BioBacta
Journal of Bioscience and Applied Research
https://jbaar.journals.ekb.eg/
SPBH

# Periodontally Involved Root Surfaces Biocompatibility following Application of Different Curcumin Concentration (In Vitro Study)

**Noorhan K. El Badawi [1], Hala H. Yassin [2], Amina F Farag [3], Mahetab M. Abdalwahab [4], Ahmed Y Gamal [5].**

[1] Department of Periodontology, Faculty of Dentistry, October 6 University.

[2] Department of Oral Medicine, Periodontology, Oral Diagnosis and Oral Radiology, College of Dentistry at Arab Academy for Science and Technology and Maritime Transport AASTMT, New Alamein Alexandria, Egypt

[3]Department of Oral and Maxillofacial Pathology Department **Attachment** Faculty of Dentistry, October 6 University, Egypt

[4] Department of Oral Medicine, Periodontology, Oral Diagnosis and Oral Radiology, Faculty of Dentistry, Russian University, Cairo, Egypt.

[5]Department of Periodontology, Faculty of Dentistry. Ain Shams University and Misr University for Science and Technology, Egypt

**Corresponding author:** Noorhan Khedr Abdel-Fattah El-Badawi. Department of Periodontology, Faculty of Dentistry, October 6 University, Egypt. Tel: 00201101807789.

E-mail: noorhanelbadawi5@gmail.com . ORCID: https://orcid.org/0009-0002-8405-9562

**DOI:10.21608/jbaar.2024.381634**

## Abstract

**This study aimed** to assess the impact of 0.12%, 1%, and 2% curcumin concentrations on the adherence of periodontal ligament cells to root surfaces damaged by periodontal disease. **Materials and Methods**: Twenty periodontally compromised teeth were removed and sectioned into root specimens. PDL fibroblasts were harvested from freshly extracted teeth for orthodontic purposes, and subsequently grown and incubated. PDL fibroblast cells were then introduced to curcumin-coated root samples at varying dosages. The study samples were categorized into four groups: Group 1 (0.12% curcumin paste), Group 2 (1% curcumin paste), Group 3 (2% curcumin paste), and Group 4 (control group). All samples were examined using a scanning electron microscope. **Results**: Group 3, with a 2% curcumin concentration, exhibited distinctly defined multilayered adhering cells that encompassed the whole surface, characterized by entirely flat polyhedral bodies featuring elongated cytoplasmic extensions and minimal to no bacterial colonization, along with sporadic isolated planktonic bacterial adherence. **Conclusion**: Curcumin at 2% concentration optimally stimulates cellular attachment and exhibits antibacterial properties on root surfaces damaged by periodontitis.

**keywords:** Periodontal ligament fibroblast, curcumin concentration, root surface.

---

## Introduction

Periodontal disease is a persistent inflammatory condition affecting the supporting tissues around the tooth. It is regarded as one of the most significant global oral health challenges, impacting over 10-15% of the world's population and 89.8% of the Egyptian population (1, 2). Pathogenic anaerobic microorganisms clinging to tooth surfaces have been identified as the causal agents of periodontitis. Untreated periodontitis commonly results in apical

Received: July 3, 2024. Accepted: September 8, 2024. Published: September 24, 2024

