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Hypericin- and mTHPC-mediated photodynamic therapy for the treatment of cariogenic bacteria

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Abstract

Objective: Dental caries is one of the most common diseases in Western countries. Its pathoetiology is multifactorial, however, bacteria including *Streptococcus mutans* and the closely related *Streptococcus sobrinus* are regarded as key factors involved in this process. The fact that therapeutic approaches to eradicate these microorganisms are still limited prompted us to investigate the treatment potential of photodynamic therapy with the photoactive compounds hypericin (HYP) and meso-tetra(hydroxyphenyl)chlorin (mTHPC) *in-vitro*.

Material and methods: *S. mutans* and *S. sobrinus* were cultivated under standard conditions and incubated with HYP (Invitrogen, Basel, Switzerland), the liposomal mTHPC derivative Foslipos (FOS, Biolitec, Jena, Germany) or a mixture of both at concentrations ranging between 0.625 and 10 μg/ml for various time points. Following a thorough washing step, bacteria were irradiated with a dental polymerization instrument (400-505 nm). All samples were subjected to serial dilutions and spiral plating on blood agar plates. Viable colony counts

were determined after 48 h in culture. Photosensitizer fluorescence of bacteria was visualized by confocal microscopic techniques.
Results: One hundred percent of *S. sobrinus* could be killed by a 15 min incubation with down to 2.5 µg/ml HYP, 5 µg/ml FOS or a mixture of 1.25 µg/ml of each photosensitizer followed by light activation of 120 s. In contrast to *S. sobrinus*, *S. mutans* displayed a significant dark toxicity for FOS (10–1.25 µg/ml) and no relevant PDT effects using HYP (10–0.625 µg/ml) under these conditions. HYP-mediated PDT effects (10 µg/ml) could be enhanced to more than 99.9% by prolonging photosensitizer incubation to 30 min and fractional illumination (2 x 120 s). Complete eradication of *S. mutans* was achieved by incubation for 15 min with a mixture of 0.625 µg/ml each FOS and HYP and illumination for 120 s.
Conclusion: For both *S. mutans* and *S. sobrinus* short PDT protocols with FOS and/or HYP could be established that completely eradicated these cariogenic bacteria in suspension. Our study, however, indicated that careful optimization of PDT conditions may be necessary for successful treatment even of closely related bacterial species. In multispecies microbial populations the application of photosensitizer combinations for PDT may be useful.

Keywords: Photodynamic therapy; Bacteria; mTHPC; Hypericin; Gram-positive

Introduction

The oral cavity harbors a plenitude of microorganisms that together constitute the normal flora. However, under pathologic conditions this complex system may be disturbed, eventually contributing to the etiology of various inflammatory diseases that may even spread outside the *cavitas oralis* [1].
The most prevalent of microorganism-related oral diseases is caries. Among the different pathogens involved, the contribution of the Gram-positive facultative anaerobic bacteria *Streptococcus mutans* and the related *Streptococcus sobrinus* are thought to play key roles for the progression of this condition [2].
It is well-known that appropriate oral hygiene strongly reduces the risk for caries, however, the current number of affected patients is considerable, creating substantial health care costs [3]. In most cases, caries treatment consists of debridement of the oral plaque (i.e. the biofilm where the cariogenic species reside within an extracellular polymer matrix) or excavation, disinfection and sealing of the infected dentin. The limitations of these non-specific and

invasive treatment options recently rekindled the interest in a method whose basic effects on microorganisms are known since over 100 years: photodynamic therapy (PDT) [4]. Briefly, PDT is a non or minimally invasive treatment method relying on the ability of a photoactive non-toxic drug (the so-called photosensitizer) to rapidly generate upon activation with light free radicals or singlet oxygen that can oxidize cellular constituents and eventually lead to cell death [5].

In the past decades, numerous studies have underlined the potential of PDT to destroy bacteria, protozoa, fungi and viruses [6]. Although published data on PDT for oral pathogens are comparably limited and most of the studies had been performed *in vitro*, it had been proposed that PDT either alone or in combination with other strategies may be a useful option to treat pathogens involved in periodontitis [7], peri-implantitis [8], endodontitis [9] and caries [10].

Of the broad spectrum of photosensitizers available today, several had been successfully applied for PDT on the cariogenic bacteria *S. mutans* and *S. sobrinus*. These included tricyclic dyes [11–20] or tetrapyrroles belonging to the classes of porphyrines [16,20], chlorins [21], and phthalocyanines [22,23]. However, to the best of our knowledge, neither meso-tetra(hydroxyphenyl)chlorin (mTHPC) nor hypericin (HYP) had been tested for treatment of *S. mutans* or *S. sobrinus* by PDT.

mTHPC is currently regarded as one of the most potent second generation photosensitizers. While the vast majority of reports concern its high efficiency to destroy cancer cells [24], effects on bacteria are anecdotal and only include successful treatment of methicillin-resistant *Staphylococcus aureus* with a liposomal mTHPC formulation [25].

Hypericin is a phenanthroperylene quinine pigment naturally occuring in plants such as St. John's Wort (Hypericum species). It is currently among the most popular drugs against mild depression in complementary medicine. In addition to its potent photo-damaging effects on cancer cells [26], several studies indicated that HYP is active against various Gram-positive bacteria [27–29].

With the aim to gain more insight into the potential and effects of new photosensitizers for clinical applications, in the present study, we investigated for the first time the antimicrobial effects of mTHPC and HYP alone or in combination in PDT on the cariogenic Gram-positive bacteria *S. mutans* and *S. sobrinus*.

Materials and Methods

Bacterial culture

S. mutans (OMZ 918) and *S. sobrinus* (OMZ 176) were obtained from the culture collection of the Institute of Oral Biology, University of Zurich, Switzerland and routinely grown aerobically over night at 37°C in fluid universal medium (FUM) as described by Gmür and Guggenheim [30] until late log phase.

Photosensitizers

Foslipos (FOS), a novel liposomal uncharged mTHPC compound was kindly donated by Biolitec, Jena, Germany. A stock solution (1.5 mg/ml) of FOS was prepared in water. HYP was purchased from Invitrogen, Basel, Switzerland and dissolved as a stock at 1 mg/ml in ethanol. All photosensitizer stock solutions were kept at 4°C in the dark and further diluted in FUM.

