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Curcumin is effective in managing oral inflammation: An in vitro study

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Abstract

Background: Oral inflammation is among the most prevalent oral pathologies with systemic health implications, necessitating safe and effective treatments. Given curcumin's documented anti-inflammatory and antioxidant properties, this study focuses on the potential of a curcumin-based oral gel in safely managing oral inflammatory conditions.

Methods: This in vitro study utilized four human cell lines: oral keratinocytes (HOKs), immortalized oral keratinocytes (OKF6), periodontal ligament fibroblasts (HPdLF), and dysplastic oral keratinocytes (DOKs). The cells were treated with Lipopolysaccharides (LPS) and curcumin-based oral gel to simulate inflammatory conditions. A panel of cellular assays were performed along with antimicrobial efficacy tests targeting *Candida albicans*, *Streptococcus mutans*, and *Porphyromonas gingivalis*.

Results: LPS significantly reduced proliferation and wound healing capacities of HOKs, OKF6, and HPdLF, but not DOKs. Treatment with curcumin-based oral gel mitigated inflammatory responses in HOKs and HPdLF by enhancing proliferation, colony formation, and wound healing, along with reducing apoptosis. However, its impact on OKF6 and DOKs was limited in some assays. Curcumin treatment did not affect the invasive capabilities of any cell line but did modulate cell adhesion in a cell line-specific manner. The curcumin-based oral gel showed significant antimicrobial efficacy against *C. albicans* and *S. mutans*, but was ineffective against *P. gingivalis*.

Conclusion: This study demonstrates the potential of the curcumin-based oral gel as a safe and effective alternative to conventional antimicrobial treatments for managing cases of oral inflammation. This was achieved by modulating cellular responses under simulated inflammatory conditions. Future clinical-based studies are recommended to exploit curcumin's therapeutic benefits in oral healthcare.

KEYWORDS
anti-inflammatory actions, anti-microbial agents, cellular assays, curcumin, oral products

1 | INTRODUCTION

Oral health has long been recognized as essential to overall well-being, influencing functional and aesthetic concerns and systemic health.[1] As one of the most prevalent oral pathologies, oral inflammation represents a pivotal aspect of the body's defense mechanism against microbial invaders, tissue injury, and irritants within the oral cavity.[2] Nonetheless, inadequate management of oral inflammation can lead to profound systemic effects, potentially contributing to conditions like inflammatory bowel disease, cardiovascular diseases, diabetes, and adverse pregnancy outcomes.[1]

Gingivitis, a prevalent form of periodontal disease, exemplifies localized inflammation of the gingiva, often associated with poor oral hygiene and plaque accumulation.[3] If left untreated, gingivitis can go beyond the gingiva to the teeth-supporting structures, leading to tissue destruction and tooth loss.[3] Beyond periodontal diseases, diverse oral inflammatory conditions, such as oral lichen planus, recurrent aphthous ulcers, and angular cheilitis, underscore the multifactorial nature of oral inflammatory disorders, highlighting the need for tailored therapies.[4]

Therapeutic remedies for oral inflammation predominantly rely on anti-microbial agents.[5] Chlorhexidine has long been established as a leading therapeutic agent due to its robust antimicrobial efficacy against common oral microbes.[5] However, the seemingly beneficial anti-microbial effects come with limitations, as their cytotoxic nature poses challenges to vital physiological processes by impairing cell proliferation essential for oral health.[6] A previous in vitro study investigated 12 oral products and found that only cosmetic-use products maintained cell viability.[7] On the contrary, therapeutic products exhibited varying degrees of cytotoxicity, ranging from moderate for those with ethanol or pegylated hydrogenated castor oil to high in products with chlorhexidine or cetylpyridinium chloride.[7] Collectively, this justifies previous results that reported significant wound healing delay linked with chlorhexidine, resulting in a "scar wound healing" pattern due to abnormal collagen deposition.[8]

Natural products offer a promising approach to oral healthcare by showcasing a balance between antimicrobial effectiveness and mitigating cytotoxicity.[9] Curcumin, a polyphenolic compound derived from the turmeric plant (*Curcuma longa*), is renowned for its anti-inflammatory and antioxidant properties.[10] The significance of curcumin in managing inflammatory conditions like inflammatory bowel disease, arthritis, psoriasis, and atherosclerosis has been documented in the literature.[11] The multifaceted mechanism of action of curcumin involves controlling inflammatory signs by suppressing immune cells such as macrophages and neutrophils, downregulating the expression of pro-inflammatory cytokines and enzymes, and counteracting oxidative stress by enhancing the activity of endogenous antioxidant enzymes.[10,12,13] Additionally, curcumin demonstrates antimicrobial efficacy, effectively disrupting the microbial integrity of microorganisms implicated in oral diseases such as *Streptococcus mutans* and *Candida albicans* by hindering their proliferation and virulence.[14,15] We hypothesize that curcumin is effective in managing oral mucosal inflammation. Hence, this in vitro study aims to investigate the curcumin-based oral gel's potential anti-inflammatory properties and assess its efficacy against a spectrum of oral microbes implicated in the pathogenesis of common oral inflammatory conditions.

2 | MATERIALS AND METHODS

2.1 | Cell lines and culture conditions

Four human cell lines were employed in this project (i) primary oral keratinocytes (HOKs) (2610, ScienCell, CA, USA), (ii) immortalized oral keratinocytes (OKF6) (CRL-3397, ATCC, VI, USA), (iii) periodontal ligament fibroblasts (HPdLF) (2630, ScienCell, CA, USA), and (iv) dysplastic oral keratinocytes (DOKs) (94122104, Sigma-Aldrich, MO, USA). HOKs, OKF6, and DOKs were cultured as previously described.[10] HPdLF cells were grown using fibroblast medium (2301, ScienCell) with 2% fibroblast growth supplement (2352, ScienCell), 2% FBS (0010, ScienCell), and 1% penicillin/streptomycin (0503, ScienCell).