migration of the epithelial attachment, degradation of connective tissue, loss of alveolar bone, and ultimately, tooth loss (3). The primary cells of periodontal ligaments (PDLs) are fibroblasts, responsible for synthesizing collagen bundles in the PDL and for the renewal and replacement of aged and damaged collagen fibrils (4, 5). Fibroblasts help periodontal tissue homeostasis by their capacity to remodel tissues and repopulate wounds (6, 7). Viable and healthy periodontal cells are needed for effective periodontal therapy and predictable regeneration, and these cells must adhere to the tooth root surfaces (8). Lucas et al. (9) proposed that endotoxins from periodontal infections infiltrate the root surface and obstruct cellular adhesion. Bacterial endotoxin adhered to tooth surfaces impeded cellular adhesion to those surfaces. Furthermore, the removal of bacterial endotoxins enhanced the cellular attachment of human gingival fibroblasts (10). Herbal extracts have been utilized for the treatment of human ailments for centuries. Numerous herbal formulations can regulate the synthesis of pro-inflammatory mediators, therefore controlling various inflammatory processes (11). Curcumin exhibits potent antibacterial properties that inhibit the proliferation of several gram-positive and gram-negative bacteria (12,13). Curcumin is a natural substance possessing antioxidant and anti-inflammatory effects (14,15). This study investigates the effect of varying curcumin concentrations on the adhesion of human periodontal ligament fibroblasts to periodontally compromised roots, aiming to assess the attachment of PDL cells to diseased roots modified by curcumin. The secondary objective was the impact of varying curcumin concentrations on bacterial colonization of periodontally compromised root surfaces.

## Materials and Methods

Screening patients receiving treatment at the outpatient clinic of the "Department of Oral Medicine and Periodontology at the Faculty of Dentistry, October 6 University" was used to enroll participants for this research. After receiving a thorough explanation of the research methods, all patients signed a permission form allowing the use of their removed teeth in the study. An ethical review board from the Faculty of Dentistry on October 6 University in Giza, Egypt, approved the research with the permission number RECO6U/25-2022. Twenty teeth, including both front and back teeth, from twenty individuals (16 males and 4 females, ranging in age from 41 to 54 years old) with periodontitis stage IV and grade III mobility were collected. Smokers, pregnant women, and those with systemic diseases affecting the periodontium were not included in the research. In addition, those who had undergone scaling or dental prophylaxis in the last three months were not included. As part of their dental treatment, every patient was asked to bring in one tooth that was free of cavities and was meant to be extracted. Additionally, to isolate periodontal ligament fibroblasts, 10 diseased third molars extracted from adolescents who were otherwise medically healthy were used. Using a calibrated periodontal probe, we assessed the clinical probing depth and attachment level to establish the clinical criterion for tooth collection. The company behind the periodontal probe is Aksim Surgical Ltd, and its address is TW3 1EA in the United Kingdom. Starting at the cementoenamel junction and working our way up to the base of the probing depth, we measured the attachment level. A clinical attachment loss of 5mm and a probing depth of at least 5mm were seen on the examined sick tooth surfaces.

To determine which tooth portions were exposed to the pocket environment, a pencil mark was applied at the gingival edge level before extractions were conducted under local anesthetic. Thus, the test locations will be little affected ("Mepecaine-L, The Alexandria Co., Alexandria, Egypt"). After the tooth was removed, a small spherical bur with a number 0 ("Brassler, Savannah, GA, USA") was used to make two grooves: one at the pencil mark and another at the bottom of the pocket, where the periodontal tissue was most attached to the root surface. After that, the areas that were being tested were cleaned using ultrasonic technology until a strong, full tooth

structure could be seen. The ultrasonic scalers used for the treatment were made by Yimei Dentistry Industry Co. Ltd of Zhengzhou, China ("Yimei UD-32"). Sample preparation, sterilization, and allocation were conducted as outlined below: Following the technique outlined by Gamal et al. (1998), a horizontal incision was executed at the groove level to detach the root from the crown of each tooth, with continuous water chilling applied. The root was thereafter incised lengthwise through the pulp to the apical tip of the test region (16). A secondary horizontal incision was executed at the pocket's base to detach the test region from the tooth. Ultimately, specimens were preserved and readied for experimentation (Figure 1) (17).

