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ORIGINAL ARTICLE

Comparative Susceptibility of Streptococcus Mutans and Lactobacilli to Antimicrobial Photodynamic Therapy Around Orthodontic Brackets: An in Vitro Study

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ABSTRACT

Background: Fixed orthodontic brackets introduce structural niches that promote the accumulation of cariogenic biofilms. While *Streptococcus mutans* initiates enamel demineralization, *Lactobacilli* drive the progression of cavitated lesions. Traditional antimicrobials present significant clinical limitations. Antimicrobial photodynamic therapy (aPDT) offers a compelling, resistance-free alternative, yet the relative, intrinsic susceptibility of these two key pathogens to natural photosensitizer-mediated aPDT around orthodontic hardware remains poorly characterized.

Objective: To systematically evaluate and compare the intrinsic susceptibility of *Streptococcus mutans* and *Lactobacilli* biofilms formed around stainless steel orthodontic brackets to aPDT mediated by curcumin or riboflavin activated by a 445 nm blue diode laser (BDL) at varying power densities.

Methods: One hundred twenty extracted human premolars bonded with stainless steel brackets were inoculated with either *S. mutans* (n=60) or *Lactobacilli* (n=60) to

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establish 72-hour mature single-species biofilms. Within each bacterial division, specimens were randomized into three core experimental blocks (each): Group I (100 μM Riboflavin), Group II (40 μM Curcumin), or Group III (Laser/Compound Controls). These were further subdivided (n=10) to undergo 30-second irradiation with a 445 nm BDL at either a low-power output (200 mW •) or high-power output (500 mW •). Surviving sessile colonies were liberated, serially diluted, cultured, and quantified as CFU/mL. Data were analyzed via Shapiro-Wilk testing, one-way ANOVA with Tukey's post hoc test, and independent *t*-tests (α =0.05).

Results: Baseline unexposed control loads were equivalent for *S. mutans* (5.98 ± 0.12$\log_{10}$ CFU/mL) and *Lactobacilli* (5.97 ± 0.13$\log_{10}$ CFU/mL). Under high-efficacy configurations, *S. mutans* consistently exhibited significantly greater susceptibility to photodynamic eradication than *Lactobacilli* ($p < 0.05$). Curcumin aPDT at 500 mW generated the maximum bacterial reduction, yielding a 99.4% clearance (3.75 ± 0.19$\log_{10}$CFU/mL) for *S. mutans* compared to a 98.9% clearance (4.02 ± 0.22$\log_{10}$ CFU/mL) for *Lactobacilli* ($p < 0.01$ relative to baseline controls). Riboflavin aPDT achieved acceptable clinical reduction only at 500 mW, resulting in a 92.6% reduction (4.85 ± 0.20$\log_{10}$ CFU/mL) in *S. mutans* versus an 88.0% reduction (5.05 ± 0.22$\log_{10}$ CFU/mL) in *Lactobacilli* ($p < 0.31$). Increasing the BDL output from 200 mW to 500 mW significantly amplified bactericidal kinetics across all protocols ($p < 0.01$), though the interspecies susceptibility gap was preserved throughout.

Conclusions: *Streptococcus mutans* is intrinsically more susceptible to blue light-mediated photodynamic inactivation than *Lactobacilli* around orthodontic brackets. *Lactobacilli* exhibit higher tolerance, requiring elevated photodynamic energy thresholds to achieve comparable logs of reduction. Curcumin-mediated aPDT at 500 mW delivers superior bactericidal efficacy against both target pathogens compared to riboflavin, supporting organism-specific, targeted aPDT regimens in orthodontic patients.

KEYWORDS

• Orthodontic Brackets • Antimicrobial Photodynamic Therapy • Curcumin • Riboflavin • Blue Diode Laser • *Streptococcus Mutans* • *Lactobacilli* • Bacterial Susceptibility

INTRODUCTION

The clinical success of fixed orthodontic therapy relies on the precise, long-term stabilization of teeth via brackets, archwires, and elastomeric auxiliaries. However, the placement of these intraoral hardwares inherently compromises the patient's capacity for mechanical plaque debridement by introducing multiple structural stagnation niches. These retentive microenvironments trigger immediate alterations in the local intraoral microbiome, shifting the ecological equilibrium from a health-associated flora to a highly acidogenic and aciduric cariogenic biofilm cluster.[12] Consequently, enamel demineralization manifests clinically as white spot lesions (WSLs) in a high percentage of patients undergoing fixed orthodontic treatment, frequently undermining the aesthetic and therapeutic outcomes of active treatment.

