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Effect of photoactivated disinfection using light in the blue spectrum

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ABSTRACT

Background: Light in the blue spectrum is well known to cure composites. This in vitro-study was aimed to analyze a potential antimicrobial activity when combined with riboflavin as a photosensitizer.
Methods: Photoactivated disinfection (PAD) using a LED lamp emitting in the blue spectrum for 30 s (PAD30) and 60 s (PAD60) after application of 0.1% riboflavin was compared with a LED lamp emitting in the red spectrum with the respective photosensitizer (PADred). Killing activity was analyzed against planktonic 14 single species and a 12-species mixture with and without 25% serum. In addition, there was a reduction of viable bacterial counts in single species and a 12-species biofilm was measured after PAD.
Results: Gram-positive bacteria were less sensitive to PAD30 and PAD60 than Gram-negatives. PAD60 decreased the counts by more than 3 $\log_{10}$ cfu in two of five Gram-positive and in six of nine Gram-negative strains, the total viable counts of the mixture were reduced by 1.04 ± 0.46 $\log_{10}$ cfu. In the presence of 25% serum a decrease by more than 2 $\log_{10}$ cfu was only found in tests with one *Porphyromonas gingivalis* strain. PADred killed all included strains except for the 12-species mixture and *Eubacterium nodatum*. PAD60 reduced the counts in *P. gingivalis* biofilms by 2–3 $\log_{10}$ cfu, however there was no activity of PAD60 and PADred on the multi-species biofilm.
Conclusions: PAD using LED emitting in the blue spectrum combined with riboflavin is active against periodontopathogenic microbial species but clearly inferior to PADred. Multi-species biofilms are not sensitive to PAD using LED.

1. Introduction

Tooth loss is mostly associated with plaque-related diseases, such as caries and periodontitis. Oral microbial-plaque communities are biofilms composed of numerous bacteria on host surfaces. Streptococci and actinomycetes are the major initial colonizers. Fusobacteria play a central role as bridges that promote coaggregation to anaerobic bacteria [1]. Periodontal disease status impacts markedly on biofilm composition [2]. It is generally accepted that a small group of predominantly Gram-negative anaerobic or microaerophilic bacteria, e.g., *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Filifactor alocis* and *Treponema denticola* [3,4] are associated with initiation and progression of periodontitis. Recently *P. gingivalis* was described as a keystone pathogen being able to change a symbiotic microbiota into a dysbiotic one [5]. Moreover, other species such as *Campylobacter rectus*, *Eubacterium nodatum*, *Fusobacterium nucleatum*, *Prevotella intermedia*, and *Parvimonas micra* might contribute to the pathogenesis of disease [3,6].

Based on the impact of certain microbes, antimicrobial efficacy is an important component in any periodontitis treatment regimen. In preventing recolonization of bacteria, chlorhexidine digluconate is a widely used agent in periodontitis treatment [7,8]. Several systematic reviews provide some evidence for amoxicillin and metronidazole as adjuncts to scaling and root planing in nonsurgical therapy of chronic periodontitis [9,10]. However, for preventing development of resistance any unnecessary use of antibiotics should be avoided [11]. Light-activated killing might be a promising approach to overcome the problems related to the use of antibiotics

[12]. In photodynamic therapy, singlet oxygen and other reactive oxygen species

being highly toxic to microorganisms are generated through the activation of photosensitizers by light [13]. Reactive oxygen species may interfere with immune response, e.g., hydrogen peroxide induces synthesis of interleukin-8 in PDL cells [14]. Light sources in antimicrobial photodynamic therapy are mainly diode lasers [13] or light-emitting diodes (LED) [15,16].

In a recently published in vitro study we determined the effect of photoactivated disinfection (PAD) using a LED in the red spectrum on both, planktonic microorganisms and periodontopathogenic biofilm,

Abbreviations: con, control; LED, light-emitting diodes; PAD, photoactivated disinfection; PAD30, photoactivating disinfection using 30 s of LED light in the blue spectrum after application of riboflavin; PAD60, photoactivating disinfection using 60 s of LED light in the blue spectrum after application of riboflavin; PADred, photoactivating disinfection using 60 s of LED light in the red spectrum after application of toluidine blue; PS, photosensitizer; TBO, toluidine blue.

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which are involved in the pathogenesis of periodontitis. The findings of that study indicated that PAD using that LED was active against periodontopathogenic microbial species even in the presence of serum and against single-species biofilms [16].

This study considered a photosensitizer which is not intensively colored combined with its relevant wavelength. The relevant wavelength is in the blue spectrum and can be used for polymerization of resins to be activated by light. The hypothesis was that photoactivated disinfection using a light-emitting diode in the blue spectrum and 0.1% riboflavin as a photosensitizer is active against oral periodontopathogenic microorganisms, also in a biofilm.

2. Materials and Methods

2.1. Device and Photosensitizer

An LED lamp emitting in the blue spectrum with a power peak at 460 nm ± 10 nm (effect approx. 1 W, so 2 W/cm^2 in intensity; FotoSan460; CMS Dental ApS, Copenhagen, Denmark) was used in the experiments. Thin tips (ENDO) were attached to the LED lamp. The time of exposure was 30 s and 60 s (2 × 30 s). The photosensitizer (PS) was Fotosan blue agent (0.1% riboflavin). When applying light all tips touched the bacterial suspensions' surfaces. For comparison, the recently investigated LED lamp emitting in the red spectrum was used with the respective photosensitizer (0.1% toluidine blue).

2.2. Microorganisms

Fourteen microbial strains were tested as single species (Table 1). Additionally a multiple species mixture consisting of 12 bacterial strains was prepared (Table 1).

