FBS) in a 25 cm^2 tissue culture flask then placed in 95 % air and 5 % CO2 in a 37 °C humidified

incubator. Cells were allowed to adhere for 24 h, after which the detached cells were washed with PBS. Moreover, fresh media was added and replenished every 3 days. Once cell cultures reached 80 % confluence, cells were transferred using a solution of 1 μmol/L ethylenediaminetetraacetic acid (EDTA) and 0.05 % trypsin to tissue culture flasks to be continuously sub-cultured in the complete growth medium, then pooled into PDL populations and stored at −70 °C. Those cells from the third passage were used now ready to be used in our study. Phase contrast microscopy was used throughout cell culture methods to track cell growth, and every cell had a shape like that of a fibroblast (Fig. 1).

2.4. Curcumin paste preparation

Curcumin pastes were prepared by Nano Gate Lab according to protocol described by Patwekar et al.[23] by suspending curcumin powder in distilled water to get the following three concentrations; 0.006 gm in 5 ml (0.12 % w/v), 0.05 gm in 5 ml (1 % w/v) and 0.1 gm in 5 ml (2 % w/v) in three sterile containers with stirring for 30 min. Then 0.25 gm of carboxymethyl cellulose (CMC) was sprinkled gently and gradually over the solution under mild temperature at 35 °C with vigorous stirring to get homogenous paste.[14,21]

2.5. Experimental design

The treated teeth samples were randomly distributed into 4 groups (5 teeth segments/group) according to the treatment protocol. Group 1 (G1), 0.12 % curcumin paste; Group 2 (G2), 1 % curcumin paste; Group 3 (G3), 2 % curcumin paste and Group 4 (G4), control/unconditioned group with no surface coating. Ten different participants' PDL cells were mixed and cultured in an EMEM with 0.5 % FBS. A hemocytometer was used to count the PDL cells, with each sample in each experimental group held 35 × 105 cells/200 mL. Each sample consisted of diseased root specimen seeded with PDL cells following its coating with different concentrations of curcumin to be incubated for 24 h at 37 °C in an atmosphere that included 95 % air and 5 % CO2.

2.6. Samples fixation and scanning electron microscope (SEM) preparation

Following the incubation period of 24 h, the segments in the culture media were collected and rinsed with phosphate buffer saline (PBS solution) to eliminate any debris, unattached cells, and culture media contents. Within their chamber slides, these samples were then fixed with 2.5 % glutaraldehyde (Sigma-Aldrich Co., St. Louis, MO, USA) in 100 mM cacodyl-ate buffer (Sigma-Aldrich Co.), then dehydrated in increasing concentrations of ethanol (30 %, 60 %, 95 % and 100 %) and subsequently soaked in hexamethyldisilane (Sigma-AldrichCo.) for 15 min and allowed to air dry. After dehydration in critical point drier (Balzers, FL9496, Liechtenstein), they were sputter coated with 200-A thick coating of gold (S150 A, Leica, Cambridge, UK).[24] Finally, SEM (Philips × 630, Philips, Eindhoven, Netherlands) was used for examination of samples at various magnifications. Investigated root specimens ranged in total surface area from 9 to 24 mm^2. Two trained-blinded examiners screened cell attachment, bacterial colonization and morphological analysis on periodontally involved root surface.

2.7. MTT assay (3–4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide, a tetrazole assay)

For quantitative evaluation of cell viability and proliferation, an MTT assay was used. Following a 24-h incubation period, the specimens were gently washed twice with sterile PBS solution to be subsequently incubated at 37 °C for another 3 h in new culture media containing 10 % MTT dye solution (0.5 mg/mL stock solution). To dissolve purple formazan crystals, the medium in each well was aspirated, replaced with an equal amount of dimethyl sulfoxide (DMSO) solvent and shake gently while being incubated for another half hour at room temperature. This was followed by transferring 100 μL of purple solution in each well to 24-well plate to only use 20-wells (5 repetitions) where an Elisa reader instrument was utilized to measure the optical density (OD) of each well at 570 and 620 nm wavelength.[25] A separate Petri dish was used for each root sample where 1 ml suspension of the isolated fibroblasts with cell density of 1 $x10^5$ cells/ml was added to each dish to be incubated for 24