The microbiological orchestration of WSL initiation and eventual progression into cavitated dentinal lesions represents a coordinated, multi-species succession dominated sequentially by *Streptococcus mutans* and *Lactobacilli* species. Streptococcus mutans functions as the primary etiological orchestrator of early cariogenesis. Utilizing glucosyltransferases, *S. mutans* synthesizes water-insoluble extracellular polysaccharides that form a dense, protective matrix.[13] This structural scaffolding facilitates adherence to stainless steel and enamel, trapping organic acids against the tooth surface and driving local pH values below the critical demineralization threshold.

Once the enamel boundary is compromised and an acidic microenvironment is established,

Lactobacilli species act as critical secondary invaders. Although *Lactobacilli* possess poor initial adherence properties to smooth enamel, their highly aciduric nature enables them to thrive within the low-pH niches created by *S. mutans*. Here, they accelerate acid production, contributing substantially to the progression and deep destruction of the underlying enamel and dentin matrix. Given these specific roles—where *S. mutans* dictates lesion initiation and Lactobacilli govern lesion advancement[11]—preventive strategies must be evaluated based on their performance against both distinct entities.

Conventional chemical adjunctive regimens, most notably chlorhexidine (CHX) mouthrinses or varnishes, are widely utilized to manage orthodontic plaque accumulation. Nonetheless, their long-term clinical utility is limited by adverse sequelae, including tooth discoloration, dysgeusia, desquamative mucosal reactions, and risks of long-term alterations of the oral microbiome.

To overcome these limitations, antimicrobial photodynamic therapy (aPDT) has emerged as a compelling, resistance-free bio-photonic intervention. The mechanism of aPDT requires the topical application of a non-toxic photosensitizer (PS) that accumulates preferentially within the target biofilm matrix and bacterial cell membranes. Upon irradiation with low-energy visible light of a matching absorption wavelength, the excited photosensitizer undergoes Type I or Type II photochemical pathways. These pathways interact with molecular oxygen to generate cytotoxic reactive oxygen species (ROS), including free radicals and singlet oxygen. These short-lived radical species induce indiscriminate, lethal oxidative damage to microbial structures, leading to lipid peroxidation of bilayers, protein denaturing, and genomic strand breaks without inducing microbial resistance.

Recent oral microbiology research has focused on natural plant-derived photosensitizers like curcumin and riboflavin to replace synthetic dyes, which can cause soft-tissue staining.[2,8] Curcumin, a polyphenolic extract from Curcuma longa, demonstrates a wide absorption band between 400 and 500 nm and possesses a highly lipophilic character that promotes penetration into microbial structures.[5] Riboflavin (Vitamin B2) exhibits major absorption peaks centered near 445 nm and undergoes Type I and Type II photochemical processing upon activation.[4] Modern semiconductor blue diode lasers (BDL) operating within the 445 nm spectrum align with the maximum absorption profiles of both compounds, offering high photon density and precise delivery.[1]

Despite extensive literature documenting the overall antimicrobial efficacy of aPDT, a critical knowledge gap remains. Most orthodontic antimicrobial trials analyze bacterial count reductions within individual species independently, omitting a direct statistical and mechanistic comparison of the intrinsic susceptibility between different cariogenic organisms under identical experimental parameters. Little is known regarding how organism-specific structural and metabolic features affect resistance to photodynamic ROS damage around fixed hardware. Understanding these susceptibility differentials is vital for transitioning from generic antimicrobial parameters to targeted, species-specific preventive aPDT protocols.

This *in vitro* study was designed to provide a systematic comparison of the photodynamic susceptibility of *Streptococcus mutans* and *Lactobacilli* biofilms formed around stainless steel orthodontic brackets using a 445 nm BDL coupled with curcumin or riboflavin. The primary research question sought to determine which cariogenic organism is intrinsically more responsive to photodynamic inactivation. The secondary questions evaluated whether these susceptibility variations remain stable across different photosensitizer types, laser power outputs (200 mW vs. 500 mW), or laser irradiation alone. The null hypothesis tested was that there is no significant difference in the post-treatment susceptibility and viable colony reductions between *S. mutans* and *Lactobacilli* across any of the evaluated photodynamic therapy configurations.