Confocal microscopy

After incubations of bacteria in the dark with FOS or HYP (5 µg/ml) for 5, 15 or 30 min respectively, all samples had been fixed by drying on microscopic slides and mounted in gycergel (Dako, Glostrup, Denmark). Analyses were performed with a confocal laser scanning microscope (TCS SP2 and SP5, Leica, Wetzlar, Germany).

PDT protocols

Planktonic bacteria were centrifuged and FUM was replaced by FOS (0.625–10 µg/ml), HYP (0.625–10 µg/ml) or a 1:1 mixture of both (0.625–10 µg/ml each). Bacteria were incubated for 1–30 min at 37°C in the dark, followed by two thorough washing steps in physiological NaCl solution. Thereafter samples were exposed at a 3 cm distance to culture surface to visible light from a halogen polymerization device (Optilux500, KerrHawe, Bioggio, Switzerland) for 120 s. According to the specifications of the manufacturer, this lamp has a wavelength range of 400-505 nm and an intensity of 1070 mW/cm^2. The light source had an illuminance of 40,000 lx at the surface and of 21,400 lx at the bottom of the incubation tube. Additional experiments were performed as a sequential light application for two times 120 s, with a 30 min vortex step in between.

Numbers of viable colony forming units (CFU) were obtained after 48 h from serial dilutions of all samples in FUM and spiral plating on Columbia blood agar base (Becton, Dickinson

and Co., Le Pont de Claix, France) supplemented with 5% hemolyzed human blood. Controls included the following: bacteria received (1) no treatment (neither light nor photosensitizer), (2) only photosensitizers, but no light ("dark toxicity") and (3) no photosensitizer, but light.

Data analyses and statistics

All experiments were at least performed in triplicate. Data are presented as means with standard deviations of the mean. Data were normalized against untreated controls (set to 100%) and expressed as a percentage surviving or killed bacteria of these untreated controls. The t-tests were regarded as significant with p-values ≤ 0.05.

Results

Streptococcus sobrinus

Microscopic analyses showed that incubations with FOS and HYP lead to a pronounced fluorescent signal at *S. sobrinus* already after 5 min. The fluorescence intensity was further increased after 15 min but did not appear to be considerably stronger after 30 min incubation (Fig. 1).

Controls indicated that growth of untreated *S. sobrinus* was not impaired by irradiation with the dental polymerization tool. Furthermore, when compared to untreated controls, no significant dark toxic effects were detected after incubations with FOS or HYP (0.625–10 µg/ml) without light activation. In untreated controls an average of 2.16E+8 CFU/ml was obtained.

When irradiated after incubation with photosensitizers, a strong PDT effect was elicited, resulting in 100% bacterial death at concentrations between 10 and 2.5 µg/ml HYP or 10 and 5 µg/ml FOS, when incubated for 15 min and light activated for 120 s. A reduced bacterial count of ≤ E+05 was still observed down to 1.25 µg/ml FOS or HYP, respectively (Fig. 2). Generally, PDT with FOS resulted in higher CFU counts (in the order of 10-fold) compared to PDT with HYP. This observation was most apparent at low photosensitizer concentrations and is exemplified in Fig. 2.

Application of MIX for 15 min and illumination for 120 s revealed that *S. sobrinus* could be 100% eradicated using concentrations between 5 and 1.25 µg/ml of each of the photosensitizers, while the lower concentrated mixture (each photosensitizer 0.625 µg/ml) led only to a 1.53E+02 fold reduction of CFU counts (Fig. 3). In comparative analyses of treatment effects with single photosensitizers we found that the bactericidal activity of the

MIX with 1.25 µg/ml of each FOS and HYP was the same as with 2.5 µg/ml HYP alone (Fig. 2). Neither PDT with FOS nor HYP at 1.25 µg/ml led to a complete destruction of *S. sobrinus* (99.988% and 99.998%, respectively).

Streptococcus mutans

In *S. mutans*, we observed a fluorescence pattern in confocal microscopy that was comparable to that in *S. sobrinus* with a maximum signal being reached after 15 min incubation with either photosensitizer (Fig. 4). Controls consisting of illumination only indicated no light sensitivity of *S. mutans*. In this bacterial strain, untreated controls reached a mean 4.16E+07 CFU/ml.

Studies dealing with possible dark toxic effects revealed that treatment with FOS between 0.625 and 10 µg/ml was already effective in killing 97.500–99.999% of bacteria without light activation (Fig. 5). Additional treatment with light further improved destruction of bacteria at all FOS concentrations investigated, ranging from 100% at concentrations between 10 and 1.25 µg/ml to 99.997% at the lowest concentration tested (0.625 µg/ml) (Fig. 5).

In contrast to FOS, not even the highest concentrations of HYP displayed a toxicity in the dark (mean bacterial survival rate of 88.923% at 10 µg/ml HYP compared to controls). However, when bacteria were irradiated (120 s) after incubations with HYP bacterial survival rates still came to high values. Even PDT with 10 µg/ml HYP only resulted in a E+02 reduction in *S. mutans,* while with low concentrations (1.25 µg/ml or 0.625 µg/ml) 65.965% or 90.894%, respectively, of bacteria survived (Fig. 6). Using the highest concentration of HYP (10 µg/ml) in combination with a prolongation of photosensitizer incubation time to 30 min and sequential light application of 2 x 120 s, a 99.988 % destruction of bacteria could be achieved (Fig 6).

Generally, the 1:1 mixture of photosensitizers displayed the same dark toxicity as the respective concentrations of FOS alone. To obtain informations about PDT using the MIX we focused only on 0.625 µg/ml of each photosensitizer, since this concentration showed a significant PDT effect for FOS when compared to its dark toxicity ($p=0.0063$). While we found that under the above conditions PDT with the MIX completely (100%) eradicated *S. mutans*, PDT with neither FOS nor HYP alone at concentrations of 0.625 µg/ml was able to do so (survival rate: 0.002% and 90.89%, respectively; Fig. 7). The same 100% effect could be obtained by PDT with 1.25 µg/ml FOS (Fig. 7).

Discussion

PDT is characterized by features that make this treatment modality especially attractive to combat microbial pathogens. These include its efficacy on antibiotic-resistant strains, its broad target spectrum as well as its very low potential for mutagenicity and photoresistance [6]. However, of the large spectrum of photoactive agents available to date only a limited number had been explored so far as regards to their antimicrobial applicability.