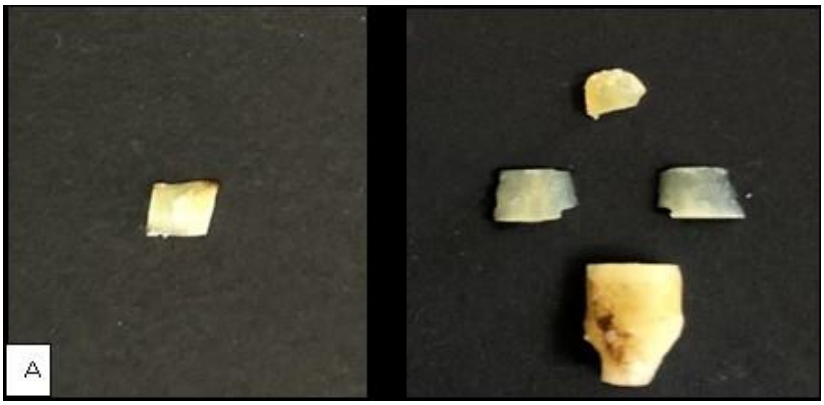


Figure 1: (A & B) root samples.

## Human periodontal ligament fibroblast isolation and culture

The PDL fibroblasts were grown in the CLSBAR culture, which is part of the Faculty of Dentistry at Ain Shams University's Center for Stem Cells and Biomaterials Applied Research. The fibroblasts used for PDL harvesting and expansion were taken from 10 impacted third molars. Extraction of teeth was carried out under strict aseptic circumstances after rinsing them many times in biopsy media. The teeth were transported to the laboratory in a sterile centrifuge tube that contained 10 ml of transfer media. To remove the periodontal tissues, the tooth was rinsed twice with phosphate-buffered saline ("Bio-Whittaker") that included 100 µg/mL streptomycin and 100 µg/mL penicillin. Then, the tooth roots were painstakingly scraped using a "sterile scalpel (No. 15)". The tissues were then cut into pieces that were 1-2 mm² in size and digested in a solution that contained 2 mg/mL of collagenase IV, 1 mg/mL of dispase II, and 0.2 µmol/L of "Eagle's minimum essential medium" (from Bio-Whittaker Inc. in Walkersville, MD, USA) in a tissue flask. The digestion was carried out for 30 minutes in an incubator at 37°C. The tissue was digested twice in the same solution after the first cell suspension was extracted, and then incubated for "90 minutes at 37°C."Once the tissues had been centrifuged, they were placed into a 25 cm² tissue culture flask along with EMEM that included 100 µg/mL penicillin, 100 µg/mL streptomycin, 1% amphotericin, 15% fetal bovine serum, and 200 µmol/L L-glutamine. The flasks were placed in a humidified incubator at 37°C with a 5% CO2 and 95% air atmosphere. After a day of adhesion, cells were washed with PBS to eliminate any remaining unattached cells. A new medium was added and replaced every three days. After coming to 80% confluence, the cells were transferred to tissue culture flasks using a mixture of 0.05% trypsin and 1 µmol/L EDTA. They were then subcultured in a complete growth medium. The cells were combined into PDL populations and kept at a

temperature of -70˚C for preservation. Our study relied on cells harvested in the third passage. Cell culture procedures routinely made use of phase contrast microscopy to track cell multiplication; each cell had a fibroblast-like appearance.

### Curcumin Paste Preparation

Curcumin paste was synthesized in the Nano Gate laboratory. As stated by Patwekar et al. (18). To create 5ml of a 2% w/v curcumin paste, 0.1g of curcumin powder was suspended in 5ml of distilled water with stirring for 30 minutes; moreover, 0.25g The effect of curcumin on the healing of tongue ulcers in albino subjects was examined by gradually and gently sprinkling Carboxymethyl cellulose (CMC) over the solution at a mild temperature of 35°C while swirling vigorously to achieve a homogeneous paste. To obtain 5ml of curcumin paste to prepare a 1% w/v solution, 0.05 g of Curcumin powder was suspended in 5 mL of distilled water with stirring for 30 minutes. Subsequently, 0.25 g of CMC was gradually and gently added to the solution while maintaining a mild temperature of 35°C, accompanied by vigorous stirring to achieve a homogeneous paste. Ultimately, 0.006 grams of Curcumin powder was dissolved in 5 milliliters of deionized water to achieve a concentration of 0.12% w/v, with continuous stirring for 30 minutes. Additionally, 0.25 g of CMC was gradually and gently dispersed into the solution at a mild temperature of 35°C while stirring vigorously to achieve a homogeneous paste (14,16).