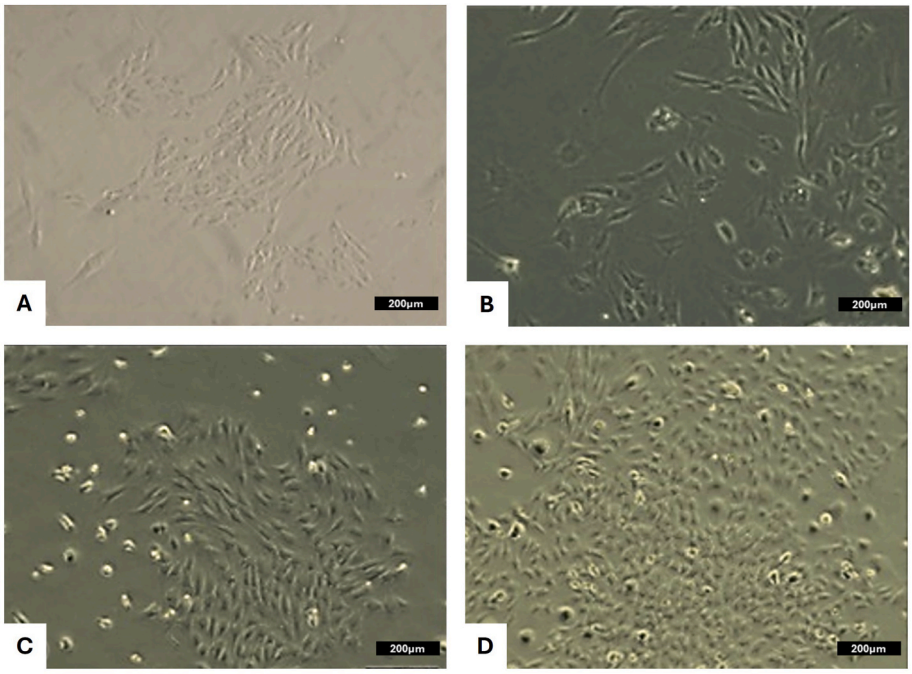

Fig. 1. Optical microscope images showing human PDL fibroblasts using phase-contrast microscopy at × 200 magnification, (A) after 3 days, (B) after 2 weeks, (C) after 3 weeks and (D) after 4 weeks of culture.

h. Curcumin paste was added for the three Petri dishes of the experimental groups with the specified concertation. Following incubation, the sections were rinsed in Hanks balanced salt solution, stained immediately with 0.01 % neutral red to assess cell viability, and then fixed in methanol-ether before being stained with 0.5 % trypan blue to highlight any adherent or attached cells.[19,21]

2.8. Data analysis

Statistical Package for Social Sciences (SPSS) *(IBM SPSS Statistics for Windows, Version 18.0. Armonk, NY: IBM Corp)* was used for data collection and analysis where collected data were presented as mean ± standard deviation (SD) values, and Kolmogrovo-Smirnov test was then performed to assess the normality of the distribution parameters where the yielded normal distribution was utilized. One-Way ANOVA test was used to compare the multiple groups, followed by Tukey's honestly significant difference (HSD) post hoc tests for pairwise comparisons. P-values < 0.05 were considered statistically significant.

3. Results

A total of 20 periodontally involved caries-free human anterior and posterior teeth with grade III mobility (16 male and 4 females, ranging in age from 41 to 54 years) diagnosed with stage 4 periodontitis were extracted and collected in sterile saline solution until preparation as mentioned in the materials and methods section.

Both G1 (0.12 %) and G2 (1 %) groups also showed multilayered arrangement of healthy flat PDL cells with long cytoplasmic extensions attached to root surfaces but with more bacterial colonization compared to G3 group (Figs. 2 and 3). These bacterial colonies formed of small, round or rod-shaped entities that appeared in separate areas in 3 out of the G1 samples and only 2 out of the G2 samples.

Compared to control and other experimental groups, the SEM examination of all samples in G3 (2 % curcumin concentration) group showed well defined multilayered adherent PDL fibroblasts with elongated flat or spindle-shaped bodies and evident long cytoplasmic extensions covering the entire root surface. Moreover, those G3 samples revealed no bacterial colonies in 4 out of 5 samples to scarce colonization with sporadic planktonic bacterial adhesion in only one sample (Fig. 4).

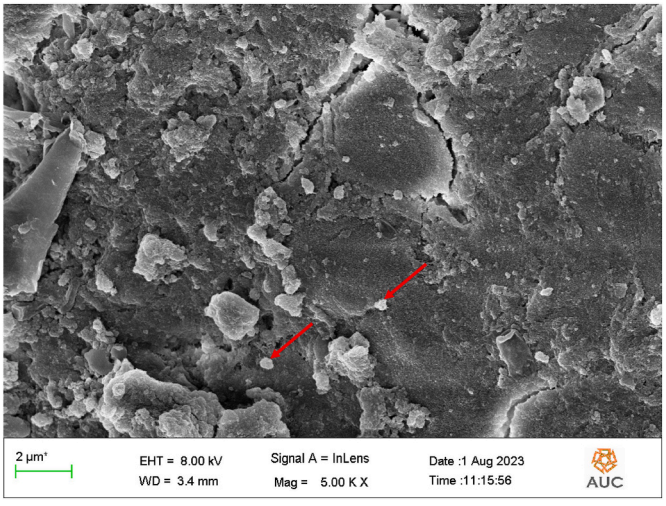

Fig. 2. SEM micrograph of G1 (0.12 % of curcumin paste) showing multilayered fibroblastic arrangement with evident cytoplasmic extensions attached to the root surface coated with curcumin paste together with bacterial colonization appeared in separate areas (red arrows), magnification 5.00 K X. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