MATERIALS AND METHODS

Study Design and Setting

This *in vitro*, controlled laboratory trial investigated the comparative bactericidal kinetics of a 445 nm blue diode laser combined with natural photosensitizers. The study was conducted across specialized dental and microbiological research departments.

Institutional Ethics Committee approval was secured for the use of extracted human elements.

Sample Selection and Bracket Bonding

A total of 120 healthy human first premolars, extracted exclusively for orthodontic indications, were collected following informed donor consent. Inclusion criteria required completely intact enamel crowns free of dental caries, structural or developmental defects, restorations, or prior endodontic interventions.

Sample size calculation was performed via G*Power software (Version 3.1.9.7) assuming a one-way ANOVA framework. To detect an effect size ($f = 0.42$) with an α error probability of 0.05 and a statistical power ($1 - \beta$) of 0.80 among three primary intervention arms, a minimum sample size of 60 teeth per distinct bacterial species arm was mandated (total $N = 120$ • allocating per specific experimental subgroup).

The tooth preparation and bracket bonding protocol followed a standardized sequence:

1. Collected premolars were thoroughly cleaned of macroscopic debris using 0.9% physiological saline, immersed in 3% hydrogen peroxide for 30 minutes to facilitate soft tissue debridement, and stored in a 0.5% chloramine-T solution for one week to achieve complete surface disinfection.
2. The buccal enamel surfaces were polished with a fluoride-free pumice-water paste for 15 seconds, rinsed, and dried thoroughly.
3. Enamel conditioning was executed by applying 35% phosphoric acid gel (3M ESPE Scotchbond Multipurpose Etchant) to the buccal zone for 20–30 seconds. The conditioned surfaces were rinsed for 10 seconds and air-dried until a frosty appearance was visible.
4. A thin layer of Transbond Adhesive Primer (3M Unitek) was applied to the etched field and light-cured for 10 seconds utilizing an LED curing unit emitting at 420 nm.
5. Standardized 0.022 × 0.028-inch slot stainless steel premolar brackets (3M Unitek) were coated with Transbond XT Light Cure Adhesive Paste (3M Unitek) and firmly positioned onto the center of the clinical crown.
6. Excess adhesive flash surrounding the bracket base margins was removed with an explorer. Photopolymerization was then executed for 40 seconds.
7. The bonded specimens were stored in 0.9% saline.

Bacterial Strain Preparation and Biofilm Inoculation

Pure reference strains of *Streptococcus mutans* and *Lactobacilli* were reconstituted. *S. mutans* was cultured in Brain Heart Infusion (BHI) broth, and *Lactobacilli* were propagated in de Man, Rogosa, and Sharpe (MRS) agar.

The bacterial suspensions were matched to a 0.5 McFarland standard (1.5×10^8 ˚CFL/ml) using a spectrophotometer at 625 nm (absorbance range: 0.08–0.1) and diluted with fresh BHI broth to establish a working concentration of 1×10^6 ˚CFL/ml).

The 120 bonded premolar units were divided into two main experimental tracks ($n = 60$ each) corresponding to the test microorganisms. Individual specimens were distributed into 24-well microplates • 60 samples received 1 mL of the standardized *S. mutans* suspension per well, while the other 60 received 1 mL of the *Lactobacilli* suspension. The microplates were incubated at 37°C for 72 hours to encourage thick biofilm consolidation around the bracket base and slot margins.

Group Randomization and Photosensitizer Formulations

Within each bacterial species track ($n = 60$), specimens were randomized into three core experimental blocks (each), which were further divided into subgroups of 10 samples each to evaluate the impact of laser power output (200 mW vs. 500 mW):

- **Group I (Riboflavin-mediated aPDT Block):**
  - *Subgroup IA:* Untreated Biofilm Baseline Control.
  - *Subgroup IB:* Riboflavin Formulation Alone (Dark Control).
  - *Subgroup IC:* Riboflavin + BDL irradiation at 200 mW.
  - *Subgroup ID:* Riboflavin + BDL irradiation at 500 mW.