To evaluate the protective properties of the curcumin-based oral gel on oral inflammatory conditions, local immune inflammatory environments were mimicked in specific groups of the investigated cell lines. This was achieved by starving cells for a 24-h by omitting the growth supplements, followed by a 24-h stimulation with 10 μM of lipopolysaccharides (LPS) from *Escherichia coli* O111:B4 (L4391, Sigma-Aldrich).[16] Stock solutions of LPS at a concentration of 1 mg/mL were prepared using the standard culture media for each cell line.

2.2 | Acid-phosphatase cytotoxicity assay (APH)

Based on our previous results of liquid chromatography–tandem mass spectrometry,[10] a stock concentration of 1 μM of curcumin was prepared by dissolving an appropriate amount of the curcumin-based oral gel (Periogold®, Bharma) in dimethyl sulfoxide (DMSO). An equal amount of the vehicle DMSO was added to control and LPS-only groups to nullify any potential impact of DMSO on cell behavior. Where necessary, cell counting was conducted by staining cells with ReadyProbes Cell Viability Imaging Kit (R37610, ThermoFisher) and then quantifying them using the Countess II FL Automated Cell Counter (AMQAF1000, ThermoFisher).

Data regarding the cytotoxicity of the curcumin-based oral gel for HOKs, OKF6, and DOKs were retrieved from our previous study (Supporting Information Methods).[10]

2.3 | Proliferation assay (MTS assay)

The proliferative ability of HOKs, OKF6, HPdLF, and DOKs under six treatment conditions was examined using an MTS assay (ab197010, Abcam, UK) over 4 days, according to the manufacturer's instructions (Supporting Information Methods).

2.4 | Clonogenic assay

A pilot study was conducted to determine the optimal seeding density for 6-well plates to yield around 100 colonies in 2 weeks. Based on this, the following densities per well were used: 250 cells for DOKs, 500 for HOKs and HPdLF, and 750 for OKF6 (full details in Supporting Information Methods).

2.5 | Wound healing assay

A wound healing assay (ab242285, Abcam) was performed following the manufacturer's instructions (Supporting Information Methods).

2.6 | Apoptosis assay (TUNEL—FITC assay)

A TUNEL assay (ab66108, Abcam) was performed in triplicate according to the manufacturer's instructions (Supporting Information Methods).

2.7 | Invasion assay

The in vitro invasion of the cell lines under various treatments was assessed using the Cell Invasion Assay—Basement Membrane Kit (ab235679, Abcam) (Supporting Information Methods).

2.8 | Adhesion assay

The Vybrant Cell Adhesion Assay Kit (V13181, ThermoFisher) assessed cell adhesion of various treatments (Supporting Information Methods).

2.9 | Antimicrobial activity and colony forming unit (CFU)

The antimicrobial efficacy of the curcumin-based oral gel was assessed in comparison to two commercially available products, Bonjela® mouth ulcer and teething gel (Choline salicylate 87 mg/g) (Reckitt, Slough, UK) and Chlorofluor® Gel (Chlorhexidine Gluconate 0.2% v/w and Sodium Fluoride 0.0033% w/v) (PDS, VIC, Australia). The products' antimicrobial efficacies were compared against three microorganisms: *C. albicans* (10 231, ATCC), *S. mutans* (700 610, ATCC), and *Porphyromonas gingivalis* (33 277, ATCC). A detailed description of the methods is presented in the Supporting Information Methods.

2.10 | Statistical analysis

The statistical analysis and the related graphs were performed using GraphPad Prism (Version 10.0.2, CA, USA). Difference between groups was assessed using One-way ANOVA and Tukey's multiple comparisons test for all assays, except for the MTS assay, where