To the best of our knowledge, our study is the first to investigate bactericidal PDT effects of mTHPC (FOS) or HYP on *S. sobrinus* and *S. mutans*. However, PDT data from previous studies in *mutans streptococci* are available for a few other photosensitizers, including toluidine blue O [11,12,14,17,18,20], methylene blue [16,19], erythrosine [15,16], hematoporphyrin derivative [16,20], native and lysine-conjugated chlorin e6 [21], aluminium disulphonated or cationic Zn(II) phthalocyanine [22,23], and Rose Bengal [13]. Our results as well as the vast majority of the above published data underlined the usefulness of PDT to destroy *mutans streptococci*, supporting the general observation that Gram-positive bacteria are susceptible to inactivation by photodynamic procedures [31]. Since many experimental parameters such as cell culture conditions, photosensitizer concentrations and their incubation times, light sources, illumination intensities and times considerably differ between studies and our setup, a comparison of experiments is only of limited value.

However, when analyzing data or establishing protocols, it should be kept in mind that with respect to the extremely fast growth of *mutans streptococci*, PDT killing rates of bacteria may be statistically significant but clinically insufficient. Our study shows that short PDT protocols with FOS and HYP can be established that have the capacity to completely kill these bacteria in suspension. Among all previously published studies, only two also used planktonic cultures (*S. mutans*) as models, showing that PDT protocols with either 0.5 μg/ml Rose Bengal or 100 μg/ml toluidine blue O resulted in a complete bacterial eradication [13,18]. All PDT reports dealing with single- or multispecies oral biofilms (with *mutans streptococci*) were only able to reduce viable bacterial counts [14–17,19,20,23]. It is well-known that pathogens organized in biofilms are more resistant towards antimicrobial modalities as compared to planktonic cultures. This may also hold true for our PDT protocols, since preliminary studies with *mutans streptococci* grown in sucrose-supplemented media also displayed a reduced bactericidal efficiency [Besic Gyenge et al., in preparation].

As light source, in our study, we used a halogen hand-held unit routinely applied for polymerization of light-cured dental materials. Successful elimination of bacteria in our setting indicated that it may be an interesting alternative to the commonly used lasers;

especially since the polymerization tool is safer, cheaper and readily available in most dentist's offices. While for cancer PDT light sources with longer wavelengths are preferred because of deeper penetration needs, the superficial sites of cariogenic bacteria are easily accessible with our device. Its wavelength range (400-505 nm) is perfectly suitable for activation of FOS, since the Soret band of this photosensitizer is at 420 nm [32]. The spectrum of HYP contains a very broad absorption band near 460 nm but, notably, main peaks are outside the wavelength range of our lamp [33]. Our study is not the first using non-laser light for PDT [34] and previously, another brand of hand-held photopolymerizer had been successfully applied for activation of Rose Bengal in *S. mutans* [13].

Both *S. mutans* and *S. sobrinus* are traditionally grouped in a common taxonomic cluster – the mutans group – based on shared phenotypic reactions. The strongly different experimental sensitivity of the two *streptococcus* species observed in our study was therefore rather unexpected and question the close relationship. Our data support earlier molecular phylogenetic analyses of glucan-producing enzymes and superoxide dismutases indicating that the two bacteria are in fact genetically separate [35–37]. From *in-vitro* cancer studies – including our own (unpublished data) – the different behavior of photosensitizers in related types of tumor cells is actually well-known, underlining the complexity of biomechanisms initiated by PDT.

Basically, the observed differences in sensitivity concerned two parameters: (1) high levels of dark toxicity of FOS in *S. mutans* vs. *S. sobrinus* and (2) ineffectiveness of HYP-mediated PDT in *S. mutans* – at least under the conditions effective in *S. sobrinus*.

Currently, no comparable data of our mTHPC formulation are available for other microorganisms. In ongoing studies we are thus investigating as to whether *S. mutans* has an extremely high tolerance or *S. sobrinus* is extraordinarily sensitive towards this compound. However, we propose that the observed dark toxic effects of FOS should not impose any clinical treatment consequences. In contrast, inefficiency of HYP-mediated PDT in *S. mutans* under the same experimental conditions as in *S. sobrinus* would necessitate the development of protocol modifications. Since our microscopic analyses indicated that the fluoresence signal of HYP was morphologically not different from that of *S. sobrinus* we conclude that lack of adherence and/or uptake was not the underlying problem. Details of these processes, however, have not been investigated here. Notably, the light source used in our setting had a sub-optimal wavelength range for activation of HYP. The improvement of PDT effects by a fractionated illumination may indicate that the failure with our standard protocols is – at least in part – related to this factor. Our data are in line with earlier studies showing that HYP-

mediated PDT effects can be enhanced by sequential light application [38]. Another critical factor may be the known complexity of HYP biological effects [39]. These appear to involve an array of light-dependent and -independent mechanisms whose roles may vary under different circumstances. It had been e.g. reported that HYP activity may be strongly and specifically protein-dependent, resulting in either promotion or inhibition of PDT effects in the presence of certain highly related isoforms of glutathione S-transferases [40]. The HYP used in our study was a plant extract of 99% purity (according to the manufacturer's specification) and the remaining 1% unspecified herbal components may have further contributed to the observed differences of bioeffects. In none of our experiments, however, we observed increased growth rates after treatment with HYP as reported for *S. aureus* [41].

In our study we also investigated the consequences of a combined application of FOS and HYP for PDT. While this is the first report on *mutans streptococci*, combinatorial photosensitizer approaches have been investigated before in a limited number of studies. In *S. aureus*, the combination of HYP and mTHPC was reported to be counterproductive since bacterial growth was stimulated and PDT effects inhibited [41], while in contrast the combination of mTHPC and hematoporphyrin derivative resulted in a superior (additive) PDT effect in this species [42]. In human endometrial cancer cells, in a breast cancer patient as well in a mouse mammary cancer model photosensitizer combinations (5-aminolaevulinic acid/HYP and Photofrin II/meso-tetra-(4-sulfonatophenyl)-porphine, respectively) led to an enhanced phototoxicity [43,44]. To explore possible advantages of a FOS/HYP combination in our model system, we focused on concentrations for photosensitizers that – when given alone – were unable to completely kill *S. mutans* or *S. sobrinus*. With both bacteria investigated here, PDT after a short incubation with the MIX completely sterilized planktonic cultures, indicating the high efficiency of this treatment procedure. Of note, PDT with 1.25 µg/ml FOS in *S. mutans* or with 2.5 µg/ml HYP in *S. sobrinus* had the same effect 100% killing effect as the respective optimal MIX. Therefore, the effects of low-dose single photosensitizers was enhanced in the MIX, but no clear advantage towards the application of the total dose by a single photoactive compounds was evident in our setup. However, in many clinical settings, multispecies microbial communities have to be treated. Given a non-uniform response of these pathogens towards certain photosensitizing agents (as in our models of cariogenic bacteria), the application of mixtures may have distinct advantages by targeting a whole array of microorganisms in one treatment session.