- **Group II (Curcumin-mediated aPDT Block):**
  - *Subgroup IIA:* Untreated Biofilm Baseline Control.
  - *Subgroup IIB:* Curcumin Formulation Alone (Dark Control).
  - *Subgroup IIC:* Curcumin + BDL irradiation at 200 mW.
  - *Subgroup IID:* Curcumin + BDL irradiation at 500 mW.
- **Group III (Laser and Synergy Controls Block):**
  - *Subgroup IIIA:* Untreated Biofilm Baseline Control.
  - *Subgroup IIIB:* Combined Dark Formulation (Riboflavin + Curcumin, no laser).
  - *Subgroup IIIC:* BDL irradiation Alone at 200 mW.
  - *Subgroup IIID:* BDL irradiation Alone at 500 mW.

The photosensitizers were formulated under strict darkroom lighting conditions to eliminate premature ambient photoactivation:

- *Curcumin:* Pure curcumin powder was dissolved in a vehicle of dimethyl sulfoxide (DMSO) in phosphate-buffered saline (PBS) to achieve a final working concentration of 40 μM.
- *Riboflavin:* Riboflavin was dissolved directly in sterile 0.9% normal saline to yield a final working concentration of 100 μM.

All solutions were handled fresh to avoid ambient photoactivation.

Photodynamic Therapy Delivery Protocol

Prior to therapeutic delivery, the biofilm coated brackets received the application of the designated photosensitizer. One milliliter of the specific photosensitizer solution (40 μM curcumin or 100 μM riboflavin) was introduced into the test wells and allowed a 5-minute dark pre-irradiation incubation period to facilitate complete molecular diffusion into the biofilm matrix.

Photo-irradiation was executed utilizing a calibrated semiconductor Blue Diode Laser unit (BDL) emitting a continuous wavelength of 445 nm. The laser energy was applied for 30 seconds. The power configurations were varied according to subgroup assignment:

- 200 mW Power Output: Generating a precise power density of 0.4˚W/cm^2.
- 500 mW Power Output: Generating a precise power density of 1.0˚W/cm^2.

Control groups were managed identically but received no laser energy or compound exposure based on group matrixing.

Microbiological Harvesting and Colony Quantification

Immediately after treatment, each tooth-bracket unit was placed into 1 mL of fresh BHI broth and mechanically vortexed for 2 minutes to liberate residual biofilms.

Ten-microliter aliquots of the liberated suspensions were serially diluted and plated onto agar plates. The plates were incubated aerobically at 37°C for 48 hours. Total colony-forming units (CFU/ml) were counted under 10 × stereomicroscopy magnification.

Statistical Testing

The raw absolute bacterial values (CFU/ml) were converted to values to stabilize variance and normalize data distribution. Normal distribution parameters across all 20 distinct experimental subgroups were validated using the Shapiro-Wilk test.

Inter-group comparative testing across the three primary treatment divisions at each independent power output setting was conducted using one-way Analysis of Variance (ANOVA) paired with Tukey's post hoc test. Intra-group evaluations analyzing the direct effect of laser power escalation (200 mW vs. 500 mW) within the same photosensitizer track were evaluated via independent Student's -tests. Significance thresholds were established at $p < 0.05$.

RESULTS

Distribution and Baseline Equivalence

Shapiro-Wilk normality testing verified that the $\log_{10}$ transformed colony parameters across all evaluated experimental subgroups conformed to normal distribution criteria (-statistic values ranged from 0.8727 to 0.9610 • $p < 0.05$). This supported the use of parametric statistical testing. The initial unexposed

baseline biofilm counts established for the untreated control clusters tracked at 5.98 ± 0.12$\log_{10}$ CFU/mL for *Streptococcus mutans* and 5.97 ± 0.13$\log_{10}$ CFU/mL for *Lactobacilli*.

Comparative Susceptibility Profiles and Interspecies Differentials

Direct interspecies analysis revealed a consistent and statistically significant susceptibility differential between the two cariogenic pathogens under high-efficacy conditions ($p < 0.05$). *Streptococcus mutans* exhibited significantly higher susceptibility to blue light photodynamic damage than *Lactobacilli* across all high-power combinations. As outlined in the susceptibility matrix (Table 3), negative mean delta values confirm that *Lactobacilli* consistently retained higher concentrations of surviving colonies than *S. mutans* when treated with identical parameters.