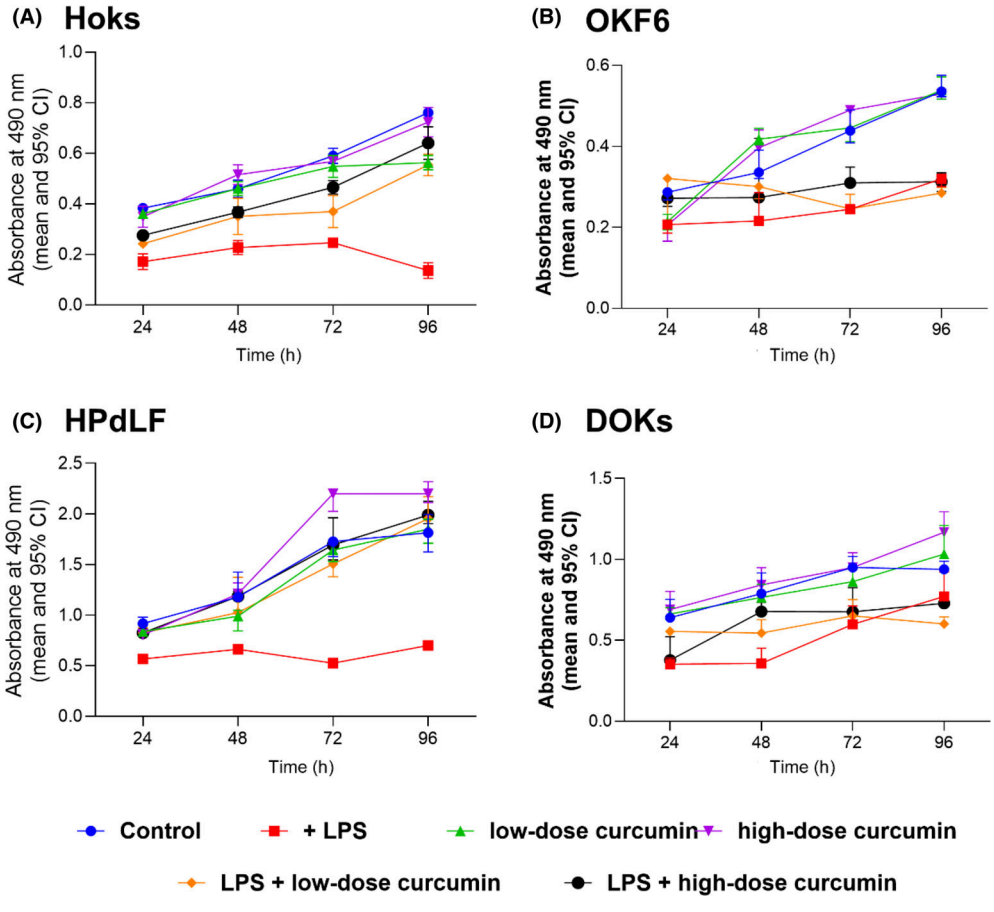

FIGURE 1 MTS proliferation assay over four time-frames under various treatment conditions for (A) HOKs, (B) OKF6, (C) HPdLF, and (D) DOKs. Data are presented as mean and 95% confidence interval (95% CI).

differences were determined using a Two-way ANOVA and Tukey's multiple comparisons test, comparing time points and treatment conditions. The level of significance was defined as $p < 0.05$.

3 | RESULTS

3.1 | Treatment with curcumin-based oral gel stimulates the proliferation of certain cell lines under local inflammatory conditions

MTS assays showed that LPS significantly reduced the proliferative ability of HOKs ($p = 0.0004$), OKF6 ($p = 0.0046$), and HPdLF ($p = 0.0323$) in comparison to controls. DOKs exhibited no significant difference in response to LPS ($p = 0.119$) (Figure 1). At the 96-h time, concurrent treatment with LPS and curcumin-based oral gel significantly increased the proliferation of HOKs and HPdLF compared to the groups treated with LPS alone ($p < 0.05$). This increase was comparable to the control groups ($p > 0.05$), as illustrated in Figure 1. There was no significant difference in the proliferative inducibility based on curcumin concentrations ($p > 0.05$).

On the contrary, treatment with curcumin did not nullify LPS's action in increasing the proliferative capacity of OKF6 and DOKs ($p > 0.05$), as illustrated in Figure 1.

3.2 | Curcumin enhances colony formation in select cell lines

HOKs and OKF6 treated with LPS showed a significant reduction in the number of formed colonies, $p = 0.0002$ and $p = 0.001$, respectively (Figure 2). There was no statistically significant difference concerning HPdLF and DOKs, $p > 0.05$.

The results also revealed that inducing cells with LPS and curcumin-based oral gel, significantly increased the number of colonies in comparison to groups treated with LPS alone for HOKs ($p < 0.0001$) and HPdLF ($p < 0.05$) cell lines (Figure 2). For OKF6, only groups treated with a high dose of curcumin-based oral gel exhibited a significant increase in colonies compared to the LPS-only treated group ($p = 0.012$). On the contrary, the DOK cell line showed no significant changes in colony-forming ability across the different conditions ($p > 0.05$).

3.3 | Curcumin-based oral gel mitigates LPS-induced inflammatory actions and enhances wound closure among certain cell lines

The conducted wound healing assay showed that treating cells with LPS significantly reduced the migratory wound healing