Conclusions

Our study provides further support that PDT with second generation photosensitizers may be an effective treatment modality to eradicate cariogenic pathogens. Short protocols within less than 20 min treatment time may be feasible. The differential results on closely related bacterial species, however, indicates how little we actually understand the factors that govern PDT-generated cell death in bacteria. For the achievement of clinically relevant effects, great care must be taken to adjust PDT parameters depending on specific microoorganisms to be targeted. Future investigations of molecular and cellular events elicited by photosensitizer incubation and PDT may reveal whether reduced doses of photosensitizers applied in a combinatorial treatment will result in favorable clinical effects, e.g. with regards to immune responses *in vivo*. However, the strength of mixtures of photosensitizers for antimicrobial PDT may reside in their potential to target complex microbial populations such as in supragingival plaque.

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Zusammenfassung

Hypericin- und mTHPC-vermittelte photodynamische Therapie zur Behandlung kariogener Bakterien

Zielsetzung: Karies gehört zu den häufigsten Erkrankungen in den westlichen Ländern. Wenngleich vermutlich multiple Faktoren eine ursächliche Rolle spielen, so wird doch den verwandten Bakterien *Streptococcus mutans* und *Streptococcus sobrinus* eine Schlüsselrolle für die Pathoetiologie von Karies zugesprochen. Da geeignete antibakterielle Behandlungsmöglichkeiten zur Zeit fehlen, haben wir in der vorliegenden Arbeit das Potential von photodynamischer Therapie (PDT) mit den photoaktiven Substanzen Hypericin (HYP) und meso-Tetra(hydroxyphenyl)chlorin (mTHPC) *in vitro* untersucht.

Material und Methoden: *S. mutans* und *S. sobrinus* wurden routinemässig kultiviert und mit HYP (Invitrogen, Basel, Schweiz), dem liposomalen mTHPC-Derivativ Foslipos (FOS, Biolitec, Jena, Deutschland) oder einer Mischung aus beiden mit Konzentrationen von 0,625–10 µg/ml unterschiedlich lang inkubiert. Nach sorgfältigen Wasch-Schritten wurden die Bakterien mit einer zahnärztlichen Polymerisationslampe bei 400-505 nm bestrahlt. Die Proben wurden dann seriell verdünnt und auf Blut-Agar-Platten ausspiralisiert. Überlebende Kolonien wurden nach 48 h ausgezählt. Ausserdem wurde die Fluoreszenz der Photosensibilisatoren an den Bakterien mit einem Konfokalmikroskop untersucht.
Ergebnisse: *S. sobrinus* konnte zu 100% abgetötet werden durch eine 15-minütige Inkubation mit 2,5 µg/ml HYP, 5 µg/ml FOS oder einer Mischung von 1,25 µg/ml beider Photosensibilisatoren und anschliessender Lichtaktivierung für 120 s. Im Gegensatz zu *S. sobrinus* zeigte *S. mutans* unter diesen Bedingungen eine signifikante Dunkeltoxizität für FOS (10–1,25 µg/ml) und keine relevanten PDT-Effekte für HYP (10–0,625 µg/ml). HYP-bedingte PDT-Effekte (10 µg/ml) konnten aber durch Verlängerung der Inkubationszeit auf 30 min und eine fraktionierte Bestrahlung (2x120 s) auf mehr als 99.9% erhöht werden. Eine vollständige Eliminierung von *S. mutans* konnte durch eine 15-minütige Behandlung mit einer Mischung von je 0,625 µg/ml FOS und HYP und einer Belichtung von 120 s erzielt werden.
Schlussfolgerungen: Sowohl für *S. mutans* wie auch für *S. sobrinus* konnten kurze PDT-Protokolle mit FOS und/oder HYP entwickelt werden, welche zur vollständigen Abtötung dieser beiden kariogenen Keime in Suspension führten. Unsere Studie zeigte aber auch, dass die Behandlung verwandter Keime eine sorgfältige Optimierung der PDT-Bedingungen notwendig machen kann. Die Verwendung von Photosensibilisator-Kombinationen könnte für die Therapie von mikrobiellen Multispezies-Populationen hilfreich sein.

Schlüsselwörter: Photodynamische Therapie; Bakterien; mTHPC; Hypericin; Gram-positiv

References

[1] Parahitiyawa NB, Jin LJ, Leung WK, Yam WC, Samaranayake LP. Microbiology of odontogenic bacteremia: beyond endocarditis. Clin Microbiol Rev 2009;22(1):46-64.

[2] Takahashi N, Nyvad B. Caries ecology revisited: microbial dynamics and the caries process. Caries Res 2008;42(6):409-18.

[3] Bagramian RA, Garcia-Godoy F, Volpe AR. The global increase in dental caries. A pending public health crisis. Am J Dent 2009;22(1):3-8.

[4] Ackroyd R, Kelty C, Brown N, Reed M. The history of photodetection and photodynamic therapy. Photochem Photobiol 2001;74(5):656-69.

[5] Plaetzer K, Krammer B, Berlanda J, Berr F, Kiesslich T. Photophysics and photochemistry of photodynamic therapy: fundamental aspects. Lasers Med Sci 2009;24(2):259-68.

[6] Jori G, Fabris C, Soncin M, Ferro S, Coppellotti O, Dei D, et al. Photodynamic therapy in the treatment of microbial infections: basic principles and perspective applications. Lasers Surg Med 2006;38(5):468-81.

[7] Meisel P, Kocher T. Photodynamic therapy for periodontal diseases: state of the art. J Photochem Photobiol B 2005;79(2):159-70.

[8] Hayek RR, Araújo NS, Gioso MA, Ferreira J, Baptista-Sobrinho CA, Yamada AM, et al. Comparative study between the effects of photodynamic therapy and conventional therapy on microbial reduction in ligature-induced peri-implantitis in dogs. J Periodontol 2005;76(8):1275-81.