Under the most potent therapeutic protocol—curcumin-mediated aPDT at 500 mW—the mean surviving load for *S. mutans* fell to 3.75$\log_{10}$ CFU/mL (a 99.4% reduction), whereas *Lactobacilli* maintained a higher residual count of 4.02$\log_{10}$ CFU/mL (a 98.9% reduction), presenting an interspecies mean discrepancy of 0.27$\log_{10}$ units ($p < 0.001$). This gap was maintained during riboflavin aPDT at 500 mW, with *S. mutans* tracking at 4.85$\log_{10}$ CFU/mL versus 5.05$\log_{10}$ CFU/mL for *Lactobacilli* ($p < 0.031$), and during 500 mW laser irradiation alone (5.00$\log_{10}$ CFU/mL vs. 5.18$\log_{10}$ CFU/mL • $p < 0.038$).

Response to Curcumin-Mediated Photodynamic Action

Curcumin formulations produced the greatest overall bactericidal impact against both target pathogens. Interestingly, curcumin alone (without laser activation) demonstrated significant dark antimicrobial action, reducing *S. mutans* to 5.32 ± 0.18$\log_{10}$ CFU/mL (a 78.1% reduction) and *Lactobacilli* to 5.45 ± 0.20$\log_{10}$ CFU/mL (a 69.8% reduction) relative to baseline controls.

When activated with 200 mW BDL, curcumin aPDT significantly accelerated bacterial clearance, reducing counts to 4.90 ± 0.24$\log_{10}$ CFU/mL (94.5% reduction) for *S. mutans* and CFU/mL (91.5% reduction) for *Lactobacilli*. Escalation to the 500 mW curcumin aPDT protocol maximized eradication, reducing survival to 3.75 ± 0.19$\log_{10}$ CFU/mL for *S. mutans* and 4.02 ± 0.22$\log_{10}$ CFU/mL for *Lactobacilli*.

Response to Riboflavin-Mediated Photodynamic Action

Riboflavin configurations demonstrated significantly lower bactericidal performance compared to curcumin and were highly dependent on power density. Riboflavin application without laser exposure showed minimal baseline activity, generating negligible reductions for *S. mutans* (5.96 ± 0.14$\log_{10}$ CFU/mL • 0.3% reduction) and *Lactobacilli* (5.93 ± 0.15$\log_{10}$ CFU/mL • 0.7% reduction).

When combined with the low-power 200 mW BDL, the riboflavin protocol remained largely ineffective, with counts settling at 5.85 ± 0.22$\log_{10}$ CFU/mL (1.8% reduction) for *S. mutans* and 5.88 ± 0.23$\log_{10}$ CFU/mL (1.9% reduction) for *Lactobacilli*. Significant riboflavin-mediated inactivation occurred only when paired with the high-power 500 mW BDL, which dropped survival to 4.85 ± 0.20$\log_{10}$ CFU/mL (92.6% reduction) for *S. mutans* and 5.05 ± 0.22$\log_{10}$ CFU/mL (88.0% reduction) for *Lactobacilli* ($p < 0.001$ relative to their 200 mW counterparts).

Response to Blue Diode Laser Irradiation Alone

Irradiation with the 445 nm BDL without exogenous photosensitizers demonstrated a notable intrinsic antimicrobial effect. BDL exposure alone at 200 mW produced a 69.4% reduction (5.50 ± 0.18$\log_{10}$ CFU/mL) in *S. mutans* and a 60.3% reduction (5.60 ± 0.20$\log_{10}$ CFU/mL) in *Lactobacilli*. Escalation to 500 mW BDL alone enhanced independent kill kinetics, yielding a 90.5% reduction (5.00 ± 0.22$\log_{10}$ CFU/mL) in *S. mutans* and an 83.8% reduction (5.18 ± 0.25$\log_{10}$ CFU/mL) in *Lactobacilli*.

We also note that the dark riboflavin-curcumin blend (without laser) achieved substantial non-photodynamic clearance, reducing *S. mutans* by 90.0% (4.98 ± 0.20$\log_{10}$ CFU/mL) and *Lactobacilli* by 85.4% (5.10 ± 0.23$\log_{10}$ CFU/mL).

Statistical Modeling and Power Influences

One-way ANOVA confirmed highly significant variations among the treatment divisions for both target pathogens at both the 200 mW and 500 mW settings ($p < 0.001$) (Table 1). Pairwise post hoc testing (Tukey's

test) confirmed that the curcumin cohort significantly outperformed the riboflavin and laser-only protocols across both power settings ($p < 0.05$). At the 500 mW threshold, riboflavin-mediated aPDT bactericidal activity converged with that of the laser-only controls, showing no statistically significant difference ($p < 0.857$ for *S. mutans* • $p < 0.621$ for *Lactobacilli*).

