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Research Article

Influence of Blue Light on a Dual-Species Biofilm in a Model of Dental Caries

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Abstract

Dental blue LED curing light (wavelengths, 400 - 500 nm), used for composite restorative materials, has shown potential to affect oral biofilm formation. However, there is limited study of its effect on caries formation. We aimed to study the sustained effects of blue light on biofilm grown on bovine enamel or cover glass in an *in vitro* caries model with mono-culture or co-culture of *Streptococcus mutans* and *Streptococcus sanguinis*. Twenty-four-hour biofilm samples were treated by blue light at a fluence of 140 J/cm^2 and incubated anaerobically for 24 hrs with no-light-treatment as control. Then, planktonic pH and calcium concentration in the supernatants, as well as biofilm biomass, bacteria levels, live/dead bacteria ratios, and biofilm structure under the confocal microscopy were evaluated.

Results showed that, under the experimental conditions, blue light treatment had a significant effect on the co-culture biofilms only, demonstrated by a significantly higher pH, less calcium release in the supernatants, and reduction of *S. mutans* levels in the biofilms, when compared to the control. In all experimental conditions, blue light did not affect the biofilm thickness or live/dead bacterial ratio.

Using this in vitro caries formation model, our results indicate that blue light may suppress cariogenic *S. mutans* growth in biofilm when co-cultured with *S. sanguinis*, which resulted in less acidity and enamel demineralization, with a potential to reverse biofilm dysbiosis for caries prevention.

Keywords: *Streptococcus mutans; Streptococcus sanguinis*; Biofilm; Bovine Enamel; Artificial Caries; Confocal Laser Scanning Microscopy

Introduction

Dental caries is an infectious disease caused by microbial dysbiosis of an over-population of cariogenic bacteria in dental biofilm. The mutans streptococci (MS), group is one of the main groups of cariogenic bacteria. In humans the main subspecies of MS are *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*). The virulence factors of MS include extracellular polysaccharide synthesis, high acid tolerance, and high acid production in dental biofilm when metabolizing fermentable carbohydrates [1-5]. In an environment of frequent carbohydrate consumption or presence of highly virulent MS, the dominance of commensal bacteria, such as *Streptococcus sanguinis* (*S. sanguinis*), in dental biofilm

is broken by over-growth of MS, leading to the predominance of demineralization over remineralization and development of dental caries lesions. Therefore, treatments that reduce cariogenic bacteria load and restore biofilm symbiosis, can play an important role in caries prevention.

The beneficial effect of conventional antimicrobial treatments by chemical agents may be limited in caries prevention because the biofilm itself creates a physical barrier for bacteria deeper in the biofilm and provides them with a means of resistance to conventional antimicrobial agents [6-10]. Therefore, better antimicrobial agent delivery systems or alternative antimicrobial therapies should be explored for caries prevention.

Photodynamic therapies (PDT), such as with laser irradiation (HeNe, YAG) and visible light including white, red, and blue light have been advocated as alternative methods to modify dental biofilms [11,12]. PDT has been shown to regulate bacterial gene expression in oral biofilm [13,14] and may penetrate deeper in biofilm than conventional chemical agents.

Blue light (wavelengths, 400 - 500 nm) used in dentistry for curing (polymerization) of composite resins in dental restorations is visible and has been shown to affect local microbial biofilm formation [15-17]. Using a dental curing blue light with light-emitting diodes (LED) as an antimicrobial treatment device, if effective, could be especially beneficial since it is ready available in every dental office and can be adopted widely. Use of blue light alone has shown a major phototoxic effect on Gram-negative periodontal pathogens [15] with a lesser effect on the Streptococci species [16,17]. However, blue-light treatment on pre-formed *S. mutans* biofilm has shown a sustained effect on these bacteria to form new biofilm with a higher dead/live ratio (more rapid apoptosis) than the no-light-treatment controls [18]. Further, the effect of blue-light treatment on new biofilm formation was more prominent when *S. mutans* were treated in biofilm than when they were treated in planktonic phase [19], indicating the potential of blue-light treatment to be a more effective treatment for biofilm compared to conventional antimicrobial solution therapy.