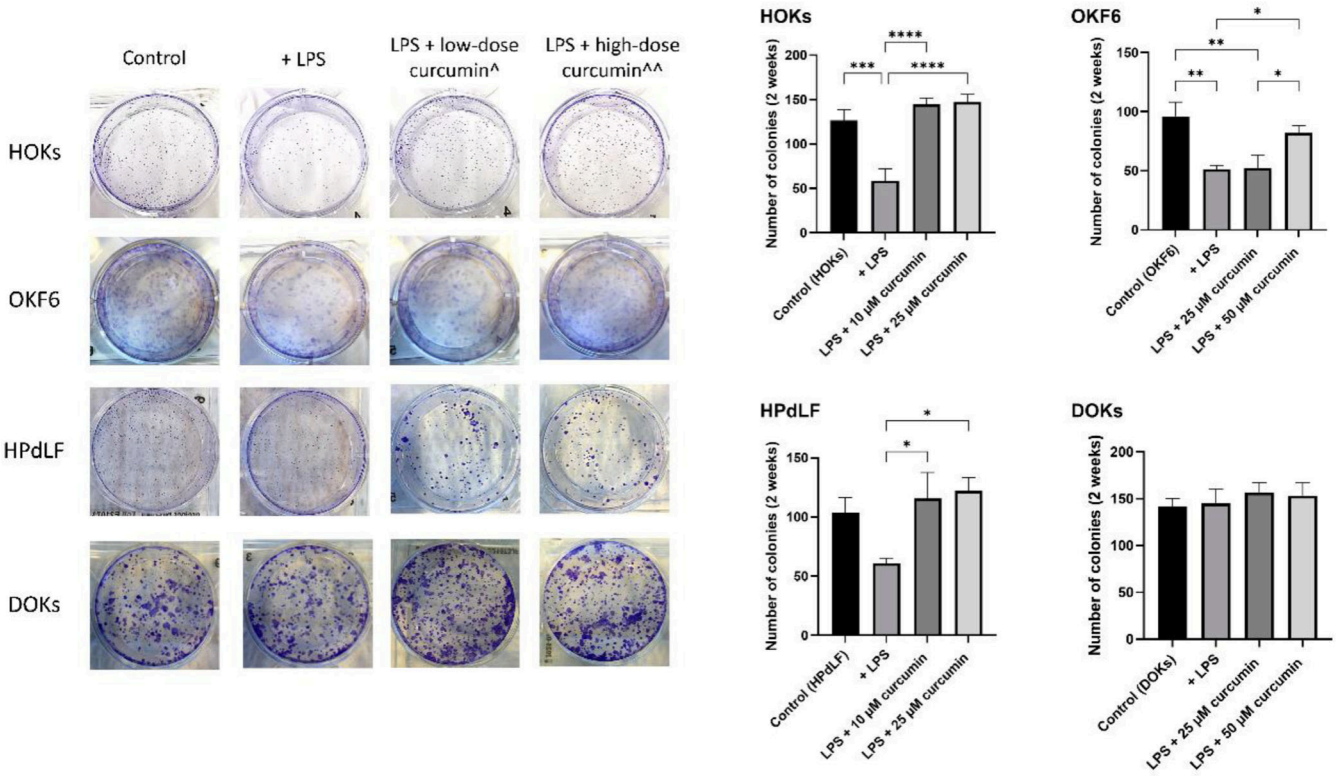

FIGURE 2 Left side; visual representations of the conducted colony forming assay over 2 weeks of incubation of the studied cell lines under various treatment conditions. ^Low-dose curcumin (10 μM for HOKs and HPdLF, 25 μM for OKF6 and DOKs), ^^High-dose curcumin (25 μM for HOKs and HPdLF, 50 μM for OKF6 and DOKs). Right side; bar graphs showing the number of formed colonies for each cell line over 2 weeks. Statistical significance is denoted as follows: *$p < 0.05$, ** $p < 0.002$, *** $p < 0.0005$, and **** $p < 0.0001$.

potential of HOKs ($p < 0.0001$), OKF6 ($p = 0.025$), and HPdLP ($p < 0.0001$) compared to the control. This was the opposite of the DOK's response to LPS, where LPS increased the wound healing capacity, although this increase was not statistically significant, $p = 0.115$ (Figure 3).

Moreover, treating HOKs and HPdLP cells with LPS and curcumin-based oral gel significantly increased their wound healing capacity in comparison to groups treated with LPS alone to levels comparable to controls ($p < 0.0001$) (Figure 3).

On the contrary, curcumin did not increase the wound-healing ability of LPS-induced OKF6 cells, either at concentrations of 25 or 50 μM (Figure 3). Noteworthy, although treating DOKs with LPS did not reduce their wound healing capacity, groups treated with LPS and high concentrations of curcumin-based oral gel (50 μM) significantly increased their wound healing capacity compared to the controls ($p = 0.013$).

3.4 | Curcumin treatment significantly reduces the number of apoptotic cells

LPS was found to exert a significant potent pro-apoptotic impact by increasing the number of apoptotic cells for HOKs, OKF6, and HPdLF ($p < 0.0001$), compared to the untreated control groups. Conversely, no such effect was observed in DOKs ($p = 0.694$) (Figure 4). Upon treating cells with LPS and varying concentrations of curcumin-based oral gel, a statistically significant reduction in the number of apoptotic cells was observed for both HOKs and HPdLF ($p < 0.0001$). However, this response was not concentration-dependent (Figure 4).

The impact of curcumin on apoptosis was concentration-dependent in OKF6 cells. Groups treated with LPS and exposed to high curcumin concentrations (50 μM) showed a significant reduction in apoptotic cells ($p = 0.017$) in comparison to groups treated with LPS only, while no difference was observed in groups of low curcumin