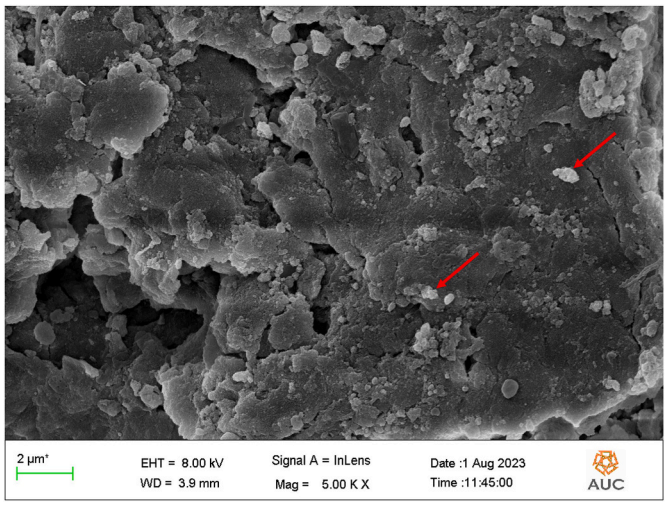

Fig. 3. SEM micrograph of G2 (1 % curcumin paste), showing flat multilayered fibroblasts attached to curcumin discs by long cytoplasmic extensions together with planktonic bacteria in few separate parts of root surface (red arrows), magnification 5.00 K X. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

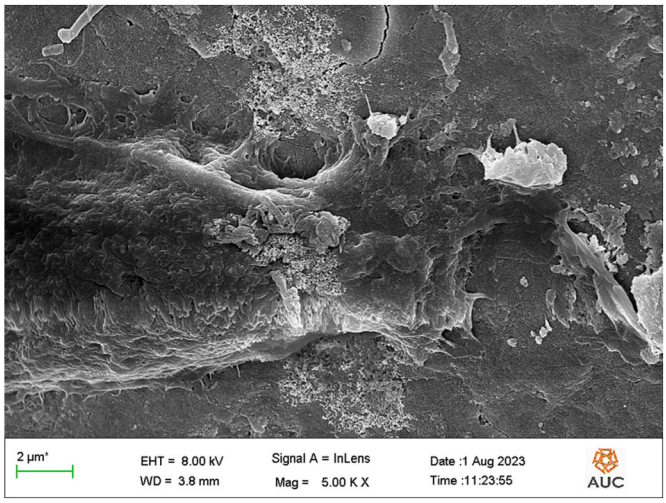

Fig. 4. SEM micrograph of G3 (2 % curcumin paste) showing well defined, multilayered adherent elongated flat or spindle-shaped PDL fibroblasts with long cytoplasmic extensions covering the entire root surface with no evident bacterial colonies, magnification 5.00 K X.

All samples of the Control G4 unconditioned group showed predominantly single layer of rounded cells with pulled up and blipped surface which indicate partial loss of cell viability and weak cellular adhesion. Moreover, the root surfaces of all control samples were completely covered with abundant bacterial colonies (Fig. 5).

MTT cell proliferation assay revealed a concentration-dependent mean optic density with G3 samples showed the highest mean (4.26 ± 0.27) followed by G2, G1 and Control G4 groups (2.55 ± 0.07, 2.37 ± 0.08 and 2.33 ± 0.17, respectively) with a highly significant difference found between all studied groups ($P < 0.001$). In addition, the pairwise comparisons between G3 and any of the studied groups (Control G4, G1 or G2) yielded statistically highly significant differences while non-significant differences were observed in comparing Control G4 vs either G1 or G2 groups and G1 vs. G2 groups (Table 1, Fig. 6A).

The mean percentage of viability for G3 samples (182.8 ± 11.5) was also significantly higher than that of G2, G1 and Control G4 groups (109.5 ± 3.2, 101.6 ± 3.6 and 99.8 ± 7.4, respectively) with a highly

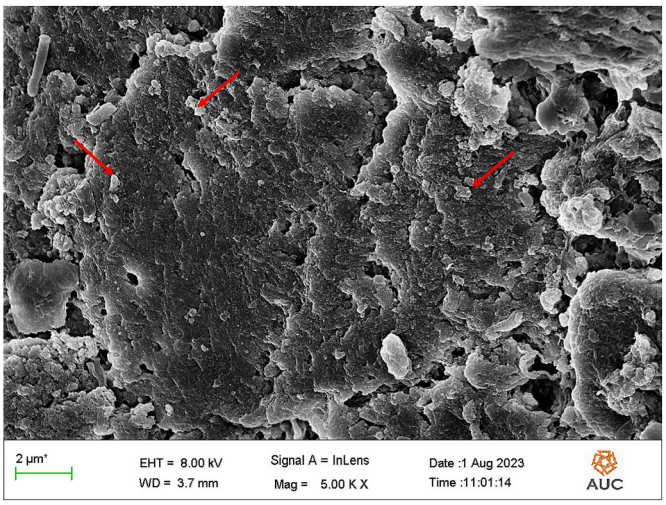

Fig. 5. SEM micrograph of G4 (control/unconditioned group) showing the surface area entirely covered with abundant bacterial colonies (red arrows) together with single layer of rounded cells with short cytoplasmic extensions and pulled up blipped surface, magnification 5.00 K X. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

significant difference found between all studied groups ($P < 0.001$). Moreover, highly significant differences were observed between G3 and any of the studied groups (Control G4, G1 or G2) but no statistically significant differences between Control G4 vs either G1 or G2 groups and G1 vs. G2 groups in pairwise comparison (Table 2, Fig. 6B).