Intra-group evaluations using independent Student's *t*-tests proved that increasing the laser power from 200 mW to 500 mW significantly enhanced bacterial reduction across all active categories ($p < 0.001$) (Table 2). The mean shift values highlight that power escalation provided an additional reduction of to 0.83 ± 1.00$\log_{10}$ CFU/mL for the photosensitizer groups, while preserving the interspecies susceptibility gap.

Statistical Tables

The table below details the variation testing across primary treatment blocks within each organism track.

Table 1: One-Way ANOVA Comparisons Between Main Groups at Specific Power Settings

Organism	Setting	Source of Variance	Sum of Squares	df	Mean Square	F-value	p-value
S. mutans	200 mW	Between Groups	2.841	2	1.421	28.4	<0.001
		Within Groups	1.347	27	0.050	2	
	500 mW	Between Groups	8.762	2	4.381	87.6	<0.001
		Within Groups	1.350	27	0.050	2	
Lactobacilli	200 mW	Between Groups	2.105	2	1.053	21.0	<0.001
		Within Groups	1.350	27	0.050	6	
	500 mW	Between Groups	5.318	2	2.659	53.1	<0.001
		Within Groups	1.350	27	0.050	8	

The table below displays Student's *t*-test determinations evaluating the impact of laser power escalation within identical cohorts.

Table 2: Intra-Group Influence of Power Outlay (200 mW vs. 500 mW)

Tested Strain	Core Group	200 mW Mean ± SD ($\log_{10}$)	500 mW Mean ± SD ($\log_{10}$)	Mean Shift	*t*-statistic	*p*-value
S. mutans	Group I: Riboflavin	5.85 ± 0.22	4.85 ± 0.20	1.00	12.87	<0.001
	Group II: Curcumi	4.72 ± 0.21	3.75 ± 0.19	0.97	13.01	<0.001
	Group III: BDL alone	5.50 ± 0.18	5.00 ± 0.22	0.50	6.78	<0.001
Lactobacilli	Group I: Riboflavin	5.88 ± 0.23	5.05 ± 0.22	0.83	10.28	<0.001
	Group II: Curcumin	4.90 ± 0.24	4.02 ± 0.19	0.88	10.71	<0.001
	Group III: BDL alone	5.60 ± 0.20	5.18 ± 0.25	0.42	5.11	<0.001

The table below systematically details the direct interspecies comparisons, proving the greater resistance of *Lactobacilli*.

Table 3: Differential Bacterial Susceptibility Under High-Efficacy Environments

aPDT Experimental Subgroup	S. mutans $\log_{10}$ Mean	Lactobacilli $\log_{10}$ Mean	Mean Delta	p-value
Riboflavin + BDL 500 mW	4.85	5.05	-0.20	0.031
Curcumin + BDL 200 mW	4.72	4.90	-0.18	0.041
Curcumin + BDL 500 mW	3.75	4.02	-0.27	<0.001
BDL irradiation alone 500 mW	5.00	5.18	-0.18	0.038

DISCUSSION

Principal Findings

This controlled laboratory investigation provides a systematic comparison of the photodynamic susceptibility of *Streptococcus mutans* and *Lactobacilli* biofilms formed around stainless steel orthodontic brackets. The primary findings demonstrate that *S. mutans* consistently exhibits significantly

greater susceptibility to blue light-mediated photodynamic inactivation than *Lactobacilli* across all high-efficacy treatment conditions ($p < 0.05$). Conversely, *Lactobacilli* demonstrate higher resistance, maintaining greater numbers of surviving colonies and requiring higher photodynamic energy thresholds to achieve comparable logs of reduction. Consequently, the null hypothesis must be rejected.

Our data show that while curcumin-mediated aPDT at 500 mW yields exceptional clearance for both groups, *S. mutans* is reduced by 99.4%, whereas *Lactobacilli* clearance is limited to 98.9% ($p < 0.01$). This pattern highlights that despite utilizing identical photosensitizer distribution profiles and laser delivery kinetics, structural or metabolic factors render *Lactobacilli* more resilient to photodynamic destruction around fixed appliances.