[9] Garcez AS, Nuñez SC, Hamblin MR, Ribeiro MS. Antimicrobial effects of photodynamic therapy on patients with necrotic pulps and periapical lesion. J Endod 2008;34(2):138-42.

[10] Walsh LJ. The current status of laser applications in dentistry. Aust Dent J 2003;48(3):146-55.

[11] Burns T, Wilson M, Pearson GJ. Sensitisation of cariogenic bacteria to killing by light from a helium-neon laser. J Med Microbiol 1993;38(6):401-5.

[12] Williams JA, Pearson GJ, Colles MJ, Wilson M. The photo-activated antibacterial action of toluidine blue O in a collagen matrix and in carious dentine. Caries Res 2004;38(6):530-6.

[13] Paulino TP, Ribeiro KF, Thedei G Jr, Tedesco AC, Ciancaglini P. Use of hand held photopolymerizer to photoinactivate Streptococcus mutans. Arch Oral Biol 2005; 50(3):353-9.

[14] Zanin IC, Gonçalves RB, Junior AB, Hope CK, Pratten J. Susceptibility of Streptococcus mutans biofilms to photodynamic therapy: an in vitro study. J Antimicrob Chemother 2005;56(2):324-30.

[15] Metcalf D, Robinson C, Devine D, Wood S. Enhancement of erythrosine-mediated photodynamic therapy of Streptococcus mutans biofilms by light fractionation. J Antimicrob Chemother 2006;58(1):190-2.

[16] Wood S, Metcalf D, Devine D, Robinson C. Erythrosine is a potential photosensitizer for the photodynamic therapy of oral plaque biofilms. J Antimicrob Chemother 2006;57(4):680-4.

[17] Zanin IC, Lobo MM, Rodrigues LK, Pimenta LA, Höfling JF, Gonçalves RB. Photosensitization of in vitro biofilms by toluidine blue O combined with a light-emitting diode. Eur J Oral Sci 2006;114(1):64-9.

[18] Bevilacqua IM, Nicolau RA, Khouri S, Brugnera A Jr, Teodoro GR, Zângaro RA, et al. The impact of photodynamic therapy on the viability of Streptococcus mutans in a planktonic culture. Photomed Laser Surg 2007;25(6):513-8.

[19] Müller P, Guggenheim B, Schmidlin PR. Efficacy of gasiform ozone and photodynamic therapy on a multispecies oral biofilm in vitro. Eur J Oral Sci 2007; 115(1):77-80.

[20] Giusti JS, Santos-Pinto L, Pizzolito AC, Helmerson K, Carvalho-Filho E, Kurachi C, et al. Antimicrobial photodynamic action on dentin using a light-emitting diode light source. Photomed Laser Surg 2008;26(4):281-7.

[21] Rovaldi CR, Pievsky A, Sole NA, Friden PM, Rothstein DM, Spacciapoli P. Photoactive porphyrin derivative with broad-spectrum activity against oral pathogens in vitro. Antimicrob Agents Chemother 2000;44(12):3364-7.

[22] Burns T, Wilson M, Pearson GJ. Killing of cariogenic bacteria by light from a gallium aluminium arsenide diode laser. J Dent 1994;22(5):273-8.

[23] Wood S, Nattress B, Kirkham J, Shore R, Brookes S, Griffiths J, et al. An in vitro study of the use of photodynamic therapy for the treatment of natural oral plaque biofilms formed in vivo. J Photochem Photobiol B 1999;50(1):1-7.

[24] Kvaal SI, Warloe T. Photodynamic treatment of oral lesions. J Environ Pathol Toxicol Oncol 2007;26(2):127-33.

[25] Bombelli C, Bordi F, Ferro S, Giansanti L, Jori G, Mancini G, et al. New cationic liposomes as vehicles of m-tetrahydroxyphenylchlorin in photodynamic therapy of infectious diseases. Mol Pharm 2008;5(4):672-9.

[26] Agostinis P, Vantieghem A, Merlevede W, de Witte PA. Hypericin in cancer treatment: more light on the way. Int J Biochem Cell Biol 2002;34(3):221-41.

[27] Avato P, Raffo F, Guglielmi G, Vitali C, Rosato A. Extracts from St John's Wort and their antimicrobial activity. Phytother Res 2004;18(3):230-2.

[28] Conforti F, Statti GA, Tundis R, Bianchi A, Agrimonti C, Sacchetti G, et al. Comparative chemical composition and variability of biological activity of methanolic extracts from Hypericum perforatum L. Nat Prod Res 2005;19(3):295-303.

[29] Cecchini C, Cresci A, Coman MM, Ricciutelli M, Sagratini G, Vittori S, et al. Antimicrobial activity of seven hypericum entities from central Italy. Planta Med 2007;73(6):564-6.

[30] Gmür R, Guggenheim B. Antigenic heterogeneity of Bacteroides intermedius as recognized by monoclonal antibodies. Infect Immun 1983;42(2):459-70.

[31] Musk DJ Jr, Hergenrother PJ. Chemical countermeasures for the control of bacterial biofilms: effective compounds and promising targets. Curr Med Chem 2006; 13(18):2163-77.

[32] Kachatkou D, Sasnouski S, Zorin V, Zorina T, D'Hallewin MA, Guillemin F, et al. Unusual photoinduced response of mTHPC liposomal formulation (Foslip). Photochem Photobiol 2009;85(3):719-24.

[33] Weitman H, Roslaniec M, Frimer AA, Afri M, Freeman D, Mazur Y, et al. Solvato-chromic effects in the electronic absorption and nuclear magnetic resonance spectra of hypericin in organic solvents and in lipid bilayers. Photochem Photobiol 2001;73(2):110-8.

[34] Brancaleon L, Moseley H. Laser and non-laser light sources for photodynamic therapy. Lasers Med Sci 2002;17(3):173-86.

[35] Shiroza T, Ueda S, Kuramitsu HK. Sequence analysis of the gtfB gene from Streptococcus mutans. J Bacteriol 1987;169(9):4263-70.

[36] Abo H, Matsumura T, Kodama T, Ohta H, Fukui K, Kato K, et al. Peptide sequences for sucrose splitting and glucan binding within Streptococcus sobrinus glucosyl-transferase (water-insoluble glucan synthetase). J Bacteriol 1991;173(3):989-96.