4. Discussion

Several regenerative periodontal therapies were developed to restore loss of supporting apparatus of teeth. These therapies include the growth factor delivery, enamel matrix derivative, guided tissue regeneration (GTR) and bone grafts in addition to the combined cells and growth factors within matrix-based scaffolds.[26] Various herbal products have received attention as adjunctive therapy to enhance the outcomes of such approaches in treating periodontitis due to their overall safety and efficacy. Curcumin, a bioactive polyphenol derived from curcuma longa, acknowledged to have analgesic, antibacterial, antioxidant and anti-inflammatory characteristics.[27] Different concentrations of curcumin are used to treat periodontitis, although the ideal concentration is not supported by literature. A vital component in the regeneration of the missing attachment apparatus is the PDL cells,[28] thus, early recruitment, adhesion and restoration of these cells in periodontal lesions is seen as crucial for periodontal regeneration. Cell adhesion to tooth surfaces is usually compromised by bacterial endotoxins adsorbed to the surfaces and their meticulous elimination were reported to enhance cellular attachment.[29]

Additionally, smoking affects cellular attachment on the root surface where nicotine binds to the root surface, changes fibroblast attachment

Table 1
MTT assay results for assessment of optical density of PDL cells treated with different concentration of curcumin after 24 h duration.

Group	N	Mean	SD	Median	Min.	Max.	F	P Value	Sig.
G1 [a]	5	2.37	0.08	2.42	2.28	2.43	151.6	<0.001	HS
G2 [a]	5	2.55	0.07	2.57	2.43	2.63			
G3 [b]	5	4.26	0.27	4.36	3.80	4.49			
G4 Control [a]	5	2.33	0.17	2.26	2.12	2.52			

[a] HS: highly significant ($P < 0.001$), superscript letters indicate statistically significant difference within the same column while same letters indicate no statistical significance.

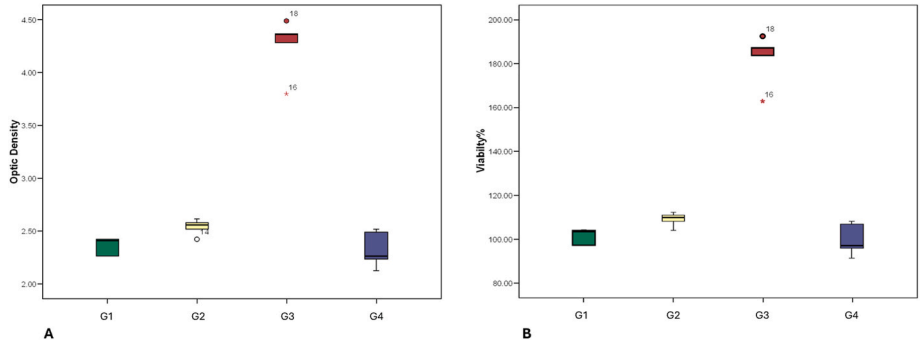

Fig. 6. Boxplots for mean values of PDL fibroblasts as measured by MTT assay after exposure to different concentrations of curcumin for 24 h regarding (A) optic density and (B) viability percentage.

Table 2
MTT assay results for assessment of viability percentage of PDL cells treated with different concentration of curcumin for 24 h.

Group	N	Mean%	SD	Median	Min.	Max.	F	P Value	Sig.
G 1 [a]	5	101.6	3.6	103.9	97.6	104.4	151.6	<0.001	HS[a]
G 2 [a]	5	109.5	3.2	110.3	104.4	112.7			
G 3 [b]	5	182.8	11.5	187.2	163.1	192.6			
G4 Control [a]	5	99.8	7.4	97.0	91.2	108.2			

[a] HS: highly significant ($P < 0.001$), superscript letters indicate statistically significant difference within the same column while same letters indicate no statistical significance.

and integrin expression, so that the present study excluded them.[30] Pregnant women were also disqualified because of elevated estrogen and progesterone levels, which were thought to significantly affect the periodontium by changing the subgingival microbial ecology, the host response, and progesterone's inhibitory effect on the rate of human GF proliferation.[31]

The current study focused on attachment, proliferation, and viability of PDL cells in response to different concentrations of curcumin on periodontally affected root surfaces to figure out the optimal concentration that leads to maximum cellular function. According to Rujirachotiwat and Suttamanatwong, curcumin treatment at concentrations ranging from 0.1 to 20 μM did not impact the viability of human gingival fibroblasts, while a concentration of 50 μM resulted in marked, concentration-dependent cytotoxicity.[32] Moreover, the curcumin concentration range reported by Mandroli et al. from 2.5 to 10 mg in 1000 μL promoted PDL fibroblasts viability, proliferation and migration with no cytotoxicity rendering it a promising agent with desired pharmacological properties.[33] Based on these results, curcumin concentrations of 0.12 %, 1 %, and 2 % were selected for investigation in present study where the authors choose to report 2 % as the optimum effective concentration as it provided the greatest beneficial effect for periodontal therapy with no further desired effects observed at any higher concentrations.

In the current *in-vitro* model, the selected periodontally affected root surfaces were treated with root debridement before application of varying concentrations of curcumin treatment. This model is more like the *in-vivo* process. Root samples were obtained from periodontally affected roots between the pocket base and gingival margin to get areas exposed to bacterial environment in the oral cavity and later undergo healing by long junctional epithelium to mimic naturally affected root surfaces after non-surgical professional mechanical root debridement.