### Experimental design

The treated tooth chip samples were randomly allocated into four groups, each consisting of five tooth segments, following the treatment methodology. PDL cells from ten distinct people were aggregated and grown in EMEM containing 0.5% FBS. The cell count was determined using a hemocytometer, with 35×10^5 cells per 200 mL allocated for each sample. Each sample comprised sick root specimens inoculated with PDL cells after being coated with varying doses of curcumin. Group 1 (G1) received 0.12% curcumin paste. Group 2 (G2), 1% curcumin paste. Group 3 (G3), 2% curcumin paste. Group 4 (G4) is a control group devoid of surface covering. The cells were cultivated on the test samples from all of the groups for twenty-four hours at 37 degrees Celsius in an atmosphere consisting of ninety-five percent air and five percent carbon dioxide.

### Samples fixation and SEM preparation

Following a 24-hour incubation period, the sample segments were washed with phosphate-buffered saline to eliminate any leftover culture medium components, debris, or unattached cells. After that, the fluid from the culture was taken. When placing the samples on their respective chamber slides, I cooled them with ice water. (BDH Chemicals, Ltd., Poole, England) for thirty minutes containing 3.3% glutaraldehyde. After being submerged in 70% ethyl alcohol ("Merck, Darmstadt, Germany") for half an hour, the specimens were immersed in 100% ethyl alcohol for two hours and then permitted to soak in an extra 100% ethyl alcohol overnight. On the very same morning, the specimens were submerged in a solution containing 100% ethyl alcohol for two hours and 100% amyl acetate for one hour. Leica Research in Cambridge, UK developed the "S 150 A" technique to sputter coat the samples with a 200 Å thick coating of gold after they were dried in the critical point drier ("Balzers, FL9496, Liechtenstein"). A scanning electron microscope ("Philips Ï630, Philips, Eindhoven, the Netherlands") was then used to analyze the specimens at a magnification of Ï750 and capture photos at different magnifications. The surface areas of the root samples analyzed ranged from 9 to 24 mm². A.Y.G., the examiner, was ignorant of the therapy but proceeded to morphologically analyze and screen for cell attachment on root surfaces afflicted by periodontal disease.

### Data analysis

This study used SPSS ("IBM SPSS Statistics for Windows, Version 18.0, Armonk, NY: IBM Corp")

for data management and statistical analysis. The ANOVA test was used to compare groups based on parametric data, which was presented as mean ± standard deviation (SD), whereas the Kruskal-Wallis test was used to evaluate non-parametric numerical variables. To examine changes in results over time within each group, the Friedman test was used. Statistical significance was determined by p-values that were less than or equal to 0.05. Previous research determined the average difference in cell adhesion in sick root samples with different amounts of curcumin, and this information was used to estimate the sample size for each of the four groups. an alpha of 0.05 and a power of 80%. The average ± standard deviation of PDLF in root specimens from the G3 and G4 groups, 8.2 ± 1.43 and 6.4 ± 1.52, respectively, are used to estimate the sample size (16).

## Results

### Morphological Analysis Using Scanning Electron Microscope (SEM):

All samples in group 3 of 2% curcumin concentration showed well-defined multilayered adherent cells covering the entire surface with totally flat polyhedral bodies with long cytoplasmic extensions and little or no bacterial colonization or sporadic separate planktonic bacterial adhesion compared to other experimental groups. SEM examination of group 1 (0.12%) samples showed healthy flat and multilayered cell arrangement with evident cytoplasmic extension covering root samples. Bacterial colonization appeared in separate areas in 2 out of the examined samples. SEM examination for group 2 samples (1%) showed similar results to group 1 of a multilayered fibroblastic attached to root samples and scarce bacterial colonization
in all samples (Figure 2).