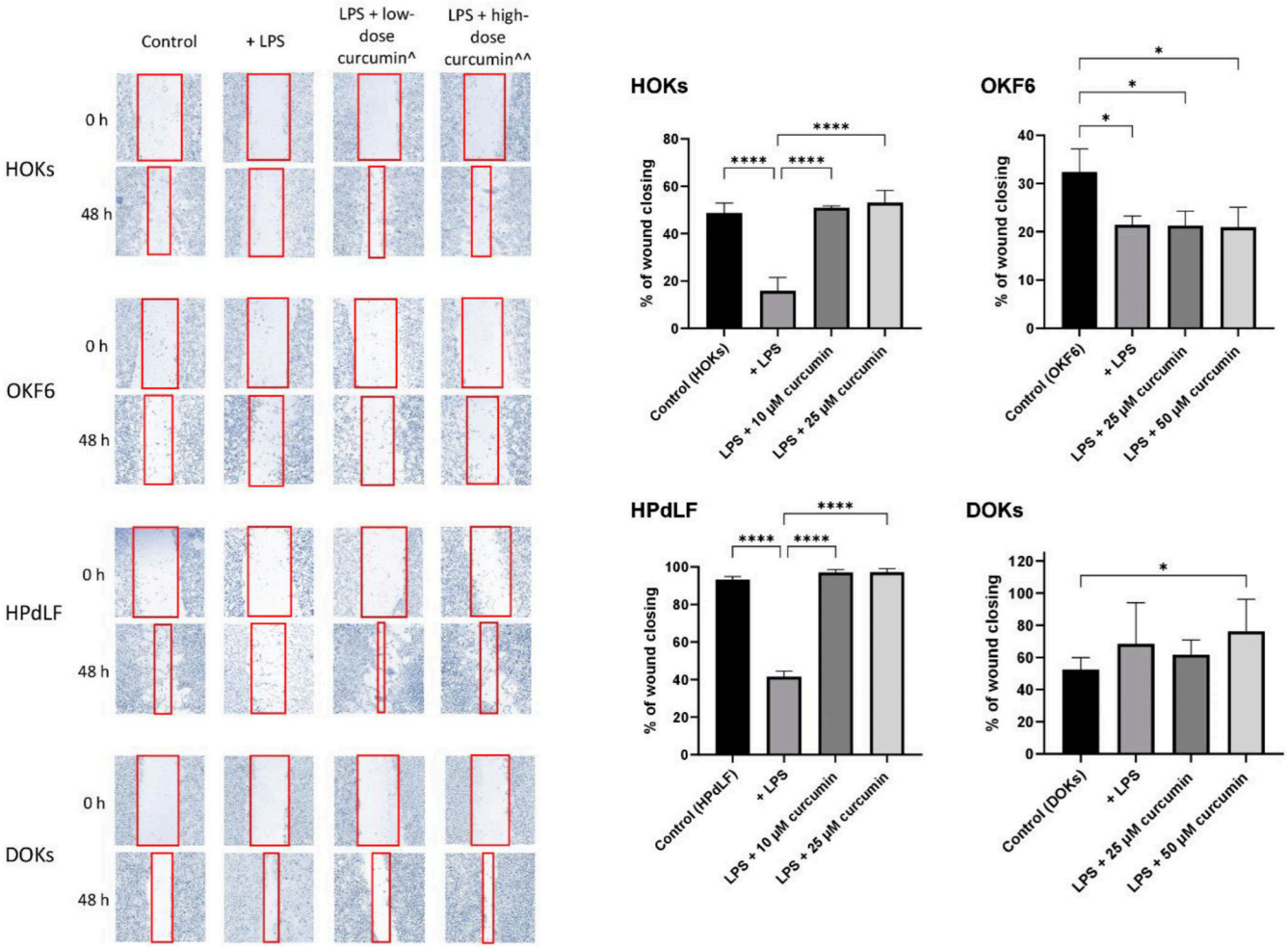

FIGURE 3 Left side; representative photomicrographs at ×20 magnification of the conducted wound healing assay among the investigated cell lines under different treatment conditions at 2-time frames, (0 h) immediately after removing the inserts, and (48 h) 48 h later. ^Low-dose curcumin (10 μM for HOKs and HPdLF, 25 μM for OKF6 and DOKs), ^^High-dose curcumin (25 μM for HOKs and HPdLF, 50 μM for OKF6 and DOKs). Right side; bar graphs illustrating the percentage of wound closure among different groups of the included cell lines. Statistical significance is represented as follows: *$p < 0.05$, ****$p < 0.0001$.

concentrations (25 μM), $p = 0.229$ (Figure 4). Conversely, curcumin did not exhibit a significant impact on apoptosis in DOKs ($p > 0.05$) (Figure 4).

3.5 | Diverse treatments show no effect on the invasive capabilities of the investigated cell lines

The basement-membrane-based invasive assay results revealed that irrespective of the cell line or the treatment conditions, there were no statistically significant differences among different groups regarding their invasive capabilities, $p > 0.05$.

3.6 | Cell line-specific modulation of adhesion capabilities in response to curcumin-based oral gel

Treatment with LPS led to a significant reduction in adhesion among HOKs ($p < 0.0001$) and HPdLF ($p = 0.007$) cell lines. LPS co-treatment with low and high doses of the curcumin-based oral gel significantly induced adhesion in the HPdLF cell line in comparison to the LPS-only induced groups ($p < 0.005$), whereas a significant effect on HOKs adhesion was only observed with the high curcumin concentration, $p = 0.007$.

In the case of the OKF6 cell line, no significant differences in adhesion were observed among groups, regardless of the treatment conditions, $p > 0.05$ (Figure 5). As for the DOKs cell line, although treatment with LPS alone did not statistically significantly change the adhesion capabilities of DOKs, the sole treatment regimen that significantly enhanced adhesion was the combination of high-dose curcumin and LPS, $p < 0.0001$ (Figure 5).

3.7 | The curcumin-based oral gel showed significant anti-microbial efficacy against *C. albicans* and *S. mutans*

The antimicrobial investigation found that for both *C. albicans* and *S. mutans*, the MIC was 50% across the tested products. This indicates a uniform threshold concentration necessary to inhibit the growth of these microorganisms. Conversely, only Chlorofluor® Gel demonstrated a MIC at 50% against *P. gingivalis*. Neither PeriGold® nor Bonjela® showed efficacy against this pathogen. Consequently, a cfu assay for *P. gingivalis* was not conducted.

In the assessment of the oral products' efficacy against *C. albicans*, the results revealed that all products were associated with a statistically significant reduction in cfu/mL in comparison to the