In this study, most of the G3 (2 % curcumin) cells exhibited flat polyhedral bodies, with long cytoplasmic extensions denoting maximum cell attachment. Most samples of this group revealed minimal bacterial colonization. This was followed by G2 (1 %) and G1 (0.12 %) respectively with more round or spindle cells, short cytoplasmic extensions, pulled up morphology and more bacterial colonization of the root surfaces. This could be attributed to the significant antibacterial effect of 2 % curcumin concentration which may induce an increased root surface energy and improved fibroblast attachment.[25] Moreover, curcumin itself enhances collagen formation by fibroblast which may improve cell attachment to the root surface. The positive outcome of 2 % curcumin pastes in reducing bacterial colonization could be an outcome of curcumin adsorption to periodontopathic bacterial cell wall and generation of reactive oxygen species (ROS), which lead to pathogens eradication in the immediate vicinity.[34] Thess findings comes in compliance with an *in-vitro* study pointed out the effect of 2 % curcumin concentration on increasing cellular proliferation and collagen synthesis at wound site with increased DNA and collagen especially type III collagen that improved fibroblasts.[35]

An *in-vivo* study found that the local application of 2 % curcumin gel enhances the management of chronic periodontitis by improving clinical parameters, including clinical attachment loss (CAL), pocket depth (PD), gingival index (GI), and plaque index (PI). This effect is attributed to its antibacterial properties, making it a viable adjunct to mechanical debridement for eliminating subgingival irritants.[36]

Cell morphology is commonly used to denote the degree of cellular attachment and viability. In the present study curcumin 2 % showed more flat cells with long cytoplasmic extension. This was consistent with the *in-vitro* study carried out by Liu et al. that that studied the benefits of laser treatment on PDL fibroblast morphology and attachment to root surfaces of teeth. Fibroblasts that were tightly attached to root sections showed numerous lamellipodia and flat appearance, in comparison with the fibroblasts poorly attached to untreated root sections which exhibited few attachment processes and were round shaped.[29] The present study Control G4 samples showed impairment in fibroblast attachment with apparently pulled up appearance and blipped surface. Additionally, this group revealed abundant bacterial colonies with no significant difference compared to G1 (0.12 % curcumin) and G2 (1 % curcumin). This data may light on the inadequate antibacterial effect of curcumin 0.12 and 1 % on bacterial endotoxins over root specimens which adversely affect cell adhesion. In addition, it also lights on the importance of using proper concentration of adjunctive antibacterial agents in combination with mechanical debridement to achieve an effective therapeutic potential consistent with what reported in the systematic review and meta-analysis by Teughels et al. where the combined use of antimicrobials following full mouth scaling and root planning showed improvement in full-mouth probing depth and clinical attachment gain over 6-month follow-up.[37] However, caution must be exerted as excessive and improper use of antimicrobial agents can lead to the emergence of specific drug-resistant and/or multi-drug-resistant periodontopathic bacterial species, which may no longer respond to these agents or necessitate higher concentrations for their effective elimination.

In a concentration-dependent manner, curcumin exhibits bacteriostatic and bactericidal properties against a wide range of periodontopathic microorganisms as being a lipophilic polyphenol, it can insert itself into the lipid bilayer of the bacterial cell wall resulting in increased cell permeability and inhibition of bacterial growth, development, and biofilms production.[38] The current study presented 2 % curcumin (G3) as the optimum effective concentration for treatment of periodontitis where lower concentrations of 0.12 % (G1) and 1 % (G2) were found less effective in agreement with observations of Jalaluddin et al. and Elavarasu et al. who stated that mouthwash containing 0.2 % chlorhexidine was more effective than mouthwash containing 1 % curcumin on all clinical measures and that the effectiveness of 0.2 % curcumin strip in conjunction with nonsurgical therapy and reported non-significant difference between both the test (scaling & root planning with curcumin) and the control (scaling & root planning alone) groups, respectively.[39,40]

The drastic changes in the extracellular environment occurring during periodontitis represents one of the major challenges in restoring damaged periodontal tissues where PDL fibroblasts in the periodontal niche undergo inflammatory immune responses in addition to several types of regulated cell death pathways including apoptosis, necroptosis, pyroptosis, NETosis, and ferroptosis.[41–44] Several studies proved that such changes can be reversed by strong antioxidant, anti-apoptotic and neuroprotective properties of curcumin added in protecting and restoring PDL fibroblasts viability and treating chronic periodontitis.

In the research performed by Wang et al. on ligature-induced periodontal-diseased mice, they concluded that curcumin alleviated periodontal damage by preventing ferroptosis and lipid peroxidation through downregulating malondialdehyde (MDA), ACSL4 and TfR1 expression levels while upregulating that of superoxide dismutase (SOD), total glutathione (GSH), SLC7A11 and GPX4.[45]

MTT assay for G3 (2 % curcumin) showed significantly higher PDL cells viability and proliferation in comparison with control group, G1 and G2. Curcumin was reported to induce more transforming growth factor-beta 1 (TGF-β1) formation which increases the proliferation and migration of fibroblasts and induces ECM synthesis increasing collagen production.[10] Moreover, Sharma et al. (2024) demonstrated that curcumin at 1 mg/ml enhances the proliferation of healthy human gingival fibroblasts and significantly increases cell viability in experimentally induced periodontitis and diabetic gingival fibroblast cell line.[46] Furthermore, Curcumin significantly increased the mRNA expression of TGF-β1, TGF-βRI, TGF-βRII, and vascular endothelial growth factor (VEGF) compared to the vehicle-treated control group in the *in-vitro* study conducted by Rujirachotiwat and Suttamanatwong who tested the effect of 1 μM curcumin on human gingival fibroblast monolayers in a 24-h wound healing model.[32]