In the real world, effects of blue-light treatment on continuous growth and caries formation of pre-formed biofilm have important clinical value in caries prevention. In this study, we assessed the effect of blue light on biofilm grown on bovine enamel surface, containing both cariogenic bacteria and commensal bacteria, in order to understand the impact of blue light on biofilm dysbiosis and caries formation. To our knowledge, there are only limited studies investigating the effect of blue curing light on initial caries formation in enamel surfaces and biofilm structure of *S. mutans* and *S. sanguinis* mono- and co-cultures in a dental caries model.

Materials and Methods

Overall study design and statistical analysis

Biofilms of mono- or co-cultures of *S. mutans* UA159 and *S. sanguinis* 10556 were formed on bovine enamel blocks in Brain Heart Infusion medium (BHI) supplemented with sucrose for 24 hrs. Then, biofilms were treatment with blue light (BL group) using no light treatment as control (CTL group). Then, the biofilms were continually grown in BHI with sucrose on bovine enamel blocks for another 24 hrs. The planktonic pH and calcium concentration as a measure of demineralization were measured prior to and 24 hrs after light treatment. The 24 hr post-light-treated biofilms were evaluated for biomass, bacterial composition and levels, thickness, and live/dead bacteria ratio. All assays were completed in triplicate and were repeated once (n = 6/group at each data point except the ones otherwise specified). Comparison of planktonic pH, planktonic calcium concentration, biomass optical density (OD), the logarithm converted colony unit/ml of *S. mutans* and *S. sanguinis*, thickness of biofilm and the live/dead bacteria ratio were compared between the BL and CTL groups using a Student's t-test between the BL group and the CTL group. The statistical analysis were two-tailed ($p < 0.05$) and performed using SPSS 10.

Biofilm formation and caries formation in an *in vitro* caries formation model

Biofilms were grown on polished bovine enamel blocks each with a 2 x 2 mm window in a 12 well culture plate (Becton Dickinson GmbH, Germany) to assess caries formation [11,20,21] and were grown on cover glass (n = 4 per group and repeated once) in a 24 well culture plate to assess the biofilm structure by confocal microscopy. The plates with bovine enamel blocks or cover glasses were sterilized by gamma irradiation overnight and pre-warmed in 4 ml of PBS before the biofilm experiments commenced.

Overnight cultures of *S. mutans* and *S. sanguinis* were grown anaerobically at 37ºC in Brain Heart Infusion broth (BHI) (Becton, Dickinson and Company, MD, USA). The cells were harvested by centrifuge and inoculated to a final concentration of OD 0.05 at 490 nm. Four ml of BHI with 0.6% sucrose were added to each well for each enamel block or 2.2 ml BHI in 24 well plates for each cover glass. The bovine enamel blocks were immersed so that the window surface was toward to the bottom, and the cover glass surface was placed at a 45 degree angle. The mono-culture or co-cultures of the above bacteria in individual cell culture plates were incubated for up 24h in 0.6% sucrose BHI broth to allow biofilm formation at 37°C facultatively for 24 hrs prior to blue light treatment. After blue light treatment, the enamel blocks or cover glasses were transferred to new culture medium and plates for another 24-hour incubation before assessments for caries and biofilm formation.

The supernatants of the culture medium were collected daily for pH and calcium measurements. The supernatant pH was measured by a pH meter (Orion 2-star pH Benchtop Meter, Thermo Scientific, USA). The calcium concentration in the supernatant was measured by atomic absorption spectroscopy assays (Atomic Absorption Spectrometer 3110, Perkin-Elmer, MA, USA) measured both prior and post blue light treatment on a daily basis at wavelengths 422 nm [22,23].