All samples of the control unconditioned group showed predominantly single layers of rounded cells with pulled-up and blipped surfaces which indicate partial loss of cell viability and cellular adhesion. Moreover, the surface was completely covered with bacterial colonies.

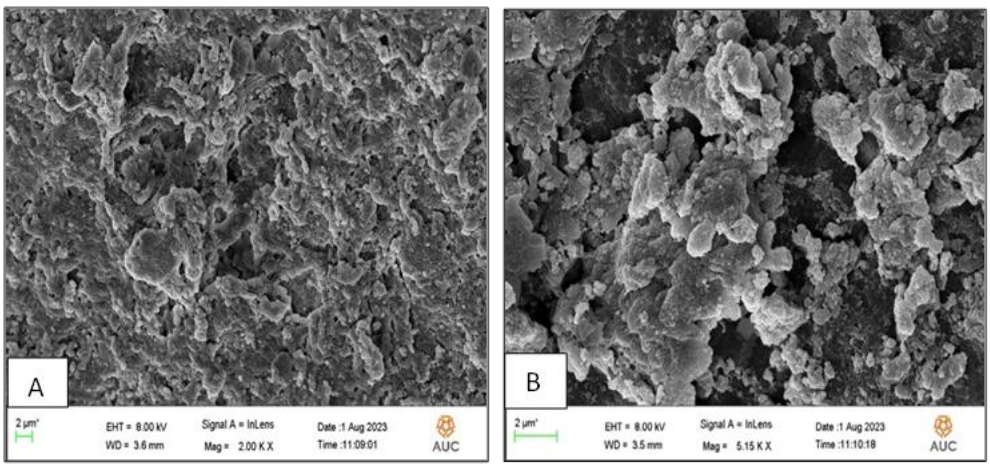


Figure 2: SEM micrograph of group 1 showing multilayered healthy fibroblast attached to the root surface with some areas of bacterial colonization, magnification (A) 2.00 K X. (B) 5.15 K X.

## Discussion

To the authors' knowledge, this study is the inaugural investigation assessing the efficacy of varying doses of curcumin on the adherence of PDL cells to periodontally compromised root surfaces. Root samples were collected from periodontally compromised roots situated between the gingival margin and the base of the pocket to access regions exposed to the bacterial milieu in the oral cavity, afterward, replicating naturally damaged root surfaces after non-surgical scaling and root planing, the afflicted areas will undergo healing via long junctional epithelium.

In this investigation, group III (2% curcumin) demonstrated that the majority of cells displayed flat polyhedral shapes, characterized by elongated cytoplasmic extensions, indicating optimal cell adhesion and reduced bacterial colonization. Subsequently, group II (1%) and group I (0.12%) exhibited a greater prevalence of rounded or spindle-shaped cells, abbreviated cytoplasmic extensions, altered morphology, and increased biofilm surface coverage. This may be ascribed to the pronounced antibacterial efficacy of 2% curcumin concentration, which enhances root surface energy and promotes fibroblast adhesion (19). Furthermore, curcumin promotes collagen synthesis by fibroblasts, hence enhancing attachment. This outcome aligns with a prior in vitro study that indicated curcumin's effect on enhancing cellular proliferation and collagen production at the wound site, particularly boosting DNA and type III collagen, which improved fibroblast attachment (20,21).