Investigations by Iova et al., Zhao et al. and Hirata et al. revealed the curcumin as an antioxidant agent inhibiting gingival inflammation and

periodontitis through its ability to scavenge excessive reactive oxygen species (ROS), modulate oxidative stress, inducing an antioxidant response and prevent nuclear fragmentation associated with regulated cell death processes.[47–49] It enhances the transcription of cytoprotective genes to eliminate ROS through triggering transcription factors such as nuclear factor erythroid 2-related factor 2 (Nrf-2) and nuclear factor-κB (NF-κB).[50]

Anti-inflammatory effects of curcumin on the lipopolysaccharide (LPS)-induced inflammatory response in rat gingival fibroblasts *in-vitro* and ligation-induced experimental periodontitis *in-vivo* were examined by Xiao et al. who observed downregulation of pro-inflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) levels in *in-vitro* fibroblasts in addition to suppressed NF-κB activation and decreased osteoprotegerin (OPG)/soluble receptor activator of nuclear factor kappa-B ligand (RANKL) ratio *in-vivo*. These events resulted in the inhibition of activation and recruitment of inflammatory cells associated with gingival inflammation, collagen destruction and alveolar bone resorption in periodontitis.[51] Similar results of outstanding impact of curcumin on periodontal inflammation were detected by Guimarães et al. and Zhou et al. where it significantly lowered the formation of inflammatory infiltrates within the periodontal lesion while boosted the number of PDL fibroblasts and collagen content within periodontium and associated lesions.[52,53]

5. Conclusion

Within the limitations of the present study, the authors concluded that PDL fibroblasts as the main architect builders of PDL, their maximum viability, proliferation and cellular attachment to periodontitis-affected root surfaces were achieved with 2 % curcumin concentration. Lower concentrations have no or limited value. Clinical effects of varying curcumin concentrations in randomized clinical trials are required to confirm in situ same SEM findings which could be affected by periodontal pocket ecology.

CRediT authorship contribution statement

AF: conception and design of the study, data acquisition and analysis, interpretation of data, manuscript draft and revision; personal accountability; HY: conception and design of the study, data acquisition, analysis, interpretation, drafted and revised manuscript; AG: conception and design of the study, interpretation of data, manuscript revision, personal accountability; NB: contributed to data acquisition, analysis, interpretation, drafted and revised manuscript; MW: conception and design of the study, data acquisition and analysis, interpretation of data, manuscript draft and revision, personal accountability. All authors reviewed and approved the manuscript.