24 hr post-blue-light treated biofilms were collected from the enamel windows or glasses by a cotton swab, re-suspended in 1 ml PBS, dispersed by intermittent 5-second sonication for 3 times, and then the optical density was measured at 490 nm with E max Precision Microplate Reader (Molecular Devices, USA) for biofilm biomass. The suspensions were also subjected to serial dilution and cultured on Mitis Salivarius agar plates (Difco Mitis Salivarius Agar, Becton Dickinson GmbH, Germany) for 72 hrs anaerobically for *S. mutans* and *S. sanguinis* enumeration (CFU/ml) measured under a dissecting microscope by colony morphology.

Blue light treatment of biofilm

A blue LED curing light (wavelengths 400 - 500 nm) (ART-L5 Curing Light, Bonart Co., LTD, Taiwan) was used to treat the biofilm in a biological hood under sterile aerobic conditions as previously described [19]. Briefly, the biofilm were irradiated by the light at a distance of 8mm from the light source for 3 minutes at 779 mW/cm^2 (equivalent to accumulated fluences of 140 J/cm^2).

Confocal scanning laser microscopy (CSLM) assessment of biofilm

CSLM (DMi8, LEICA, Germany) was used to assess the biofilm thickness and bacterial vitality measured by live/dead ratio. The biofilm was washed with PBS and fixed by 300μL of Paraformaldehyde (4%) for 5 minutes at room temperature followed by washing with PBS again. Then, the biofilms were stained with 300 μl of LIVE/DEAD BacLight Bacterial Viability dye (Invitrogen, USA) at 1:500 dilution for 15 minutes in the dark at room temperature followed by washes with PBS. The samples were then mounted on slide glass with Dako Faramount Aqueous Mounting Medium Ready-to-use (Dako North America Inc., USA) and dried overnight in the dark [24]. This staining allows differentiating the live organisms from the dead bacteria. Green fluorescence of SYTO9 was measured following excitation by an argon-ion laser, while red fluorescence of Propidium Iodide was obtained after excitation by He-Ne laser. Optical sections were acquired at spacing steps from the surface through the depth of the biofilm. The intensity of all sections (separately for SYTO9 and Propidium Iodide) was measured by the ImageJ-win64 Program [25]. The thickness of each biofilm was measured by reading the Z-axis value from the outer layer to the bottom. In each experiment, exciting laser intensity, background level, contrast and electronic zoom size were maintained at the same level. Five random fields were analyzed in each experiment. All samples were analyzed for 12 layers from the outer surface to the bottom of the biofilm adhering to the glass, through the vertical axis of the specimen, using a computer-controlled motor drive. The ratio of live/dead bacteria was calculated as green intensity/red intensity at every field of the biofilm layer.

Results

In mono-cultures biofilm samples of *S. mutans* or *S. sanguinis*, there were no statistically significant differences in supernatant pH and calcium concentrations between CTL group and BL group 24 hrs after blue light treatment (Figure 1A and 1B, $P > .05$, Student t Test). However, in the co-culture biofilms of *S. mutans* and *S. sanguinis*, the suspension pH (Figure 1A) was significantly higher and calcium concentration (Figure 1B) was significantly lower in the BL groups than that of the CTL groups ($p < .05$, Student t test).

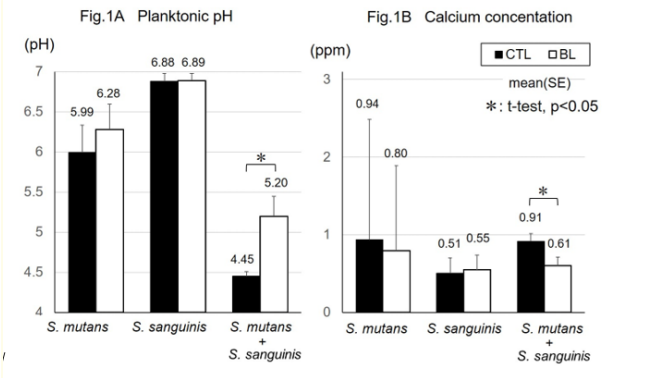

Figure 1A and 1B: The pH (A) and calcium concentration (B) measured in supernatants of mono- and co-culture *S. mutans* and *S. sanguinis* biofilm samples 24-hr after blue light treatment using 140 J/cm^2 in the dental caries model (n = 12).