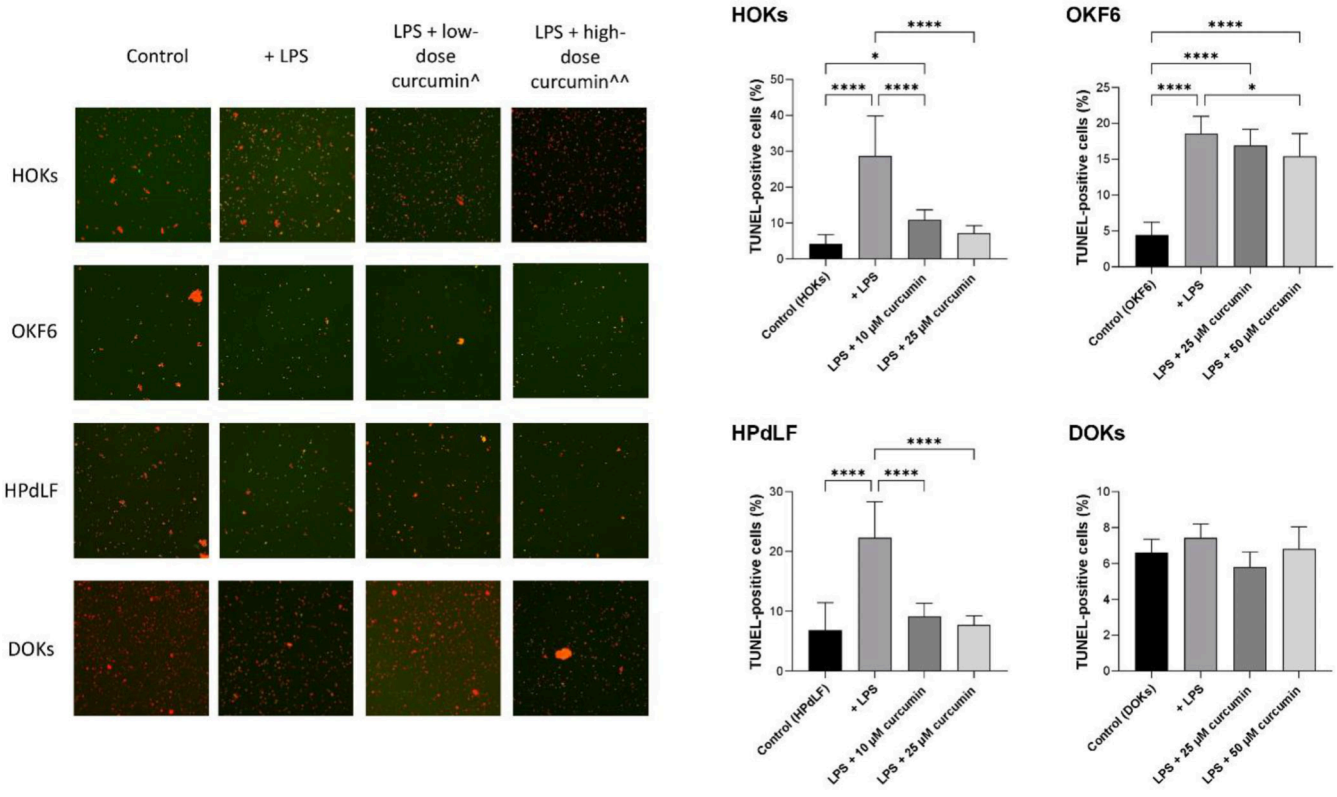

FIGURE 4 Left side; representative photomicrographs of employing TUNEL assay to quantify the number of apoptotic cells under various treating conditions for the investigated cell lines. The orange-red PI counterstain represents normal cells; green stain represents apoptotic cells. ^Low-dose curcumin (10 μM for HOKs and HPdLF, 25 μM for OKF6 and DOKs), ^^High-dose curcumin (25 μM for HOKs and HPdLF, 50 μM for OKF6 and DOKs). Right side; bar graphs showing the percentage of apoptotic cells based on the conducted TUNEL assay under different treatment conditions. Statistical significance is denoted as follows: *$p < 0.05$, ****$p < 0.0001$.

