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Photo-disinfection of orthodontic brackets contaminated with *Lactobacillus acidophilus* with blue laser

Edris Pordel,[1] Trife Ghasemi,[2] Stefano Benedicenti,[3] Luca Solimei,[3] Nasim Chiniforush,[3,4] Shima Afrasiabi[5]

[1]Department of Pediatric Dentistry, Dental School, Sabzevar University of Medical Sciences, Sabzevar, Iran; [2]Independent Researcher, Mashhad, Iran; [3]Department of Surgical Sciences and Integrated Diagnostics, University of Genoa, Italy; [4]Dentofacial Deformities Research Center, Research Institute for Dental Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran; [5]Laser Research Center of Dentistry, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran

ABSTRACT

Decontamination of teeth with Chlorhexidine (CHX) in the treatment of dental disease is associated with some concerns. The objective of the current study was to ascertain whether the Blue Diode Laser (BDL), as a new approach in combination with riboflavin and curcumin as photosensitizers, would have any impact on the number of *Lactobacillus acidophilus* around orthodontic brackets. A total of 36 orthodontic brackets were contaminated with *L. acidophilus* and categorized into six different groups, including the negative control, riboflavin alone or riboflavin + BDL with a radiant power of 500 mW, and curcumin alone or curcumin + BDL with a radiant power of 500 mW, and 0.2% CHX as positive control. Orthodontic brackets were irradiated with a BDL (wavelength of 450 nm) and radiant exposure of 30 J/cm^2 for 30 s. Colony-forming units per milliliter (CFUs/ml) were determined. One-way Analysis Of Variance (ANOVA) followed by Tukey's post-hoc tests were performed to compare CFU/ml between groups. All groups were better at eliminating *L. acidophilus* around orthodontic brackets than the negative control group, but this was not significant for riboflavin alone. The curcumin groups were more effective than the riboflavin groups at reducing CFU/ml of *L. acidophilus*. In addition, CHX was able to completely eliminate the colonies of *L. acidophilus* (p <0.0001). This study showed that curcumin and riboflavin plus BDL significantly reduced the amounts of *L. acidophilus* around the orthodontic brackets.

Key words: biofilms; blue diode laser; *Lactobacillus acidophilus*; orthodontic brackets; photo-disinfection.

Corresponding author:
Shima Afrasiabi, Laser Research Center of Dentistry, Dentistry Research Institute, Tehran University of Medical Sciences, Tehran, Iran
Tel.: +98-21-88384331
E-mail: shafrasiabi@sina.tums.ac.ir

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Introduction

Patients who have undergone orthodontic treatment may develop caries, leading to inadequate chewing function and premature tooth loss, which impairs quality of life. In addition, caries in the anterior region, especially in the early stages of demineralization, undermine the esthetic improvements achieved by orthodontic therapy.[1,2] Fixed orthodontic appliances allow the accumulation of dental plaque, which promotes enamel demineralization and causes dental caries.[3] Dental caries is a multifactorial, dynamic, and biofilm-associated disease that destroys teeth and the development and progression of dental caries. Dental caries remains one of the most prevalent diseases in the world.[4]

Lactobacillus acidophilus has been shown to be closely associated with the occurrence of dental caries due to its colonization and biofilm formation on tooth surfaces.[5] *L. acidophilus* is a Gram-positive bacterium with acidogenic and aciduric properties.[6] It is a bacterium with excellent acid tolerance and the ability to survive at a pH of 4.5 or below.[7] The S-layer on the outermost cell surface of the bacterium causes hydrophobicity of the cell surface, which, in combination with the production of exopolysaccharides, leads to a good adhesion capacity for biofilm formation.[6]

In the treatment of dental caries, current approaches are based on mechanical scaling and root planing to eliminate microbial deposits, antibacterial components, and fluoride-containing agents.[8] However, due to the difficult access to some parts of the teeth, side effects and efficacy, they need to be replaced by more efficient and harmless methods.[9] The main side effects of chlorhexidine (CHX) mouthwash to date include parotid swellings, tooth discoloration, hypersensitivity reactions, taste changes, burning, ulceration or erosion of the oral mucosa, and paresthesia. In the oral environment, eradicating all microbes is undesirable, as they benefit from cooperation with the microbiome. In addition, bacteria can develop resistance to antiseptics when exposed to less lethal concentrations, and they can also develop cross-compatibility with antiseptics and antibiotics.[10]