For biofilm biomass, no statistically significant differences were found between BL groups and the appropriate CTL groups of mono- and co-cultures of *S. mutans* and *S. sanguinis* (Figure 1C, p > .05, Student t test). There was no significant difference in biomass of dispersed biofilms and bacterial levels of *S. mutans* and *S. sanguinis* between the mono-cultures biofilms of BL groups and the appropriate CTL groups (P > .05, Student t test). However, in co-culture groups, significantly less *S. mutans* were detected in BL group than that in the CTL group (Figure 1D, P < .05, Student t test), but no significant differences in the levels of *S. sanguinis* and in the biofilm biomass were detected between two groups.

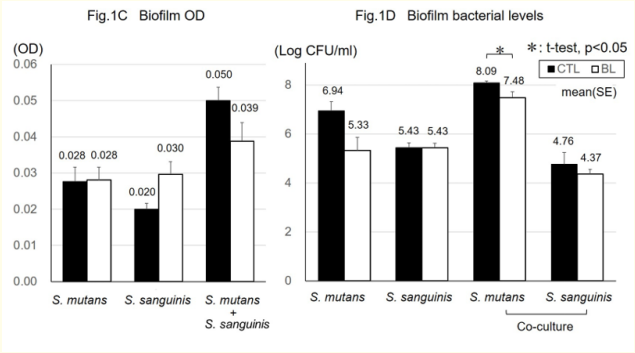

Figure 1C and 1D: Biomass of dispersed biofilms (C) and bacterial levels (D) of mono- and co-culture *S. mutans* and *S. sanguinis* biofilm samples 24-hr after blue light treatment using 140 J/cm2 in the dental caries model (n = 12).

From the CSLM results, there were no statistically significant differences in the thickness of biofilm samples after blue light treatment between the BL and CTL groups in neither the mono- nor the co-culture experiments (Figure 2A, P > .05, Student t test).

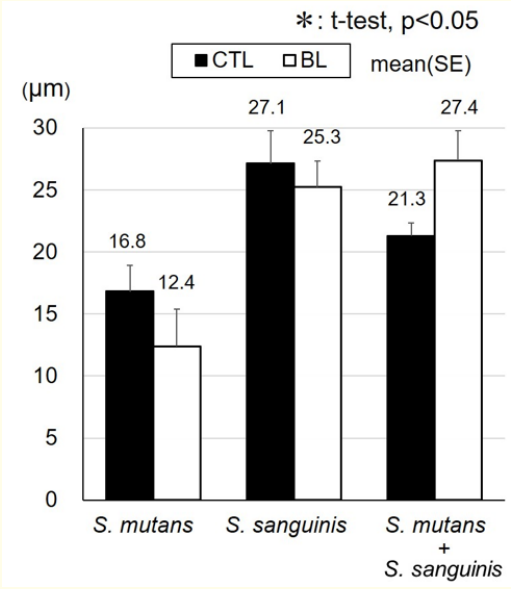

Figure 2A: Biofilm thickness of CTL groups (black columns) and BL groups (white columns) 24-hr after blue light treatment using 140 J/cm^2 as measured by CSLM assays (n = 6).

In mono-culture of *S. mutans* and co-culture of *S. mutans* and *S. sanguinis* (Figure 2B), the ratio of live/dead bacteria of BL groups was lower than that of the appropriate CTL groups, especially towards the surface layers of *S. mutans* biofilms, however, it was not statistically significant. Similar results were observed in ratio of live/dead bacteria in mono-culture biofilms of *S. sanguinis* between BL and CTL groups, with higher dead bacteria in the very out layers of the BL group biofilms (Figure 2B).