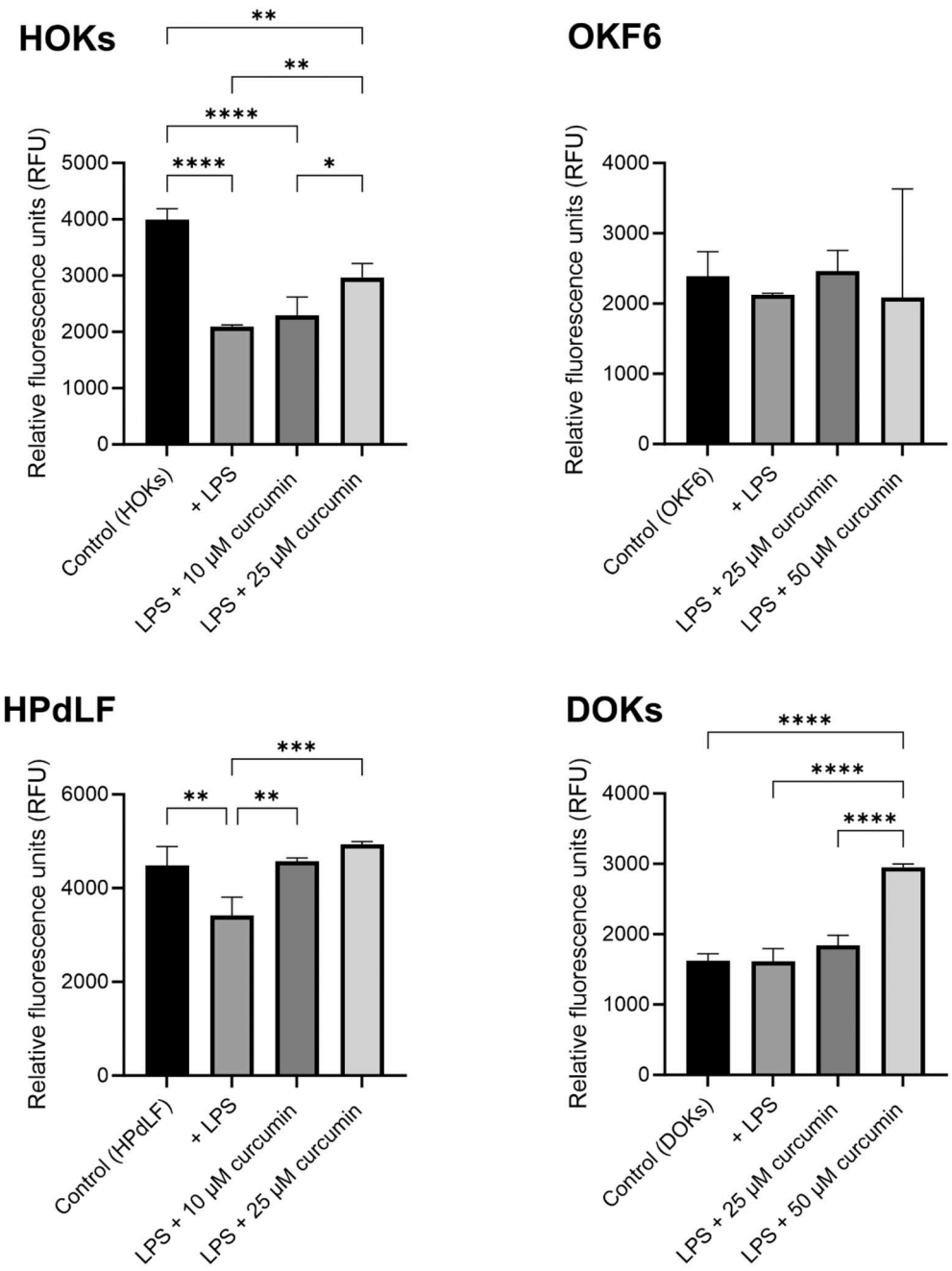

FIGURE 5 Bar graphs illustrating cell adhesion in response to various treatment conditions. Statistical significance is indicated by asterisks, where *$p < 0.05$, **$p < 0.002$, ***$p < 0.0005$, and ****$p < 0.0001$.

control group (PeriGold® and Chlorofluor® Gel $p < 0.001$, and Bonjela® Gel $p = 0.0093$) as shown in Figure 6. By comparing the products to each other, the efficacy of PerioGold® against *C. albicans* was statistically significantly higher than Bonjela® Gel ($p < 0.0001$), yet statistically significantly lower than Chlorofluor® Gel ($p = 0.0131$) (Figure 6).

Likewise, all products showed statistically significant efficacy against *S. mutans* compared to the control (PeriGold® Gel $p = 0.0001$, Chlorofluor® Gel $p < 0.0001$, and Bonjela® Gel $p = 0.0008$). The efficacy of PeriGold® Gel against *S. mutans* was statistically significantly lower than Chlorofluor® Gel ($p = 0.0064$) and similar to Bonjela® Gel ($p = 0.2128$) as shown in Figure 6.

4 | DISCUSSION

This study supports the therapeutic potential of a curcumin-based oral gel in cases of oral inflammation. This was achieved by mimicking oral inflammatory conditions based on established protocols and employing a panel of cellular and microbial assays to investigate diverse cellular responses thoroughly.

LPS is a component of the outer membrane of Gram-negative bacteria and is recognized by Toll-like receptor 4 (TLR4). This recognition initiates a cascade of intracellular signaling events similar to those in natural immune responses.[16,17] Employing LPS in vitro studies to simulate and analyze inflammatory reactions has been extensively

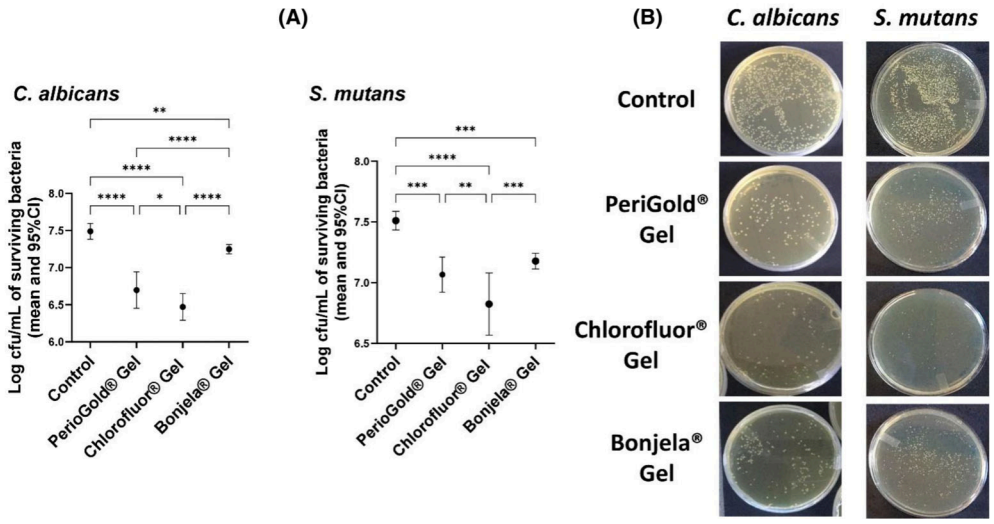

FIGURE 6 Results of the antimicrobial assays. (A) Graphs show the colony-forming unit (cfu) of the survival microorganisms after the application of the investigated products presented as cfu/mL. Statistical values are indicated as $^{*}p < 0.05$, $^{**}p < 0.002$, $^{***}p < 0.0005$, and $^{****}p < 0.0001$. (B) Representative photographs of the number of microorganism colonies for both *C. albicans* and *S. mutans* after the application of the investigated products.

validated by molecular-based studies.[16] The significant reduction in the proliferative and wound healing capacities, accompanied by the significant increase in the number of apoptotic cells among human HOKs, OKF6, and HPdLF in response to LPS, underscores the detrimental effects of bacterial endotoxins on oral cell viability. These findings align with existing literature emphasizing the capacity of LPS to induce cell cycle arrest and impair cellular proliferation, which in turn contributes to compromised tissue homeostasis during inflammatory conditions.[17]

