

Review Article

The Advantages of the Photolysis of Hydrogen Peroxide Utilizing LED Light as a Hydroxyl Radical-Based Disinfection Methodology for Photoeradication of Dental Plaque Biofilms

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Abstract

The selective “targeting” of cells utilizing dye photosensitizers inherent in the conventional PDT paradigm was originally developed for eukaryotic (mammalian) cells and tissues. However, this methodology and the confusing array of protocols associated with its use may not be the best approach in eradicating prokaryotic (bacterial) cells in dental biofilm microenvironments.

The Photolysis of H₂O₂ utilizing LED light produces a more “global” eradication of microbes via the production of the lethal hydroxyl radical. This may be a more desirable approach, in part; due to the unique and structured way “pioneer” microbes form and grow in the oral plaque. Several dental researchers have investigated this methodology and have had notable success.

It is proposed that the search for different dye photosensitizers, corresponding light sources and their affinities for different phenotypes of bacteria in oral plaque biofilm is overcome by utilizing the Photolysis of H₂O₂ to create hydroxyl radicals. The advantages of the use of the photolysis of H₂O₂ with LED light versus conventional Photodynamic Therapy (PDT) as it applies to oral plaque biofilms are discussed.

Keywords: Photodynamic therapy; Antibacterial photodynamic therapy; Photodynamic antimicrobial chemotherapy; Photodynamic disinfection; Photolysis; Hydrogen peroxide

Introduction

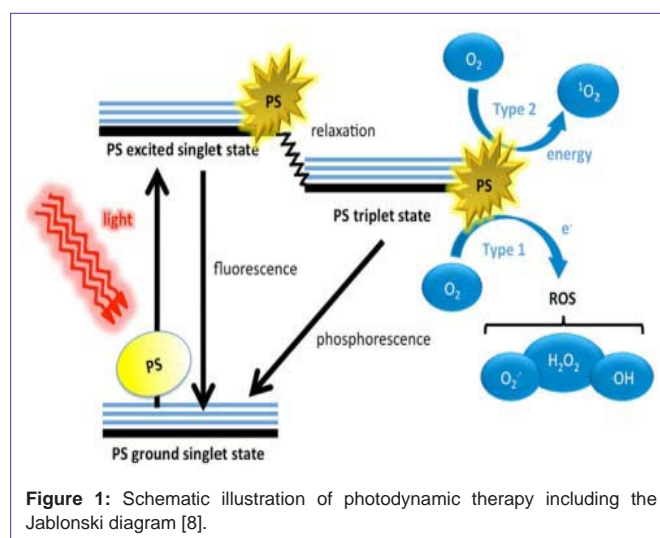
Photodynamic Therapy (PDT) involves the activation of certain chemicals or dyes (photosensitizers) by light in the presence of oxygen producing reactive radicals that are capable of inducing cell death [1] and eradicating cariogenic bacteria due to the systems antimicrobial properties [2-7].

There are two mechanisms in the conventional PDT methodology by which the excited state photosensitizers can react with bimolecules [1]. The Type I mechanism results in the production of highly Reactive Oxygen Species (ROS) (superoxide, hydroxyl radicals, hydrogen peroxide) [8]. Type II reactions produce the highly reactive state of oxygen known as singlet oxygen [1,8] (Figure 1). Type II reactions have been historically accepted as the major pathway in microbial cell destruction.

Modern PDT protocols were developed for medical uses to target tissues in mammalian (eukaryotic) cells primarily used to treat tumors [9]. PDT has mostly developed as a cancer therapy [10]. Since it was found that some photosensitizers bind rapidly and selectively to microbial cells, it was suggested that PDT could be used as an anti-infective approach; this became a reality in the mid-1990s [11].

The aim of many dental investigators has been an attempt to standardize a system of PDT by determining which photosensitizers,

usually dyes, used in the medical model and corresponding wavelengths of light are most effective in the control or inactivation of a wide range of pathogenic oral bacteria. Typical photosensitizing dyes are toluidine blue, phthalocyanine, methylene blue, rose bengal, erythrosine, indocyanine green, and others [5].



Historically, various laser wavelengths of light have been examined, however, recently non-laser light sources such as Light-Emitting Diodes (LEDs) used in composite curing lights composites emitting light in approximately the 400-500 nm range [12] have been found to be an alternative to the use of lasers [13-17]. Due to these overwhelming variances in the conventional PDT system, a reliable and effective method of treatment of microorganisms has yet to be confirmed [18] which has diminished the success of PDT [19].