Antimicrobial Photodynamic Therapy (aPDT) is an innovative therapeutic, non-invasive method, and is recommended for hard-to-reach areas.[11] The technique is repeatable, inexpensive, and easy to use. This method works by activating photosensitizers with a specific wavelength that generate Reactive Oxygen Species (ROS), which cause cell death.[12-14] Previous studies have shown the effectiveness of aPDT in reducing the number of oral bacteria from planktonic cultures, dental plaque, and biofilm.[15] One of the photosensitive and antibacterial natural components is curcumin. This colorful substance is extracted from the spice turmeric, and studies have shown that it has anti-inflammatory, antioxidant, and antibacterial properties.[16] Curcumin absorbs light in the 300-500 nm range. The disadvantages of clinical application include the discoloration of teeth and resin restorations, as well as the long irradiation time required for activation.[17] Riboflavin, another natural compound, is extremely biocompatible. It requires the maximum absorption ranges of 445, 336, and 270 nm.[18] Arajo *et al.* showed that 0.75 and 1.5 g/L curcumin in combination with a blue Light-Emitting Diode (LED) at irradiation of 5.7 J/cm^2 could significantly reduce the number of *L. acidophilus*.[19] According to Méndez *et al.*, curcumin alone had no effect on intact biofilms or microbial survival. Nevertheless, the total number of lactobacilli was reduced after treatment with 75 J/cm^2 LED. The combination of curcumin and LED significantly reduced the number of all bacterial groups and biofilm survival.[20] The low cost, naturalness, and efficacy of riboflavin and curcumin make them attractive for clinical use.

Blue Diode Laser (BDL) emits light with a wavelength of 450 nm and stands for coherent light radiation. BDL is used in various fields such as dental surgery, restorative dentistry, reduction of various bacterial strains, excision, incision, tooth whitening, coagulation, and photobiomodulation.[21-23] Although there are studies on the efficacy of blue light treatment with LED on caries-causing bacteria,[19,20] there is a lack of evidence for the efficacy of aPDT with BDL as a blue light source in reducing CFU/ml of *L. acidophilus*. Nowadays, the use of BDL in the form of aPDT has shown several applications in the field of dentistry.[22,23] However, special attention must be paid to the selection of the correct laser parameters.[24]

The aim of the present study was to investigate the effects of BDL on the disinfection of orthodontic brackets contaminated with *L. acidophilus*. This was done considering the importance of the situation of patients receiving orthodontic treatment and the potential of aPDT as a non-invasive, safe, and simple technique.

Materials and Methods

Sample preparation

The study protocol was reviewed and approved by the Ethics Committee of the Tehran University of Medical Sciences (IR. TUMS. DENTISTRY.REC. 1400. 187). Thirty-six extracted teeth were prepared and bonded with 0.022 stainless steel brackets (TSHdental, Tehran, Iran). All teeth were extracted because of orthodontic reasons and the owners of the teeth agreed to the donation. There were no cracks, fractures, or enamel restorations on the buccal surface of the teeth, and the teeth had normal structure. The teeth had no history of bleaching or aPDT pretreatment. The orthodontic brackets were viewed under a stereomicroscope (SMZ800, Nikon, Japan) at 10× magnification. All dentin surfaces were polished for 15 s with a low-speed handpiece (Coxo, Guangzhou, China), rubber bowls (Microdont, Sao Paulo, Brazil), and pumice paste (Cina, Tehran, Iran) before being thoroughly rinsed under running water. The remaining soft tissue around the teeth was removed with a periodontal scaler. All teeth were disinfected with a 0.5% (weight/volume) chloramine T solution at 3°C for one week and then placed in saline.

Culture condition and biofilm formation

L. acidophilus (IBRC-M 10815) was provided by the Iranian Biological Resource Centre (Tehran, Iran). Bacteria were inoculated in 10 ml de Man Rogosa Sharpe (MRS) broth (Ibresco, Iran) and incubated under aerobic atmosphere with 5% CO_2 at 37°C overnight. A bacterial suspension of 1.5×10^8 CFU/ml was prepared, corresponding to 0.5 McFarland. Enamel slabs with bonded brackets were placed in a 24-well microplate (Guangzhou JET Bio-Filtration Co., Guangzhou, China) and contaminated with 1 ml of *L. acidophilus* (10^6 CFU/ml). The microplates were incubated for 72 h at 37 °C and 5% CO_2 for biofilm formation.

Experimental groups

A total of 36 orthodontic brackets were allocated to 6 groups, each with six teeth bonded with 0.022 stainless steel brackets, including: i) control group, ii) riboflavin alone; iii) riboflavin + BDL with a radiant power of 500 mW; iv) curcumin alone; v) curcumin + BDL with a radiant power of 500 mW; vi) CHX (0.2%).