[37] Poyart C, Quesne G, Coulon S, Berche P, Trieu-Cuot P. Identification of streptococci to species level by sequencing the gene encoding the manganese-dependent superoxide dismutase. J Clin Microbiol 1998;36(1):41-7.

[38] Saw CL, Heng PW, Olivo M. Potentiation of the photodynamic action of hypericin. J Environ Pathol Toxicol Oncol 2008;27(1):23-33.

[39] Kober M, Pohl K, Efferth T. Molecular mechanisms underlying St. John's wort drug interactions. Curr Drug Metab 2008;9(10):1027-37.

[40] Lu WD, Atkins WM. A novel antioxidant role for ligandin behavior of glutathione S-transferases: attenuation of the photodynamic effects of hypericin. Biochemistry 2004;43(40):12761-9.

[41] Kubin A, Wierrani F, Jindra RH, Loew HG, Grünberger W, Ebermann R, et al. Antagonistic effects of combination photosensitization by hypericin, meso-tetra-hydroxyphenylchlorin (mTHPC) and photofrin II on Staphylococcus aureus. Drugs Exp Clin Res 1999;25(1):13-21.

[42] Wierrani F. Experimental investigations and clinical use of photodynamic therapy (PDT) in the Rudolf Foundation Hospital. Gynakol Geburtshilfliche Rundsch 1999; 39(4):217-25.

[43] Nelson JS, Liaw LH, Lahlum RA, Cooper PL, Berns MW. Use of multiple photo-sensitizers and wavelengths during photodynamic therapy: a new approach to enhance tumor eradication. J Natl Cancer Inst 1990;82(10):868-73.

[44] Schneider-Yin X, Kurmanaviciene A, Roth M, Roos M, Fedier A, Minder EI, et al. Hypericin and 5-aminolevulinic acid-induced protoporphyrin IX induce enhanced phototoxicity in human endometrial cancer cells with non-coherent white light. Photodiagnosis Photodyn Ther 2009;6(1):12-8.

Figure legends

Fig. 1. *Streptococcus sobrinus* after incubation with Foslipos (a-c) and hypericin (d-f) in the dark. Confocal microscopy was performed after incubation times of 5 min (a, d), 15 min (b, e) and 30 min (c, f). Images were acquired with a x63, 1.4 numerical aperture oil immersion objective lens providing an optical section thickness of 200 nm.

Fig. 2. Photodynamic effects in *Streptococcus sobrinus*. Photosensitizers (Foslipos and/or hypericin) at the concentrations indicated had been incubated for 15 min and light (400-505 nm) was applied for 120 s.

Fig. 3. Photodynamic effects in *Streptococcus sobrinus* after 15 min incubation with varying concentrations of a 1:1 mixture of the photosensitizers Foslipos and hypericin and illumination (400-505 nm) for 120 s.

Fig. 4. *Streptococcus mutans* after incubation with Foslipos (a-c) and hypericin (d-f) in the dark. Confocal microscopy was performed after incubation times of 5 min (a, d), 15 min (b, e) and 30 min (c, f). Images were acquired with a x63, 1.4 numerical aperture oil immersion objective lens providing an optical section thickness of 200 nm.

Fig. 5. Incubation of *Streptococcus mutans* with varying concentrations of Foslipos for 15 min with consecutive light activation (120 s) or without light activation.

Fig. 6. Effects of hypericin incubated for either 15 min or 30 min in *Streptococcus mutans.* Bacterial cultures had been either kept in the dark (no light), or illuminated for 15 min (1 x light) and two times 15 min with an intermittant vortex step of 30 s (2 x light).

Fig. 7. Photodynamic effects in *Streptococcus mutans*. Photosensitizers (Foslipos and/or hypericin) at the concentrations indicated had been incubated for 15 min and light (400-505 nm) was applied for 120 s.