During the photolysis of H_2O_2 , visible light exposure in the presence of H_2O_2 results in accumulation of ROS in the bacterial surroundings [20]. It has been shown when H_2O_2 is exposed to light or laser radiation, the amount of hydroxyl radical generated changed according to the concentration of H_2O_2 and irradiation time [21].

“Global” Photoeradication of Microorganisms in Dental Microenvironments May Be a Better Approach Than Conventional PDT

Evidence indicates relatively non-specific bacterial killing provided by the Photolysis of H_2O_2 and LED light could actually be beneficial and not something to be avoided in dental microenvironments. This is due to 1) the unique and structured colonization of dental plaque 2) the easy penetration of hydrogen peroxide into even mature biofilm 3) the hydroxyl radical-based disinfection technique 4) the lack of production of resistant dental pathogens, and 5) the lack of “self-shielding” of conventional dye photosensitizers.

1) Dental Plaque is the term commonly used for the biofilm that is formed on the tooth surface and consists of a complex microbial community embedded in a matrix of polymers of bacteria and salivary origin [22]. The number of bacterial species in dental plaque is expected to rise into the thousands with the advances in mass sequencing techniques [23,24].

The formation of dental plaque involves a highly ordered pattern of sequential colonization (microbial succession) by a particular species of bacteria over time and it is the same for everyone [25,26]. Early biofilm consists of morphologically distinct palisading columns of cocci [27,28]. Studies have shown *Streptococcus sanguis*, *Streptococcus gordonii* and *Streptococcus oralis* are indigenous commensal species that are among the first to colonize clean tooth surfaces [29-32].

Saliva acts as a vehicle for transporting the bacteria to the different surfaces of the condensed layer of salivary pellicle that formed at the base of the plaque. This is the biologically active substrate to which these “pioneer” organisms must attach [33]. Microbial aggregates

akin to “planktonic biofilms,” would be present in saliva, and their attachment to teeth would form dental plaque [34].

It is suggested that a more global eradication of dental plaque utilizing the production of the hydroxyl radical would allow “pioneer” organisms in the saliva to repopulate the salivary pellicle in the same orderly “lock and key” fashion that occurs as in a freshly erupted or clean tooth.

2) The low capability of conventional PDT photosensitizing agents to penetrate the deep layers of the biofilm is considered a pivotal factor [35]. Conventional dye photosensitizers are relatively much larger molecules when compared to H_2O_2 (Figure 2). H_2O_2 has been shown to penetrate even the deepest areas of a mature biofilm in an *in vitro* system [35]. By nature, H_2O_2 is a covalent and uncharged molecule that readily mixes with water and is treated as such in the body; thus it diffuses readily across cell membranes with ease where it has the capability to form highly reactive free radicals such as OH^\cdot radical [23]. The advantages of this interaction have been underestimated [35- 38] (Figure 2).

Interestingly, clinical evidence emerges that some of the H_2O_2 producing oral streptococci seem to be reduced in their abundance in subjects having oral diseases like caries or periodontal disease [39-41]. It is proposed this reintroduction of an oxygenated environment and ROS into the deepest levels of oral plaque biofilm could be advantageous in the eradication putative anaerobes and may help in re- establishing the predominance of the oral streptococci associated with health.

3) Although the singlet oxygen generated by the type II mechanism described in Conventional Photodynamic Therapy (PDT) can kill bacteria in biofilm [1,5,42], it is now believed that the reactivity and oxidizing power of hydroxyl radicals are higher than those of singlet oxygen [43]. Therefore, the hydroxyl radical disinfection system probably exerts a greater bactericidal effect than conventional PDT [44].

Interestingly, it is known that the hydroxyl radical is generated in the immunological response in the body to kill invading bacteria [45,46]. Furthermore, all three major classes of bactericidal drugs utilize a common mechanism stimulating the production of lethal amounts of hydroxyl radicals via the Fenton reaction [47].

Although hydrogen peroxide can be detoxified by endogenous antioxidants (enzymatic and non-enzymatic), no enzyme can detoxify the hydroxyl radical or singlet oxygen making them extremely toxic and acutely lethal, especially to microorganisms [20].

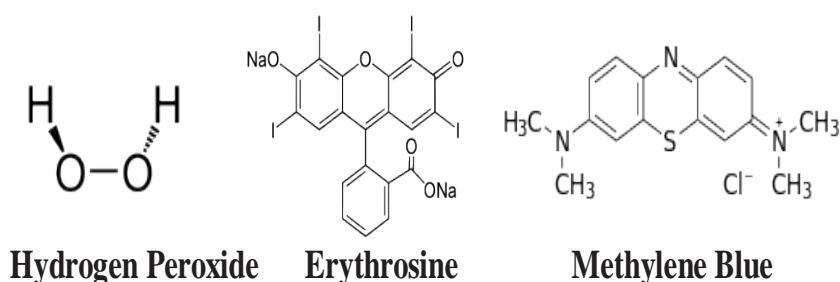


Figure 2: Molecules of Hydrogen Peroxide, Erythrosine and Methylene Blue [9-11].