The findings of this investigation align with the current results, indicating that the curcumin 2% group exhibited low bacterial colonization and significant antibacterial efficacy against gram-negative bacteria (22). The antibacterial activity was augmented with the rising concentration of curcumin in group III (2% curcumin). This aligns with other in vivo studies that concluded that 2% curcumin gel, utilized as a local delivery method in the management of chronic periodontitis, improved clinical parameters such as plaque index (PI), gingival index (GI), pocket depth (PD), and clinical attachment loss (CAL) due to its antibacterial properties, rendering it a viable and effective adjunct to mechanical debridement as a subgingival irritant (23). The favorable result of 2% curcumin paste in diminishing bacterial colonization in group III may be attributed to curcumin's adherence to the bacterial cell walls of periodontal pathogens and the generation of reactive oxygen species, which can eradicate the pathogens in the surrounding area (24). Cell morphology is frequently utilized to indicate the extent of cellular adhesion and vitality. In the current investigation, 2% of curcumin exhibited a greater number of flattened cells with elongated cytoplasmic extensions. An in vitro study examining the impact of laser treatment on fibroblast attachment to root surfaces revealed that fibroblasts firmly adhered to treated root sections displayed numerous lamellipodia and a flattened morphology, whereas fibroblasts poorly attached to untreated root sections exhibited minimal attachment processes and a rounded morphology (25). The results from the control group, which lacked curcumin coating, demonstrated compromised fibroblast attachment and exhibited a more pronounced raised appearance with a blipped surface. The control group exhibited the highest level of bacterial contamination and the lowest antibacterial activity, with no significant difference observed compared to group I (0.12% curcumin) and group II (1% curcumin). The results may stem from the insufficient antibacterial efficacy of curcumin at 0.12% and 1%, resulting in the presence of contaminated bacterial endotoxins on root specimens, which negatively impacts cell adhesion. The results aligned with another in vitro investigation that evaluated the impact of endotoxin adsorbed to dental surfaces on the attachment of human gingival fibroblasts, revealing that bacterial endotoxin impeded cell adhesion to these surfaces. Furthermore, the removal of bacterial endotoxins enhanced attachment (26).

## Authors contributions

NB [1]: conception and design of the study; data acquisition and analysis; interpretation of data;

manuscript draft and revision; personal accountability, HY [2]: design of the study; interpretation of data; manuscript revision; personal accountability. AF [3]: contributed to data acquisition, analysis, interpretation, drafted and revised manuscript, MW [4:] contributed to data acquisition, analysis, interpretation, drafted and revised manuscript, AG [5]: 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.

## Acknowledgment

This work was based on facilities in the Central Lab of Stem Cells and Biomaterial Applied Research (CLSBAR) in the Faculty of Dentistry, Ain Shams University, which is supported by Science, Technology & Innovation Funding Authority (STDF) under grant [CB project ID (21747)].

**Funding:** NONE

## Conflicts of interest

No conflicts of interest to disclose.

## References

1. 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 Nov-Dec;15(6):699-703. doi: 10.5005/jp-journals-10005-2468. PMID: 36866144; PMCID: PMC9973076.

2. Abbass MMS, Rady D, Radwan IA, El Moshy S, AbuBakr N, Ramadan M, et al. Prevalence of periodontal diseases and its correlation with different risk factors among an

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 Dec 1; 20:28–34.

4. Beertsen W, Mcculloch CAG, Sodek J. The periodontal ligament: A unique, multifunctional connective tissue. Periodontol 2000. 1997;13(1):20–40.

5. Lekic P, Mcculloch CAG. Periodontal ligament cell populations: The central role of fibroblasts in creating a unique tissue. Anatomical Record. 1996 Jun;245(2):327–41.

6. Kalson NS, Starborg T, Lu Y, Mironov A, Humphries SM, Holmes DF, et al. Nonmuscle myosin II powered transport of newly formed collagen fibrils at the plasma membrane. Proc Natl Acad Sci U S A. 2013 Dec 3;110(49).

7. Abdal-Wahab M, Abdel Ghaffar KA, Ezzatt OM, Hassan AA, El Ansary MM, Gamal AY. Regenerative potential of cultured gingival fibroblasts in treatment of periodontal intrabony defects (randomized clinical and biochemical trial). Journal of periodontal research. 2020 Jun;55(3):441-52.

8. Kitamura M, Akamatsu M, MacHigashira M, Hara Y, Sakagami R, Hirofuji T, et al. FGF-2 stimulates periodontal regeneration: Results of a multi-center randomized clinical trial. J Dent Res. 2011 Jan;90(1):35–40.