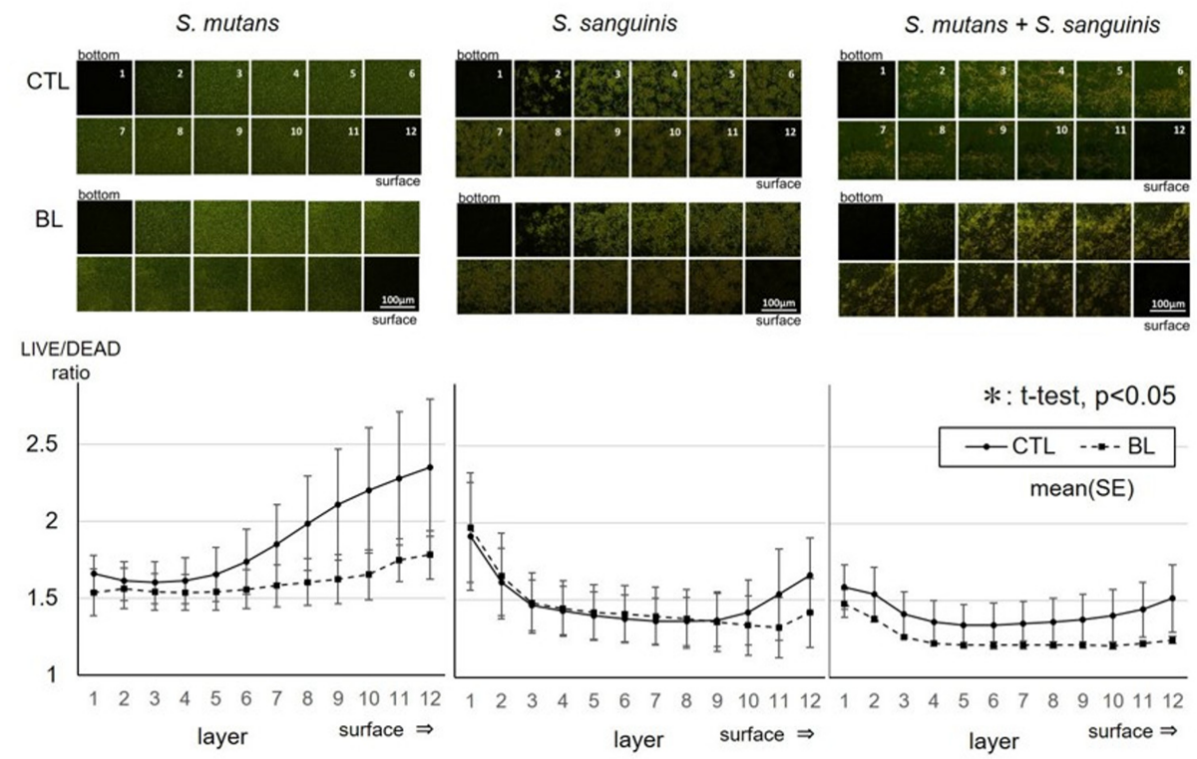

Figure 2B: Live/dead ratio of mono and co-culture biofilms of *S. mutans* and *S. sanguinis* of CTL groups (round dots) and BL groups (square dots) 24-hr after blue light treatment using 140 J/cm^2 as observed by CSLM scans, from the bottom of the biofilm (Layer 1) to the surface (Layer 12).

Discussion

Our study investigated the effects of blue light treatment on the continuous growth and composition of the biofilms as well as acid formation and the resulting enamel demineralization of mono- and co-culture biofilms of *S. mutans* and *S. sanguinis* formed on bovine enamel in an *in vitro* caries model.

We found that, under this study's experimental conditions, 24h after blue light treatment, a significant effect on the co-culture biofilms only, demonstrated by a relative increase in pH and a resulting decrease in calcium concentration in the biofilm supernatants, accompanied by a decrease in *S. mutans* levels in the biofilms but did not alter the cell apoptosis or biomass, when compared to the control. Interestingly, we did not find blue light treatment significantly affected continuous biofilm formation, acid formation and caries formation on mono-cultured biofilms of *S. mutans* or *S. sanguinis*. This is different from findings in previous studies showing that blue-light treatment of *S. mutans* in biofilm accelerated bacterial death in the regrown biofilm although it did not interfere the bacterial capability to re-form new biofilm, indicating a delayed antibacterial effect [18,19,26]. Also, Cohen-Berneron., *et al*. [19] showed that the acidogenicity from the regrown *S. mutans* biofilm was decreased as fluences of light increased. In another study with our collaborator with similar condition with us except for less medium volume [27], blue-light treatment also affected mono-species *S. mutans* biofilm growth with increased cell apoptosis while the bacterial biomass reduced, with no significant change in viability when compared to control in co-cultured biofilm of *S. mutans* and