However, while acknowledging LPS as a potent inflammatory stimulant, it is important to consider potential limitations in responses to LPS among cell lines. This is seen as the lack of significant differences in the response of DOKs to LPS. Taking into account the underlying oncogenic changes associated with DOKs as an oral dysplastic cell line, this could be attributed to several factors, including (i) cellular adaptations and altered apoptotic pathways, dysplastic cells often exhibit dysregulation in the expression and function of key apoptotic regulators, this alteration involves upregulating anti-apoptotic members of the Bcl-2 family, such as Bcl-2 and Bcl-xL,[18] and (ii) differences in TLR expression, dysregulated TLR expressions are often associated with dysplastic cells which often altered the immune response towards chronic inflammation, a hallmark of dysplasia, creating a microenvironment that promotes cell survival and hinders programmed cell death.[19]

This study also showed that concurrent treatment with curcumin-based oral gel mitigates the deteriorated tissue hemostasis response to LPS, especially among HOKs and HPdLF, as evidenced by proliferation assay, colony forming assay, wound healing assay, and apoptosis assay. Our results were also consistent with previous studies that reported the anti-apoptotic roles of curcumin in various inflammatory conditions such as intestinal epithelial inflammatory damage,[20] osteoarthritis,[21] neuronal inflammation,[22] and atherosclerosis.[23] Likewise, curcumin was reported for its ability to accelerate cutaneous wound healing through several biological pathways.[24]

Although OKF6 showed primary responses to LPS, treatment with curcumin-based oral gel had no significant effect in mitigating LPS-induced changes. As OKF6 is an immortalized cell line while HOKs and HPdLF are primary cells, these diverse responses to LPS can be elucidated by inherent genetic, phenotypic, and functional disparities across different cell types.[25] Especially because phenotypic heterogeneity in primary cells, mirroring the tissue's diversity, contributes to varied responses compared to more homogenous cell lines.[25] Moreover, OKF6 is immortalized by Telomerase (hTERT), known for involving accumulated genetic alterations, which may impact the OKF6 response to inflammatory stimuli.[26] In support of that, a previous study found that hTERT-immortalized gingival fibroblasts failed to mimic primary cell responses to external stimulation.[27] This emphasizes the significance of selecting appropriate models that best represent the specific aspects of the biological system under investigation and considering the limitations associated with each cell type.

On the other hand, curcumin stands out for its well-documented anti-inflammatory properties, exerting its effects through intricate modulation of several molecular pathways. This includes inhibiting key inflammatory mediators, including prostaglandins and leukotrienes, by suppressing enzymes like cyclooxygenase-2 (COX-2) and 5-lipoxygenase.[28] Additionally, curcumin downregulates pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), contributing to the mitigation of inflammatory responses.[29] Another pivotal aspect of curcumin's mechanism involves inhibiting the activation of Nuclear Factor-kappa B (NF-κB), a crucial transcription factor orchestrating inflammation-related gene expression, through phosphorylation suppression and degradation of IκBα.[12] The compound's antioxidant property further aids in alleviating inflammation by scavenging free radicals and reducing oxidative stress, a process intricately linked to inflammatory pathways.[10,13]

The results of the antimicrobial assay provide insights into the efficacy of PerioGold® Gel, particularly against *C. albicans* and *S. mutans*. It is worth noting that these results were not surprising as they are consistent with previous studies that assessed the antimicrobial efficacy of curcumin.[14,15,30] More importantly, a previous study found that curcumin does not only inhibit mono-species biofilms from

either *S. mutans* or *C. albicans*, but also has efficacy against dual-species biofilm formed by those two species.[14] Additionally, curcumin has been shown to hinder acid production and reduce biofilm metabolism for up to 24 h in *S. mutans*.[30] Research has also demonstrated curcumin's potent antifungal activity against *C. albicans* by inhibiting the formation of fungal hyphae and disrupting the integrity of the fungal cell membrane by inducing oxidative stress within the fungal cells, leading to their death.[15]

Several limitations were associated with this study. The study's reliance on cell lines may not fully capture the complexities of human oral tissue interactions. Developing 3-dimensional cellular models derived from patient samples would provide a better understanding of the efficacy of the curcumin-based oral gel in managing inflammatory conditions. Second, the absence of molecular assays limited the ability to assess changes in protein expressions, which is crucial for targeted therapy development. Finally, the long-term effect of the curcumin-based oral gel treatment was not addressed in this study, which is essential to understanding clinical applications for chronic oral conditions. Nonetheless, the outcomes encourage future clinical investigations to fully understand the therapeutic benefits of curcumin in oral health applications.

In conclusion, this study highlights the potential utility of the curcumin-based oral gel in modulating cellular response under simulated inflammatory conditions. This may provide a safer and a viable alternative to conventional antimicrobial topical treatments.

AUTHOR CONTRIBUTIONS

Conceptualization: O.K.; *Methodology*: M.I. and O.K.; *Validation*: M.I. and O.K.; *Investigation*: M.I. and O.K.; *Data curation*: M.I. and O.K.; *Writing—original draft preparation*: M.I. and O.K.; *Project administration*: O.K.; *Funding acquisition*: O.K. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/jop.13547.

DATA AVAILABILITY STATEMENT

The data supporting this study's findings are available on request from the corresponding author. The data are not publicly available due to privacy.

ETHICS STATEMENT

This study is purely undertaken in the lab using commercially available cell lines. Neither human nor animal samples have been used. Hence, no ethics approval was required.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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