Biological Basis of Differential Susceptibility

The distinct structural variations in cell wall architecture between *S. mutans* and *Lactobacilli* offer a logical biological explanation for their differing photodynamic susceptibilities. Both species are Gram-positive organisms, but their peptidoglycan layers differ significantly in thickness, density, and cross-linking composition. *Lactobacilli* species possess an exceptionally thick, multi-layered peptidoglycan envelope containing high concentrations of teichoic and lipoteichoic acids, overlaid in many strains by a crystalline surface layer (S-layer) of proteins. This dense macromolecular framework acts as a highly protective physical barrier that limits the inward diffusion of exogenous photosensitizers and neutralizes reactive oxygen species (ROS) before they can reach vulnerable plasma membrane lipids or internal nucleic acids.

In contrast, the peptidoglycan matrix of *S. mutans* is more porous and loosely cross-linked, allowing rapid penetration of lipophilic compounds like curcumin directly into its cellular boundary. Consequently, when laser activation triggers a localized burst of singlet oxygen and free radicals, *S. mutans* suffers immediate lipid peroxidation, wall deformation, and lysis. In *Lactobacilli*, the thicker wall envelope absorbs a significant portion of this oxidative energy, protecting vital structural components.

Beyond structural barriers, differences in oxidative stress defense mechanisms may explain the greater resilience of *Lactobacilli*. Although both species are aerotolerant anaerobes lacking true catalase enzymes, *Lactobacilli* have developed efficient non-enzymatic detoxification strategies to survive oxidative stress. Many *Lactobacilli* species accumulate high intracellular levels of manganese, which react with superoxide radicals to form functional biological scavengers. They also express specialized NADH oxidases and peroxidases that rapidly reduce intracellular oxygen derivatives, helping minimize radical-induced cell damage.

Furthermore, the inherent aciduric nature of *Lactobacilli* alters their biofilm matrix composition. Within a mature 72-hour biofilm, *Lactobacilli* synthesize dense packing elements that restrict oxygen transport and photosensitizer penetration compared to *S. mutans* biofilms. This matrix resistance, combined with effective internal antioxidant mechanisms, helps shield *Lactobacilli* from photodynamic eradication.

Influence of Photosensitizers

A key finding was that curcumin significantly outperformed riboflavin against both bacterial species across all experimental groups ($p < 0.05$). This consistent superiority is primarily due to the distinct photophysical properties and chemical structures of these natural compounds. Curcumin exhibits a high light absorption between 400 and 500 nm, aligning well with the 445 nm blue diode laser emission spectrum. This matching alignment ensures optimal photon absorption, resulting in a high quantum yield of singlet oxygen and reactive superoxides directly at the biofilm site.[3,5]

Furthermore, curcumin's lipophilic structure allows it to partition into lipid membranes and penetrate microbial outer structures during the 5-minute pre-irradiation period. This close binding ensures that generated short-range ROS can immediately disrupt the target cellular boundary. Curcumin also demonstrated a significant light-independent "dark" reduction of 78.1% for S. mutans and 69.8% for Lactobacilli. This effect is driven by its polyphenolic structure, which allows it to directly intercalate and disrupt bacterial lipid barriers even without light activation.[7] This dual action makes curcumin a highly versatile option for clinical biofilm management.

Conversely, riboflavin was ineffective at the low-power 200 mW setting, showing negligible antimicrobial action (<2% reduction). Significant bacterial reduction occurred only at the high-power 500 mW threshold. This clear power dependence stems from riboflavin's lower molar extinction coefficient at 445 nm compared to curcumin, which reduces its overall ROS generation per unit of delivered light energy. Additionally, riboflavin is highly hydrophilic, which limits its ability to penetrate dense biofilm matrices or bind tightly to lipid-rich bacterial walls. Instead, it tends to remain in the surrounding fluid, where generated ROS decay safely before reaching vital cellular targets. Riboflavin therefore requires a higher photon intensity (500 mW • 1.0˚W/cm^2) to produce enough radical concentrations to overcome its poor penetration and achieve significant bacterial reduction.[4]

Influence of Laser Power

Escalating the 445 nm BDL output from 200 mW to 500 mW significantly enhanced bactericidal efficacy across all experimental combinations ($p < 0.05$) while consistently preserving the interspecies susceptibility gap. This linear increase highlights a direct relationship between dose and response. Increasing the power density from 0.4˚W/cm^2 to 1.0˚W/cm^2 increases the photon delivery rate per second. This rapid photon influx accelerates the excitation of available photosensitizer molecules, producing a surge of ROS that overwhelms microbial antioxidant defenses.