S. sanguinis. A possible reason for those discrepancies in results may be attributed to the differences in study design and growth conditions for the biofilm. As biofilm growth conditions may affect biofilm reaction to light exposure, further studies should be conducted to investigate whether or not biofilm matrix formation and the nutritional environment affect the biofilm reaction to blue light treatment.

Our study found that, in co-cultured *S. mutans* and *S. sanguinis* model, blue light treatment significantly reduced *S. mutans* levels in the biofilm with no impact on *S. sanguinis* levels, resulting in reduction of acid production and demineralization. Because *S. mutans* is a well-known cariogenic pathogen while *S. sanguinis* is a main commensal bacteria combating against most oral pathogens, these results indicate that blue-light treatment may affect the competition of growth between *S. mutans* and *S. sanguinis* in the biofilm and may facilitate the restoration of microbiome symbiosis from dysbiosis instead of acting as a broad-spectrum antibiotic. This action is anticipated to be very beneficial in maintaining oral health.

There are a variety of caries models available to study the dental caries process *in vitro* [28]. We used an *in vitro* caries formation model that enables the bacterial biofilm to induce demineralization of enamel by light-exposed bacteria immobilized in mono and co-culture biofilms and compare results to that of control non-exposed bacteria that allow us to quantify the modeled caries outcome of changes in enamel demineralization and virulence of the bacteria in the biofilm. Using this model, we also demonstrated the influence of co-existing bacteria in multi-species biofilms when exposed to blue light. Oral biofilm *in vivo* is characterized by a structure of multispecies communities [29,30]. Different types of bacteria can have different susceptibility to visible light [16,31]. The phototoxic effect of blue light was lower on *S. mutans* than that on *P. gingivalis* and *F. nucleatum* that may be related to the protection of *S. mutans* by its antioxidant defense enzymes such as superoxide dismutase [32]. Vaknyn., *et al*. [27] suggested that the opposite effects of blue light on *S. mutans* bacteria when grown in mixed biofilm or separately could be explained by the influence of the H_2O_2 production by the coexisting *S. sanguinis* bacteria. Furthermore, their results showed difference in gene expression related to biofilm formation, demonstrating that *S. mutans* grown in mixed biofilm behave differently than in separated growth states [27]. Further studies are needed to explore the mechanism of blue light's suppression of *S. mutans* while at the same time not affecting *S. sanguinis* in the co-culture assays.

In the present study, we used 3 minutes of blue light treatment with cumulative fluences of 140 J/cm^2. Although those light parameters were effective in the co-culture model for reducing a potential caries formation and restoring biofilm dysbiosis, the exposure time to light is too long for clinical application. However, we used the light source at a distance of 8 mm from the exposed sample, and since the intensity of light is inversely proportional to the square of the distance from the source, with a smaller distance from the light source to the biofilm a much shorter time period should be sufficient. Additionally, previous studies have shown that PDT coupled with the addition of specific light absorbers such as toluidine blue, methylene blue, erythrosine or the chemical hydrogen peroxide these conditions enhance the antimicrobial effect of the blue light and shorten treatment times [26,33-36]. A synergistic effect between blue light and H_2O_2 have been demonstrated to shortened the exposure time to 1 minute for effective antimicrobial effects on oral biofilm [33]. The use of light irradiation in combination with chemical treatment should be explored for ecological advantages in future studies.