Photosensitizers, light source, and aPDT

The photosensitizers used in the study were curcumin (UltraCur, weber medical, Germany) at a concentration of 40 µM and riboflavin (Harman Finochem Ltd., Mumbai, India) at a concentration of 100 µM. For treatment, each contaminated orthodontic bracket was placed in a 24-well microplate and was exposed with 1 ml of the riboflavin or curcumin solution and was incubated in dark for 5 min. Then, the orthodontic brackets were irradiated at room temperature with a BDL (Wiser, Doctor smile, Italy) with a wavelength of 450 nm and a radiant power of 500 mW. Irradiation from BDL was directed to the orthodontic brackets for 30 s with radiant exposure of 30 J/cm^2, respectively. The diameter of the tip was 8 mm, the surface area was assumed to be 0.5 cm^2, and the distance between the tip and the orthodontic brackets was 1 mm. After treatment, each orthodontic bracket was placed in a 1.5 ml microtube containing 1 ml of phosphate buffered saline (PBS) and vortexed for 2 min. Then 10 µl of each solution was diluted (10^{-1}-10^{-5}) and transferred to MRS agar (Ibresco, Iran) plates. The plates were incubated aerobically at 37°C and 5% CO_2 for 48 h. The colony-forming units per milliliter (CFUs/ml) were calculated by multiplying the average of the observed colonies by the dilution factor and the volume of the diluted suspension on the plate.

Statistical analysis

All computations used IBM SPSS 25 statistics (Armonk, NY, USA). Using a one-way analysis of variance (ANOVA) and a post hoc Tukey test, the mean and average of $\log_{10}$ CFU/ml were compared between groups. The *p*-value <0.05 was used as significance cutoff.

Results

Among the groups, there was a significant difference in CFU/ml after the intervention (p<0.0001). Treatment of contaminated orthodontic brackets with CHX resulted in complete elimination of *L. acidophilus* around orthodontic brackets. Among the groups, riboflavin and cur-

cumin + BDL with a radiant power of 500 mW had the highest efficacy in reducing *L. acidophilus* around orthodontic brackets (p<0.0001). Curcumin alone was able to significantly reduce CFU/ml of *L. acidophilus* (p=0.001), whereas, only riboflavin + BDL with a radiant power of 500 were able to significantly reduce CFU/ml of *L. acidophilus* (p<0.0001). The groups with riboflavin alone were able to reduce the CFU/ml of *L. acidophilus*, but not significantly (p=0.84). All corresponding curcumin groups to riboflavin groups were significantly more capable in eliminating *L. acidophilus* around orthodontic brackets. The Log_{10} CFU/ml of the study groups before and after intervention is shown in Figure 1 and Table 1.

Discussion

The main finding of this study is that curcumin plus BDL as a new device was able to significantly reduce the number of CFU/ml of *L. acidophilus* around orthodontic brackets. Our study showed no discernible difference in the elimination of *L. acidophilus* between riboflavin alone and the control group, while riboflavin + BDL with a radiant power of 500 mW was able to significantly reduce CFU/ml of the bacteria. However, Araújo *et al.* found no differential decrease in viable *Streptococcus mutans* and *L. acidophilus* in the groups treated with curcumin (5.0 g/L) plus LED with a radiant exposure of 5.7 J/cm^2.[25] In contrast to our results, one study showed that curcumin did not significantly reduce CFU/ml of *S. mutans* and *L. acidophilus* without light activation.[26] The curcumin dosage, different culture media, different statistical analyses, and the timing of the process might have influenced the results. Another main difference between these two studies is the different light sources. In agreement with the studies on BDL, Merigo *et al.* also showed that BDL with a radiant exposure of 30 J/cm^2 and curcumin resulted in 99.26% growth inhibition of *S. mutans*.[27]

The photosensitizing properties of curcumin are well known and have been successfully used in aPDT.[28] Moradi *et al.* observed that aPDT at a wavelength of 460 nm and a mean radiant exposure of 60 J/cm^2 with 0.05% curcumin and aPDT with the same LED and 0.1% riboflavin significantly reduced CFU/ml of *Enterococcus faecalis*.[29] The phototoxicity of curcumin to microbial systems is mainly related to the production of ROS and its interaction with the organelles of the target cells. According to some studies, curcumin still produces a significant amount of ROS even at shorter illumination duration.[30,31] It is possible that the difference in the time required to reach maximum efficacy explains the different results between riboflavin and curcumin.[32]

The concentration of photosensitizers used is another critical component of aPDT efficacy. After sensitization with curcumin and irradiation with BDL, Arajo *et al.* demonstrated that *S. mutans* and *L. acidophilus* grew as multispecies in the biofilm phase and were sensitive to aPDT in carious dentin lesions, which is consistent with