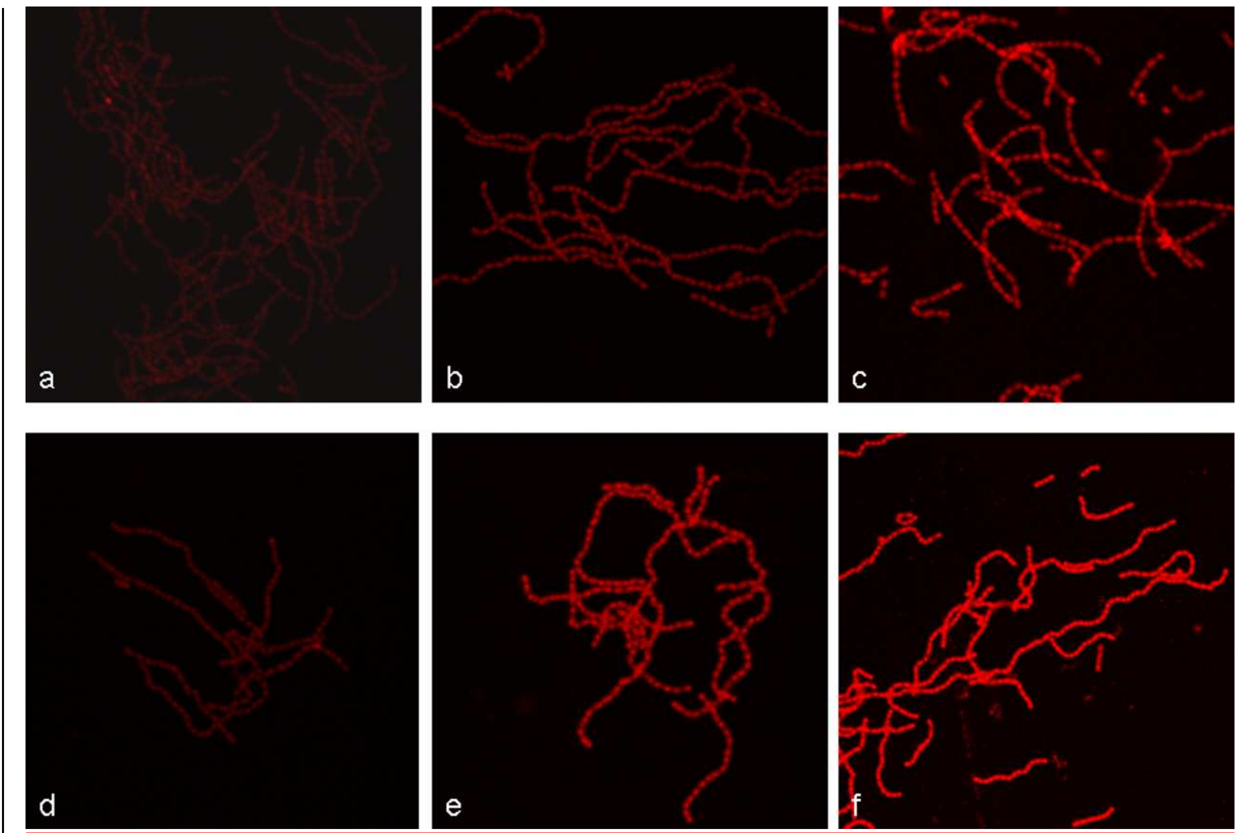


Fig. 1

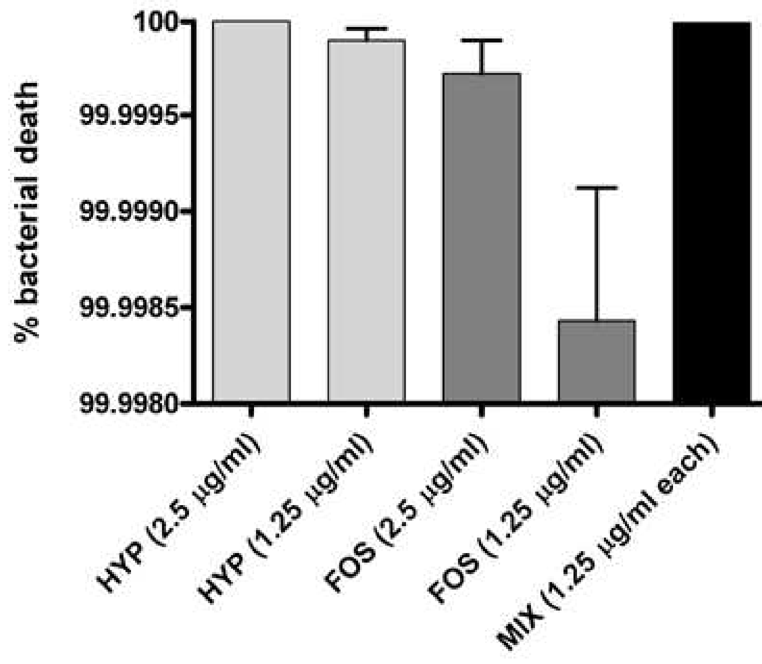
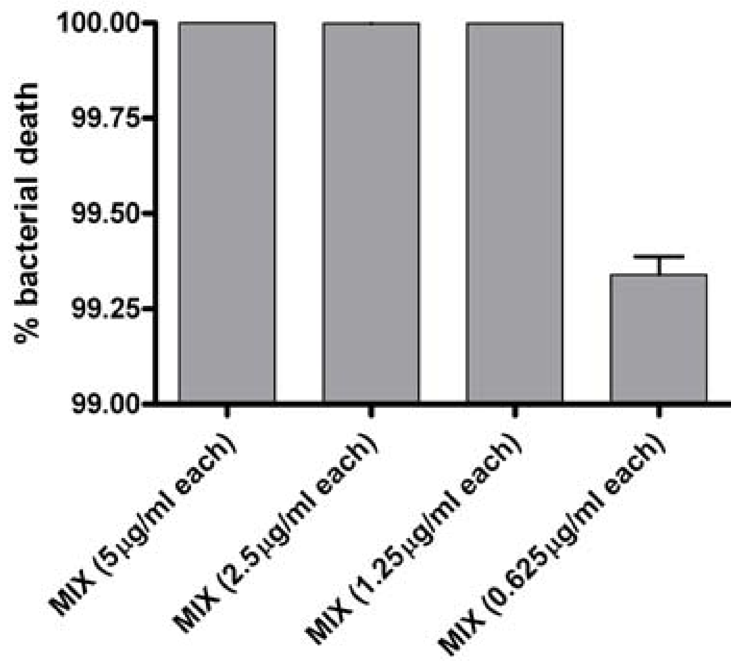


Fig. 2



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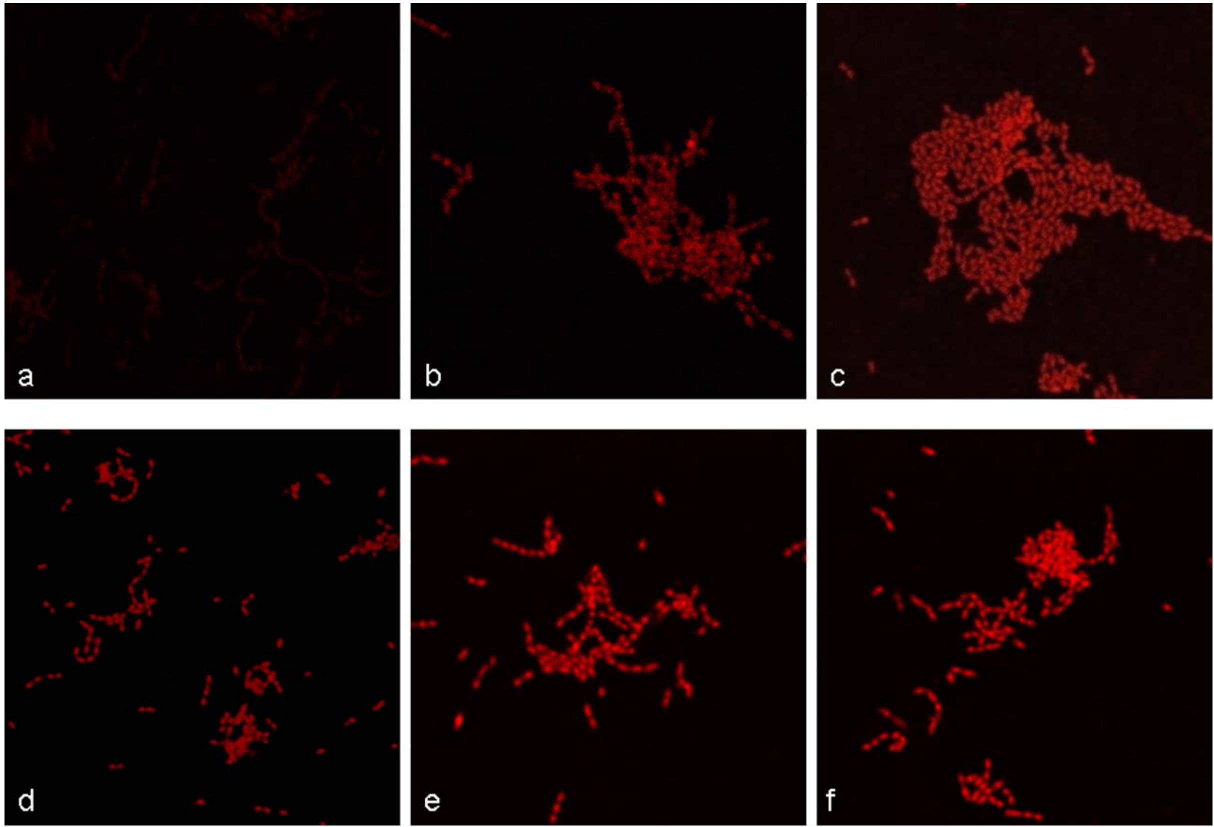