Conclusion

In conclusion, using an *in vitro* caries formation model, we found that blue light treatment significantly affected the biofilm composition by lowering *S. mutans* levels in mixed biofilms. Thus, blue light treatment may move biofilm from dysbiosis to a state that leads to reduced acidity and caries formation, which effect is sustained for at least 24h after treatment. *S. mutans* and *S. sanguinis* co-culture results indicate that this effect may involve mechanisms related to the competition between these two bacteria species. Our study confirmed the sustained potential effect of blue light treatment in suppression of the cariogenic *S. mutans* growth in co-culture biofilm and in reducing the biofilm virulence characteristics. Further studies are needed to elucidate the mechanism of light effect when different bacteria are co-exist in the biofilm and the efficiency of blue light treatment *in vivo* and in combination with other photo enhancers or chemical agents.

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Bibliography

1. W Krzyściak, A Jurczak, D Kościelniak, B Bystrowska, A Skalniak. The virulence of *Streptococcus mutans* and the ability to form biofilms. Eur J Clin Microbiol Infect Dis. 2014;33:499-515.
2. G Svensater, M Borgstrom, GH Bowden, S Edwardsson. The acid-tolerant microbiota associated with plaque from initial caries and healthy tooth surfaces. Caries Res. 2003;37:395-403.
3. J Van Houte. Role of micro-organisms in caries etiology. J Dent Res. 1994;73(3):672-682.
4. D Steinberg. Studying plaque biofilms on various dental surfaces. In: An YH, Friedman RJ (eds) Handbook of bacterial adhesion: principles, methods, and applications. Humana Press, Totowa, 2000:353-370.
5. G Khoo, L Zhan, C Hoover, JD Featherstone. Cariogenic virulence characteristics of mutans streptococci isolated from caries-active and caries-free adults. J Calif Dent Assoc. 2005;33(12):973-980.
6. PS Stewart. Biophysics of Biofilm infection. Pathog Dis. 2014;70(3):212-218.
7. JW Costerton, PS Stewart, EP Greenberg. Bacterial biofilms: a common cause of persistent infections. Science 1999;284(5418):1318-1322.
8. M Wilson. Bacterial biofilms and human disease. Sci Prog. 2001;84(3):235-254.
9. CTF Mah, GA O'toole. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. 2001;9(1):34-39.
10. M Shemesh, A Tam, D Steinberg. Differential gene expression profiling of *Streptococcus mutans* cultured under biofilm and planktonic conditions. Microbiology. 2007;153(5):1307-1317.
11. JDB Featherstone, NA Barrett-Vespone, D Fried, Z Kantorowitz, W Seka. CO_2 laser inhibition of artificial caries-like lesion progression in dental enamel. J Dent Res. 1998;77(6):1397-1403.
12. T Maisch. Anti-microbial photodynamic therapy: useful in the future? Lasers Med Sci 2007;22:83-91.
13. M Garcı´a-Domı´nguez, MI Muro-Pastor, JC Reyes, FJ Florencio. Light-dependent regulation of cyanobacterial phytochrome expression. J Bacteriol. 2000;182(1):38-44.
14. L Scha¨fer, M Sandmann, S Woitsch, G Sandmann. Coordinate up-regulation of carotenoid biosynthesis as a response to light stress in Synechococcus. Plant Cell Environ. 2006;29(7):1349-1356.
15. NS Soukos, S Som, AD Abernethy, K Ruggiero, J Dunham, C Lee, AG Doukas, JM Goodson. Phototargeting oral blackpigmented bacteria. Antimicrob Agents Chemother. 2005;49(4):1391-1396.
16. Feuerstein, N Persman, EI Weiss. Phototoxic effect of visible light on *Porphyromonas gingivalis* and *Fusobacterium nucleatum*: an *in vitro* study, Photochem Photobiol. 2004;80(3):412-415.
17. Feuerstein, I Ginsburg, E Dayan, D Veler, EI Weiss. Mechanism of visible light phototoxicity on *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. Photochem Photobiol. 2005;81(5):1186-1189.
18. D Chebath-Taub, D Steinberg, JDB Featherstone, O Feuerstein. Influence of light on *Streptococcus mutans* re-organization in biofilm. Photochem Photobiol. 2012;116:75-78.
19. J Cohen-Berneron, D Steinberg, JDB Featherstone, O Feuerstein. Sustained effects of blue light on *Streptococcus mutans* in regrown biofilm. Lasers Med Sci. 2016;31:445-452.
20. JDB Featherstone, JR Mellberg. Relative rates of progress of artificial carious lesions in bovine, ovine and human enamel. Caries Res. 1981;15:109-114.
21. S Nagaoka, HJ Liu, K Minemoto, M Kawagoe. Microbial induction of dentinal caries in human teeth *in vitro.* J Endod. 1995;21(11):546-551.