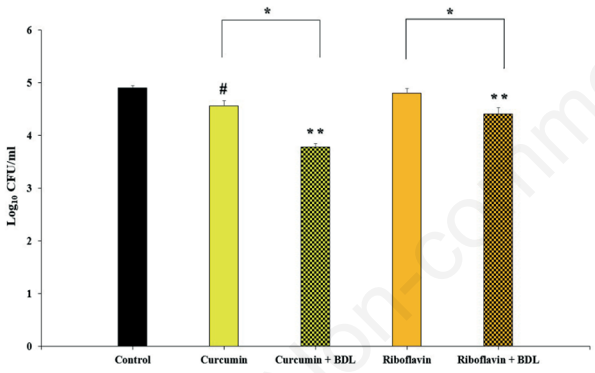

Figure 1. The log_{10} CFU/ml of the study groups; *it is statistical significance ($^{\#}p$<0.05, $^{**}p$<0.001); BDL, blue diode laser.

Table 1. Logarithm of the colony count of *Lactobacillus acidophilus*.

Groups	Mean	SD	Lower bound	Upper bound	Minimum	Maximum
Control	4.90	0.05	4.76	5.05	4.84	4.94
Riboflavin	4.80	0.09	4.57	5.02	4.70	4.88
Curcumin	4.56	0.10	4.30	4.81	4.45	4.64
Riboflavin + BDL	4.41	0.12	4.09	4.72	4.28	4.53
Curcumin + BDL	3.78	0.07	3.59	3.96	3.71	3.86

BDL, blue diode laser; SD, standard deviation.

our results. They found that the amount of curcumin influenced the phototoxic effect. Curcumin at a concentration of 5.0 g/L was able to significantly reduce the number of living cells, in contrast to other concentrations of curcumin (0.75, 1.5, 3.0, and 4.0 g/l).[28]
The chemical properties of the individual photosensitizers, such as hydrophilicity, amphiphilicity, and electrical charge, determine how effective they are in aPDT.[33] Since curcumin is hydrophobic, it does not dissolve well in water. On the other hand, riboflavin has a hydrophilic character and is, therefore, soluble in sodium chloride. Kamran *et al.* demonstrated that aPDT with LED and riboflavin was as effective as CHX in reducing *S. mutans*.[34] The results of the study by Araújo *et al.* showed that *S. mutans* was more susceptible to aPDT than *L. acidophilus* after sensitization with curcumin and irradiation with LED. A reduction in sensitivity of up to 99.9% was observed in *S. mutans*, but the reduction was much lower in *L. acidophilus* (37.6%).[19] The increasing radiant power of BDL was associated with a higher antibacterial potential of aPDT. Recently, a study compared the efficacy of riboflavin at different doses and BDL with different radiant powers (200-500 mW). They found a greater reduction of *E. faecalis* CFU/ml when using BDL with a radiant power of 500 mW.[22]
The brackets used in this study on the tooth model with the *L. acidophilus* biofilm coating simulated the oral cavity slightly better. However, the single-species biofilm used is not representative of the polymicrobial infection that occurs around orthodontic brackets. Prospective antibacterial efficacy cannot be demonstrated by examining the antimicrobial capacity at a specific point in time. Future research should test a variety of microorganisms and evaluate the value of the curcumin or riboflavin content of each strain as well as the various parameters of BDL. As this study is an *in vitro* study, the applicability of the results to clinical environments and situations is limited. Environmental conditions such as diet, limited accessibility, plaque formation, salinity, temperature, influence of the immune system, etc. cannot be determined by *in vitro* experiments. Future clinical studies are needed to investigate the use of natural photosensitizers plus BDL as adjunctive therapy to reduce the presence of cariogenic bacteria and improve clinical outcomes. The rather small sample size of the study may also impose some limitations. Our results encourage further research into the efficacy of state-of-the-art disinfection techniques using curcumin and riboflavin as well as 450 nm BDL.

Conclusions

The results of the study show that *L. acidophilus*, the second most common cariogenic bacterium, is susceptible to curcumin and riboflavin plus BDL at a radiant power of 500 mW. These characteristics make BDL a potential option for clinical use, but the efficacy of aPDT using BDL with curcumin and riboflavin needs to be investigated in further clinical studies.

Conflict of interest

The authors declare no potential conflict of interest, and all authors confirm accuracy.

Contributions

NC, SA, conceptualization; SA, data curation; SB, LS, investigation, validation, visualization; EP, TG, NC, SA, methodology; EP, TG, SB, NC, resources; EP, TG, writing–original draft; SA, writing–review editing.

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Ethics approval and consent to participate

The study protocol was reviewed and approved by the Ethics Committee of the Tehran University of Medical Sciences (IR. TUMS. DENTISTRY.REC. 1400. 187). All teeth were extracted because of orthodontic reasons and the owners of the teeth agreed to the donation.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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