Data availability statement

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

Ethical clearance

This Study was approved by Research Ethics Committee at Faculty of Dentistry, October 6 University, Giza, Egypt, with approval number (RECO6U/25–2022) obtained in its meeting held on November 12, 2022.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Bijlani S, Shanbhog RS. An in vitro evaluation of ice apple as a novel storage medium to preserve the viability of human periodontal ligament fibroblasts. *Int J Clin Pediatr Dent*. 2022;15(6):699–703.
2. Abbass MMS, Rady D, Radwan IA, et al. The occurrence of periodontal diseases and its correlation with different risk factors among a convenient sample of adult Egyptian population: a cross-sectional study. *F1000Res*. 2019;8:1740.
3. Oruba Z, Łabuz P, Macyk W, Chomyszyn-Gajewska M. Periopathogens differ in terms of the susceptibility to toluidine blue O-mediated photodynamic inactivation. *Photodiagnosis Photodyn Ther*. 2017;20:28–34.
4. Beertsen W, McCulloch CA, Sodek J. The periodontal ligament: a unique, multifunctional connective tissue. *Periodontol 2000*. 1997;13:20–40.
5. Lekic P, McCulloch CAG. Periodontal ligament cell populations: the central role of fibroblasts in creating a unique tissue. *Anat Rec*. 1996;245(2):327–341.
6. Abdal-Wahab M, Abdel Ghaffar KA, Ezzatt OM, Hassan AAA, El Ansary MMS, Gamal AY. Regenerative potential of cultured gingival fibroblasts in treatment of periodontal intrabony defects (randomized clinical and biochemical trial). *J Periodontal Res*. 2020;55(3):441–452.
7. Kalson NS, Starborg T, Lu Y, et al. Nonmuscle myosin II powered transport of newly formed collagen fibrils at the plasma membrane. *Proc Natl Acad Sci U S A*. 2013;110 (49):E4743–E4752.
8. Kitamura M, Akamatsu M, Machigashira M, et al. FGF-2 stimulates periodontal regeneration: results of a multi-center randomized clinical trial. *J Dent Res*. 2011;90 (1):35–40.
9. Lucas RM, Chen SY, Aleo JJ. Histochemical study of strain L fibroblasts exposed to endotoxin. The effect on cellular organelles. *J Periodontol*. 1979;50(1):20–22.
10. Izui S, Sekine S, Maeda K, et al. Antibacterial activity of curcumin against periodontopathic bacteria. *J Periodontol*. 2016;87(1):83–90.
11. Sukumaran SK, Vadakkekuttical RJ, Kanakath H. Comparative evaluation of the effect of curcumin and chlorhexidine on human fibroblast viability and migration: an in vitro study. *J Indian Soc Periodontol*. 2020;24(2):109–116.
12. Hussain Y, Alam W, Ullah H, et al. Antimicrobial potential of curcumin: therapeutic potential and challenges to clinical applications. *Antibiotics (Basel)*. 2022;11(3).
13. Dai C, Lin J, Li H, et al. The natural product curcumin as an antibacterial agent: current achievements and problems. *Antioxidants*. 2022;11(3).
14. Bhatia M, Urolagin SS, Pentyala KB, Urolagin SB, K BM, Bhoi S. Novel therapeutic approach for the treatment of periodontitis by curcumin. *J Clin Diagn Res*. 2014;8 (12):Zc65–Zc69.
15. Wendorff-Tobolla LM, Wolgin M, Wagner G, Klerings I, Dvornyk A, Kielbassa AM. A systematic review and meta-analysis on the efficacy of locally delivered adjunctive curcumin (Curcuma longa L.) in the treatment of periodontitis. *Biomedicines*. 2023; 11(2).
16. Konain K, Saddique N, Samie M, et al. Curcumin-loaded nanofibrous matrix accelerates fibroblast cell proliferation and enhances wound healing via GSK3-β inhibition. *J Compos Sci*. 2023;7(8).
17. Cianfruglia L, Minnelli C, Laudadio E, Scire A, Armeni T. Side effects of curcumin: epigenetic and antiproliferative implications for normal dermal fibroblast and breast cancer cells. *Antioxidants*. 2019;8(9).
18. Zheng BZ, Liu TD, Chen G, Zhang JX, Kang X. The effect of curcumin on cell adhesion of human esophageal cancer cell. *Eur Rev Med Pharmacol Sci*. 2018;22(2): 551–560.
19. Gamal AY, Bayomy MM. Effect of cigarette smoking on human PDL fibroblasts attachment to periodontally involved root surfaces in vitro. *J Clin Periodontol*. 2002; 29(8):763–770.
20. Caton JG, Armitage G, Berglundh T, et al. A new classification scheme for periodontal and peri-implant diseases and conditions - introduction and key changes from the 1999 classification. *J Clin Periodontol*. 2018;45(Suppl 20):S1–S8.
21. Gamal AY, Mailhot JM, Garnick JJ, Newhouse R, Sharawy MM. Human periodontal ligament fibroblast response to PDGF-BB and IGF-1 application on tetracycline HCl conditioned root surfaces. *J Clin Periodontol*. 1998;25(5):404–412.
22. Mailhot JM, Schuster GS, Garnick JJ, Hanes PJ, Lapp CA, Lewis JB. Human periodontal ligament and gingival fibroblast response to TGF-beta 1 stimulation. *J Clin Periodontol*. 1995;22(9):679–685.
23. Patwekar S, Khavane K, Chainpure P, Payghan S, Shivpuje S, Shivpuje S. A review on different preparation methods used for development of curcumin nanoparticles. *Int J Creat Res Thoughts*. 2021;9(1):4088–4101.
24. Murtey MD, Ramasamy P. *Sample Preparations for Scanning Electron Microscopy – Life Sciences. Modern Electron Microscopy in Physical and Life Sciences*. 2016.