22. M Welna, A Szymczycha, P Pohl. Quality of the trace element analysis: sample preparation steps. In: Akyar I (ed) Wide spectra of quality control, 1st edition. InTech, Croatia, 2011:53-70.

23. M Thompson, S Ellison, R Wood. Harmonized guidelines for single-laboratory validation of methods of analysis (IUPAC Technical Report). Pure Appl Chem. 2002;74(5):835-855.

24. A Radaic, MB de Jesus. Solid lipid nanoparticles release DNA upon endosomal acidification in human embryonic kidney cells. Nanotechnology. 2018;29:315102.

25. J Schindelin, I Arganda. Fiji: an open-source platform for biological-image analysis. Nat Methods. 2012;9(7):676-682.

26. D Steinberg, D Moreinos, JDB Featherston, M Shemesh, O Feuerstein. Genetic and physiological effects of noncoherent visible light combined with hydrogen peroxide on *Streptococcus mutans* in biofilm. Antimicrob Agents Chemother. 2008;52(7):2626-2631.

27. M Vaknin, D Steinberg, JD Featherstone, O Feuerstein. Exposure of *Streptococcus mutans* and *Streptococcus sanguinis* to blue light in an oral biofilm model. Lasers Med Sci. 2019;35:709-718.

28. JDB Featherstone. Modeling the caries-inhibitory effects of dental materials. Dent Mater 1996;12(3):194-197.

29. PE Kolenbrander, RJ Palmer Jr, S Periasamy, NS Jakubovics. Oral multispecies biofilm development and the key role of cell-cell distance. Nat Rev Microbiol. 2010;8:471-480.

30. L Netuschil, E Reich, G Unteregger, A Sculean, M Brecx. A pilot study of confocal laser scanning microscopy for the assessment of undisturbed dental plaque vitality and topography. Arch Oral Biol. 1998;43(4):277-285.

31. MTM Freitas, TT Soares, MGB Aragão, RA Lima, S Duarte, ICJ Zanin. Effect of Photodynamic Antimicrobial Chemotherapy on Mono- and Multi-Species Cariogenic Biofilms: A Literature Review. Photomed Laser Surg. 2017;35(5):239-245.

32. K Nakayama. Nucleotide sequence of *Streptococcus mutans* superoxide dismutase gene and isolation of insertion mutants. J Bacteriol. 1992;174:4928-4934.

33. Feuerstein, D Moreinos, D Steinberg. Synergic antibacterial effect between visible light and hydrogen peroxide on *Streptococcus mutans.* J Antimicrob Chemoth. 2006;57(5):872-876.

34. ICJ Zanin, RB Gonçalves, AB Junior, CK Hope, J Pratten. Susceptibility of *Streptococcus mutans* biofilms to photodynamic therapy: an *in vitro* study. J Antimicrob Chemother. 2005;56(2):324-330.

35. CR Fontana, AD Abernethy, D Som, K Ruggiero, S Doucette, RC Marcantonio, CI Boussios, R Kent, JM Goodson, ACR Tanner, NS Soukos. The antibacterial effect of photodynamic therapy in dental plaque-derived biofilms. J Periodontal Res. 2009;44(6):751-759.

36. D Steinberg, I Heling, I Daniel, I Ginsburg. Antibacterial synergistic effect of chlorhexidine and hydrogen peroxide against *Streptococcus sobrinus*, Streptococcus faecalis and *Staphylococcus aureus*. J Oral Rehab. 1999;26(2):151-156.

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