9. Lucas R, Cohen S, Aleo J. Histochemical study of strain I. Fibroblasts exposed to endotoxin. The effect on cellular organelles. J Periodontol 1979; 50:20-25.

10. Izui S, Sekine S, Maeda K, Kuboniwa M, Takada A, Amano A, et al. Antibacterial Activity of Curcumin Against Periodontopathic Bacteria. J Periodontol.2016 Jan;87(1):83–90.

11. Sukumaran S, Vadakkekuttical R, 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 Mar 1;24(2):109–16.

12. Dai C, Lin J, Li H, Shen J, Shen Z, Wang Y, et al. The Natural Product Curcumin as an Antibacterial Agent: Current Achievements and Problems. Vol. 11, Antioxidants. MDPI; 2022.

13. Hussain Y, Alam W, Ullah H, Dacrema M, Daglia M, Khan H, et al. Antimicrobial Potential of Curcumin: Therapeutic Potential and Challenges to Clinical Applications. Vol. 11, Antibiotics. MDPI; 2022.

14. Bhatia M, Urolagin SS, Pentyala KB, Urolagin SB, Menaka KB, Bhoi S. Novel therapeutic approach for the treatment of periodontitis by curcumin. Journal of Clinical and Diagnostic Research. 2014 Dec 1;8(12): ZC 65–9.

15. Soffar, A., Matta, C., Albatati, S. Curcumin suppresses cellular adhesion and migration of A549 lung cancer cells via a LIMK1/MLCK dependent mechanism. *Journal of Bioscience and Applied Research*, 2019; 5(3): 285-296. doi: 10.21608/jbaar.2019.147118

16. Gamal AY, Bayomy MM, Garnick JJ. Effect of cigarette smoking on human PDL fibroblasts attachment to periodontally involved root surfaces in vitro. J Clin Periodontol. 2002 Aug;29(8):763–70.

17. Gamal AY, Mailhot JM, Garnick JJ, Newhouse R, Sharawy MM. Human periodontal ligament fibroblast response to PDGF-BB and IGF-1 application on tetracycline HCI conditioned root surfaces. Journal of Clinical Periodontology. 1998 May;25(5):404-12.

18. Patwekar SL, Khavane KB, Chainpure PR, Shivpuje SA. A review on different preparation methods used for development of curcumin nanoparticles. International Journal of Creative Research Thoughts. 2021;9(1):4088-101.

19. Talebi-Ardakani MR, Torshabi M, Karami E, Vajar N, Esfahrood ZR, Meimandi M, 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: S51–5.

20. Adamczak A, Ożarowski M, Karpiński TM. Curcumin, a natural antimicrobial agent with strain-specific activity. Pharmaceuticals. 2020 Jul 1;13(7):1–12.

21. Abd, H., Ahmed, H. Nano-Curcumin Mitigates Against Hydroxyapatite Nanoparticle-Induced DNA Damage and KIM-1, LCN-2 Gene Responses in Kidney Rats. *Journal of Bioscience and Applied Research*, 2023; 9(4): 366-375. doi: 10.21608/jbaar.2023.332050

22. Panchatcharam M, Miriyala S, Gayathri VS, Suguna L. Curcumin improves wound healing by modulating collagen and decreasing reactive oxygen species. Mol Cell Biochem. 2006 Oct;290(1–2):87–96.

23. Teow SY, Liew K, Ali SA, Khoo ASB, Peh SC. Antibacterial Action of Curcumin against Staphylococcus aureus: A Brief Review. Vol. 2016, Journal of Tropical Medicine. Hindawi Limited; 2016.

24. 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.

25. Wang H, Xu Y, Sun J, Sui Z. The Novel Curcumin Derivative 1g Induces Mitochondrial and ER-Stress-Dependent Apoptosis in Colon Cancer Cells by Induction of ROS Production. Front Oncol. 2021 Jun 14;11.

26. 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 Mar 1;38(3):145–50.