Irradiation with the 445 nm BDL alone at 500 mW achieved a substantial 90.5% reduction in *S. mutans* and an 83.8% reduction in L*actobacilli*. This independent efficacy indicates the presence of endogenous bacterial chromophores, such as porphyrins and flavins, within the bacterial cells. These intracellular complexes absorb blue light at 445 nm, triggering an intrinsic photodynamic reaction that contributes to cell death without exogenous photosensitizers.[6,10,15] However, even under this independent effect, *Lactobacilli* maintained a higher survival rate than *S. mutans*, confirming its greater intrinsic tolerance to blue light-induced oxidative stress.

Clinical Implications

These findings have significant clinical relevance for preventing white spot lesions during fixed orthodontic treatment. Because fixed hardware creates plaque retention zones that are difficult to clean mechanically, aPDT offers an excellent, non-antibiotic method for targeted plaque control. However, the discovery that *S. mutans* and *Lactobacilli* have different photodynamic susceptibilities indicates that a single, generic aPDT protocol may not be ideal for all patients.

During the early stages of orthodontic treatment, when plaque accumulation is minimal and *S. mutan*s dominates to initiate demineralization, a low-energy or riboflavin-mediated aPDT protocol may be sufficient to clear the pathogen and protect enamel. However, in patients with poor oral hygiene or established WSLs, where aciduric *Lactobacilli* have colonized to accelerate lesion depth, a more intensive treatment is required. For these advanced cariogenic biofilms, clinicians should utilize highly effective protocols, such as curcumin-mediated aPDT at 500 mW, to ensure adequate eradication of the more resistant *Lactobacilli*. Tailoring aPDT parameters based on the patient's microbial status allows for more precise and effective preventive care.

COMPARISON WITH PREVIOUS LITERATURE

Our findings align with and build upon previous oral microbiology studies. Prior investigations have demonstrated that curcumin-assisted photodynamic inactivation using a 445 nm BDL at 500 mW (1.0˚W/cm^2) can eliminate cariogenic biofilms around metallic brackets with high efficacy.[1,4] Similarly, studies using alternative wavelengths often report minimal independent bactericidal effects unless paired with specific synthetic dyes.[9] This highlights the clinical advantage of the 445 nm blue diode laser wavelength, which can activate both natural photosensitizers and endogenous bacterial chromophores simultaneously to maximize bacterial reduction.[10]

Crucially, while past studies evaluated these pathogens independently, this investigation uniquely compares both organisms under identical experimental conditions. This direct comparison reveals the specific resistance gap of *Lactobacilli*, filling a key gap in the design of targeted orthodontic therapies.

Limitations

This study has certain limitations that should be considered when interpreting the results for clinical application. First, as an *in vitro* laboratory trial, it utilized single-species biofilm models grown under static conditions. This approach does not fully replicate the dynamic ecosystem of the human oral cavity, which includes continuous salivary flow, temperature shifts, salivary pellicle formation, and complex multi-species interactions. Salivary proteins can compete for light absorption or alter photosensitizer binding, and multi-species biofilms often display higher resistance due to protective cross-species matrices.

Second, while the evaluated power settings (200 mW and 500 mW) for a 30-second duration are known to keep intrapulpal temperature increases safely below the critical threshold, these safety boundaries must be thoroughly validated in vivo to prevent thermal damage to the dental pulp. Therefore, randomized controlled clinical trials are necessary to confirm these findings in patients.

CONCLUSIONS

Within the limitations of this *in vitro* study, *Streptococcus mutans* demonstrated consistently greater susceptibility to antimicrobial photodynamic therapy than *Lactobacilli* across all treatment conditions, with *Lactobacilli* requiring higher photodynamic energy thresholds to achieve comparable reductions in viable colonies. Curcumin-mediated photodynamic therapy provided superior antibacterial activity against both organisms compared with riboflavin.

These differential susceptibility patterns suggest that organism-specific factors should be considered when designing antimicrobial photodynamic therapy protocols for orthodontic patients, rather than relying on a single generic treatment parameter for all cariogenic species.

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Competing Interest Declaration

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