In the assays with serum *F. nucleatum* ATCC 25586, *P. gingivalis* ATCC 33277, *T. forsythia* ATCC 43037, *A. actinomycetemcomitans* J7, *E. corrodens* ATCC 23834, and the multiple species mixture were examined. *P. gingivalis* ATCC 33277 and M5-1-2 as well as *A. actinomycetemcomitans* Y4 and J7 were used to establish single-species biofilms. Further a biofilm consisting of the 12 different species was formed.

All strains were precultivated 42 h before the experiments in the appropriate atmosphere. Modified tryptic soy agar [17] was used as cultivation media. Thereafter, microbes were suspended in 0.9% (***w***/***v***) NaCl, washed once, mixed by repeated vortexing and adjusted to the 10^8 bacteria/ml.

2.3. Assessment of the Activity of Photoactivated Disinfection

Experiments were conducted similarly as described recently [16]. Defined inoculates of microorganisms (10^6 in 10 μl NaCl 0.9% each) were given into 1.5 ml tubes of dark color. After short centrifugation, 25 μl of photosensitizer was applied for 1 min and then exposed to the LED light for 30 s (PAD30) or 60 s (PAD60), respectively. Controls were 25 μl NaCl 0.9% solution without exposure to light (negative control — con), 25 μl NaCl 0.9% solution followed by 60 s of light exposure (light control — con LED) and 25 μl of PS without light exposure (con PS) as well as photoactivating disinfection using 60 s of LED light in the red spectrum after application of toluidine blue (PADred). The numbers of colony forming units (cfu) were determined after addition of 1 ml of NaCl 0.9% solution.

Table 1
Tested microbial strains and mixtures.

#	Microbial strains tested as single strains	Microbial strains of the mixture
1	*Actinomyces naeslundii* ATCC 12104	*A. naeslundii* ATCC 12104
2	*Aggregatibacter actinomycetemcomitans* Y4	*A. actinomycetemcomitans* Y4
3	*A. actinomycetemcomitans* J7	
4	*Campylobacter rectus* ATCC 33238	*C. rectus* ATCC 33238
5	*Eikenella corrodens* ATCC 23834	*E. corrodens* ATCC 23834
6	*Eubacterium nodatum* ATCC 33099	
7	*Filifactor alocis* ATCC 35896	*F. alocis* ATCC 35896
8	*Fusobacterium nucleatum* ATCC 25586	*F. nucleatum* ATCC 25586
9	*Parvimonas micra* ATCC 33270	*P. micra* ATCC 33270
10	*Porphyromonas gingivalis* ATCC 33277	*P. gingivalis* ATCC 33277
11	*P. gingivalis* M5-1-2	
12	*Prevotella intermedia* ATCC 25611	*P. intermedia* ATCC 25611
13	*Streptococcus gordonii* ATCC 10558	*S. gordonii* ATCC 10558
14	*Tannerella forsythia* ATCC 43037	*T. forsythia* ATCC 43037
15		*Treponema denticola* ATCC 35405

2.4. Assessment of the Activity of Photoactivated Disinfection in the Presence of Serum

The experiments were repeated for selected species in the presence of 25% serum (final concentration). The human serum was purchased from Sigma-Aldrich (St. Louis, MO, USA). To inactivate the complement, the serum was heated for 30 min at 60 °C. Serum was added in a final concentration of 25%. All other steps of the experiments were made as described above.

2.5. Influence of Photoactivated Disinfection on Interaction of Monocytic Cells With Bacteria

Monocytic THP-1 cells were used for these experiments. The cells were maintained in RPMI 1640 medium (Invitrogen Corporation, Carlsbad, CA, U.S.A.) supplemented with 10% fetal bovine serum (Invitrogen Corporation). The THP-1 cells were washed once with RPMI 1640 and adjusted to 10^6 cells/ml before exposing to photoactivated disinfection. Bacteria (*A. actinomycetemcomitans* J7, *P. gingivalis* ATCC 33277, 12-species mixture) were prepared as described before and in part exposed to photoactivated disinfection, too. Both systems (light in the blue spectrum combined with riboflavin and light in the red spectrum combined with toluidine blue) were applied. Finally, 5 × 10^3 THP-1 cells were mixed with 10^5 bacteria and incubated for 30 min at 37 °C with 5% CO_2 before determining cfu counts.

2.6. Biofilm Assays

96-well-plates were covered with 10 μl/well 25% serum in NaCl 0.9% solution for 1 h. Suspensions of bacterial strains were made and mixed with nutrient broth. Each 200 μl was transferred per well. The plates were incubated in appropriate conditions (*A. actinomycetemcomitans* biofilms with 5% CO_2, all other biofilms with anaerobic conditions) for 48 h. Then the medium was exchanged. In the case of the multispecies-biofilm *P. gingivalis* ATCC 33277, *T. forsythia* ATCC 43037 and *T. denticola* ATCC 35406 were added again. After an additional incubation of 48 h, the medium was removed and the biofilms were exposed to treatment with 50 μl PS, LED, PAD30, PAD60, and PAD red. Finally 250 μl 0.9 NaCl was added to the biofilms. Those were removed from the bottom by scraping, mixing by pipetting and additional vortexing before a serial dilution was made and the total cfu counts and the counts for *P. gingivalis*/well were determined.

2.7. Statistical Analysis

A bactericidal activity is defined by a reduction of 99.9%, meaning 3 $\log_{10}$ cfu. Following, any statistical analysis is of limited value. The analysis was made by using one way ANOVA test followed by LSD analysis. The influence of the presence of serum within one species (mixture) was determined by using the Student's t-test for independent samples. In all statistical analyses $\log_{10}$ cfu values were used. At least four independent results were evaluated in each experiment.