25. Talebi-Ardakani MR, Torshabi M, Karami E, et al. Comparison of Er:YAG laser and hand instrumentation on the attachment of cultured human gingival fibroblasts to periodontally involved root surfaces. *J Lasers Med Sci*. 2017;8(Suppl 1):S51–S55.
26. Liang Y, Luan X, Liu X. Recent advances in periodontal regeneration: a biomaterial perspective. *Bioact Mater*. 2020;5(2):297–308.
27. Forouzanfar F, Forouzanfar A, Sathyapalan T, Orafai HM, Sahebkar A. Curcumin for the management of periodontal diseases: a review. *Curr Pharm Des*. 2020;26(34):4277–4284.
28. Gottlow J, Nyman S, Karring T, Lindhe J. New attachment formation as the result of controlled tissue regeneration. *J Clin Periodontol*. 1984;11(8):494–503.
29. Liu J, Zhou Z, Zhang S. Effects of Er:YAG laser on the attachment of human periodontal ligament fibroblasts to denuded root surfaces simulating delayed replantation cases: an in vitro study. *Photobiomodul Photomed Laser Surg*. 2020;38(3):145–150.
30. Malhotra R, Kapoor A, Grover V, Kaushal S. Nicotine and periodontal tissues. *J Indian Soc Periodontol*. 2010;14(1):72–79.
31. Christoffers AB, Kreisler M, Willershausen B. Effects of esradiol and progesterone on the proliferation of human gingival fibroblasts. *Eur J Med Res*. 2003;8(12):535–542.
32. Rujirachotiwat A, Suttamanatwong S. Curcumin upregulates transforming growth factor-beta1, its receptors, and vascular endothelial growth factor expressions in an in vitro human gingival fibroblast wound healing model. *BMC Oral Health*. 2021;21(1):535.
33. Mandroli PS, Prabhakar AR, Bhat K, Krishnamurthy S, Bogar C. An in vitro evaluation of cytotoxicity of curcumin against human periodontal ligament fibroblasts. *Ayu*. 2019;40(3):192–195.
34. Yassin H, El Khouli A. Efficacy of locally delivered curcumin gel in the management of chronic periodontitis. A clinical and biochemical study. *Egypt Dent J*. 2014;60:1–13.
35. Adamczak A, Ozarowski M, Karpinski TM. Curcumin, a natural antimicrobial agent with strain-specific activity. *Pharmaceuticals*. 2020;13(7).
36. Teow SY, Liew K, Ali SA, Khoo AS, Peh SC. Antibacterial action of curcumin against Staphylococcus aureus: a brief review. *J Trop Med*. 2016;2016, 2853045.
37. Teughels W, Feres M, Oud V, Martín C, Matesanz P, Herrera D. Adjunctive effect of systemic antimicrobials in periodontitis therapy: a systematic review and meta-analysis. *J Clin Periodontol*. 2020;47(Suppl 22):257–281.
38. Dani S, Prabhu A, Chaitra KR, Desai NC, Patil SR, Rajeev R. Assessment of Streptococcus mutans in healthy versus gingivitis and chronic periodontitis: a clinico-microbiological study. *Contemp Clin Dent*. 2016;7(4):529–534.
39. Jalaluddin M, Jayanti I, Gowdar IM, Roshan R, Varkey RR, Thirutheri A. Antimicrobial activity of Curcuma longa L. extract on periodontal pathogens. *J Pharm Bioallied Sci*. 2019;11(Suppl 2):S203–S207.
40. Elavarasu S, Suthanthiran T, Thangavelu A, Alex S, Palanisamy VK, Kumar TS. Evaluation of superoxide dismutase levels in local drug delivery system containing 0.2% curcumin strip as an adjunct to scaling and root planing in chronic periodontitis: a clinical and biochemical study. *J Pharm Bioallied Sci*. 2016;8(Suppl 1):S48–S52.
41. Zhao Y, Li J, Guo W, Li H, Lei L. Periodontitis-level butyrate-induced ferroptosis in periodontal ligament fibroblasts by activation of ferritinophagy. *Cell Death Discov*. 2020;6(1):119.
42. Song B, Zhou T, Yang WL, Liu J, Shao LQ. Programmed cell death in periodontitis: recent advances and future perspectives. *Oral Dis*. 2017;23(5):609–619. %@ 1354-523X.
43. Li J, Shi J, Pan Y, et al. Transcription modulation by CDK9 regulates inflammatory genes and RIPK3-MLKL-mediated necroptosis in periodontitis progression. *Sci Rep*. 2019;9(1), 17369.
44. Liu J, Wang Y, Meng H, et al. Butyrate rather than LPS subverts gingival epithelial homeostasis by downregulation of intercellular junctions and triggering pyroptosis. *J Clin Periodontol*. 2019;46(9):894–907.
45. Wang Y, Lin H, Huang W, et al. Curcumin attenuates periodontal injury via inhibiting ferroptosis of ligature-induced periodontitis in mice. *Int J Mol Sci*. 2023;24(12).
46. Sharma A, Bains VK, Chandra C, et al. Impact of photoactivated curcumin on proliferation of experimentally induced periodontitis and diabetic gingival fibroblast cell line. *J Pharm Bioallied Sci*. 2024;16(Suppl 5):S4844–S4849.
47. Iova GM, Calniceanu H, Popa A, et al. The antioxidant effect of curcumin and rutin on oxidative stress biomarkers in experimentally induced periodontitis in hyperglycemic Wistar rats. *Molecules*. 2021;26(5).
48. Zhao Y, Zheng Z, Zhang M, et al. Design, synthesis, and evaluation of mono-carbonyl analogues of curcumin (MCACs) as potential antioxidants against periodontitis. *J Periodontal Res*. 2021;56(4):656–666.
49. Hirata Y, Okazaki R, Sato M, Oh-Hashi K, Takemori H, Furuta K. Effect of ferroptosis inhibitors oxindole-curcumin hybrid compound and N,N-dimethylaniline derivatives on rotenone-induced oxidative stress. *Eur J Pharmacol*. 2022;928, 175119.
50. Ashrafizadeh M, Ahmadi Z, Mohammadinejad R, Farkhondeh T, Samarghandian S. Curcumin activates the Nrf2 pathway and induces cellular protection against oxidative injury. *Curr Mol Med*. 2020;20(2):116–133.
51. Xiao CJ, Yu XJ, Xie JL, Liu S, Li S. Protective effect and related mechanisms of curcumin in rat experimental periodontitis. *Head Face Med*. 2018;14(1):12.
52. Guimaraes MR, Coimbra LS, de Aquino SG, Spolidorio LC, Kirkwood KL, Rossa Jr C. Potent anti-inflammatory effects of systemically administered curcumin modulate periodontal disease in vivo. *J Periodontal Res*. 2011;46(2):269–279.
53. Zhou T, Chen D, Li Q, Sun X, Song Y, Wang C. Curcumin inhibits inflammatory response and bone loss during experimental periodontitis in rats. *Acta Odontol Scand*. 2013;71(2):349–356.