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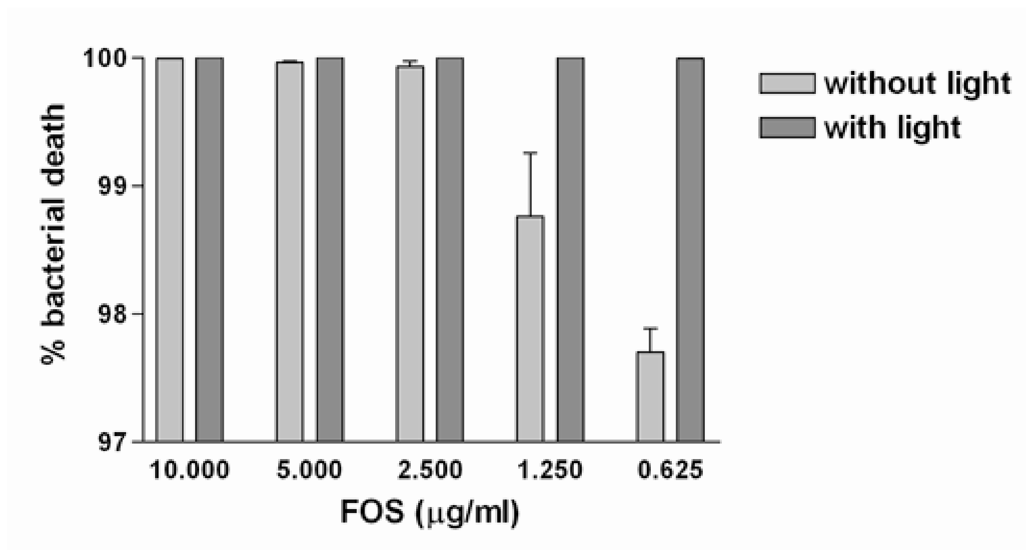

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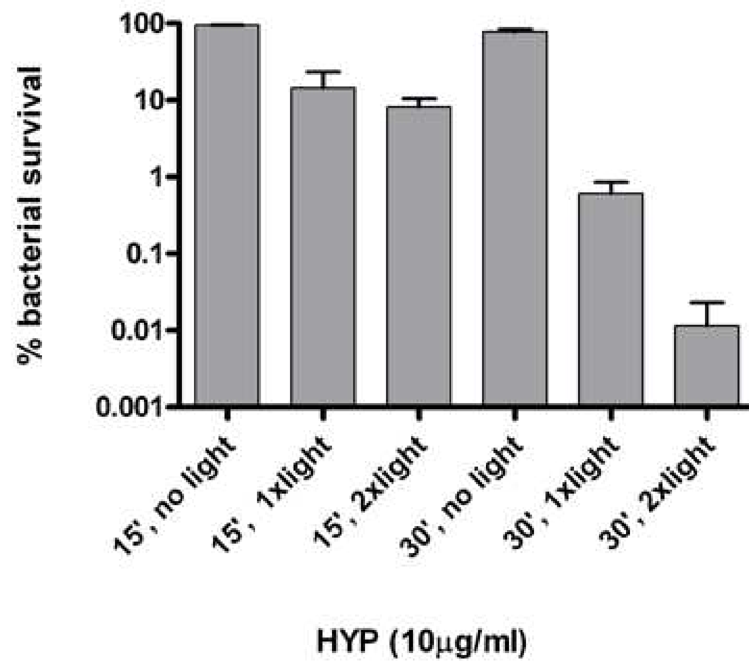

Fig. 6

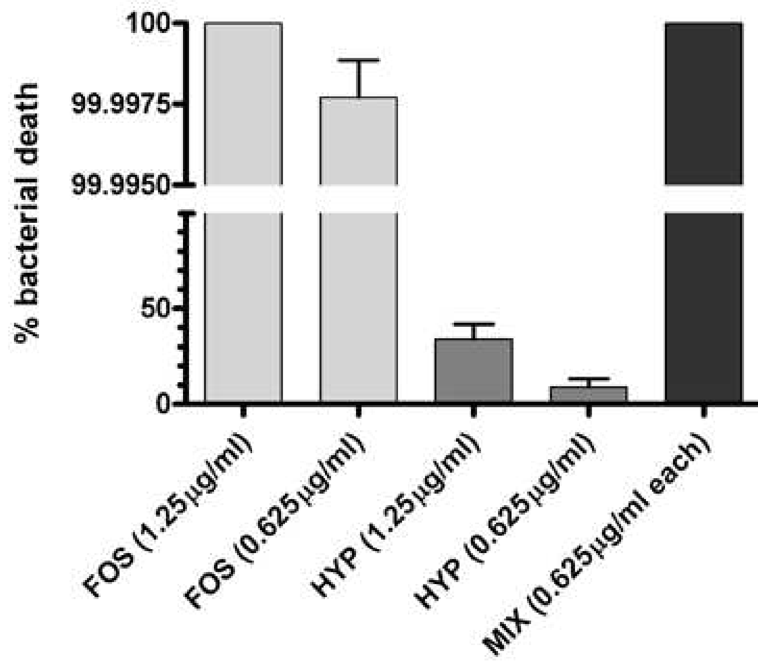

Fig. 7