4) In Conventional PDT studies, PACT is equally effective against antibiotic-resistant and antibiotic-susceptible bacteria, and repeated photosensitization has not induced the selection of resistant strains [48,49]. In particular, hydroxyl radicals and singlet oxygen are thought to be free from induction of resistance because no defense mechanisms against these two ROS have been reported in living cells. From a clinical point of view, this is a major advantage of this disinfection system [44,50,51].

5) "Self-shielding" describes the absorption of light by the dye photosensitizer which itself can limit light penetration. It follows since hydrogen peroxide is a clear liquid, this phenomenon is not an issue [52,53].

Effects of Biological Enzymatic Activity of Microorganisms

It is well known that some species of microorganisms are equipped with enzymatic defense mechanisms that suppress oxidative stress caused by ROS [50,54]. As for defense mechanisms against H₂O₂, catalase, one such enzyme, is mainly responsible for these defense mechanisms, catalyzing a reaction in which H₂O₂ decomposes into water and oxygen [55-57]. Hence, the catalase activity of microorganisms might affect the microbiocidal effect of photolysis of H₂O₂ [50]. However, the "flood" of concentrations of H₂O₂ used in the photolysis of H₂O₂ and LED light may overwhelm these defenses.

Applications to dentistry

The following investigators (Table 1) have provided significant

Study	Year	Bacteria/Biofilm	H ₂ O ₂ Conc.	Light Source/ Wavelength Irradiation time(s)
Tanaka MK, [21]	2003	none	13.5mM "various"	PL* 400-500 nm 90 sec. various, HL-400nm, HN-594 (yellow)nm, HN-632.8nm
Feuerstein O, [58]	2006	<i>S. mutans</i>	0.3m 3.0mM 30mM	XE* 450-490nm 20, 30, 40 sec. 10min.
Steinberg D, [59]	2008	Biofilms- <i>S. mutans</i>	3 to 300mM	400-500 nm 30 or 60 sec.
Ikai H, [51]	2010	<i>S.aureus</i> , <i>A.actinomycetemcomitans</i> , <i>S.mutans</i> , <i>E.faecalis</i> ** and biofilms of each	1M, 200mM 300mM	405nm 3 min.
Feuerstein O, [60]	2012	<i>S. mutans</i> -Biofilm (and tooth enamel)	30mM 300mM	non-coherent blue light 30, 60 sec. (400-500 nm) LED 400nm+- 20nm 1,5,10 min. Output power- 300mW
Nakamura K, [50]	2012	<i>S. aureus</i> <i>C. albicans</i>	250mM	LED 400+- 20nm 15,30,45,60 sec. (different temp.: 25,35,45,55 degrees C)
Shirato M, [61]	2012	<i>S. mutans</i> <i>E. faecalis</i>	250,500 750, 1000mM	dual wave length LED 2 min. 400 and 465 nm
Hayasshi E, [62]	2012	<i>S.aureus</i>	1 mol ⁽⁻¹⁾	

*PL: Plasma Lamp; HL: Halogen Lamp; HN: He-Ne laser; XE: Xenon Lamp.

evidence that this methodology is an advantageous and potent disinfection system for the Photoeradication of dental pathogens.

Evidence for safety of H₂O₂ and OH radical production

As for the safety of this system, 1.0 M H₂O₂ which is almost equal to the 3% H₂O₂ used as an oral disinfectant [63,64], was used as a substrate for hydroxyl radical generation. A subcommittee of the US Food and Drug Administration [65], also concluded that hydrogen peroxide is safe in concentrations of up to 3% and is used in medical treatment for disinfection. Even if H₂O₂ remains in the oral cavity, it quickly decomposes to water and oxygen [44].

There is the concern the hydroxyl radical-based disinfection technique presented in the Yamada, Kanno study might cause acute oxidative damage to the oral mucosa [66]. However, this study showed that three treatments of their disinfection technique had no detrimental effects in terms of histological examination.

ESR analysis demonstrated that the generation of hydroxyl radicals stopped immediately after the cessation of laser irradiation. Thus, the generation of hydroxyl radicals is controllable and the disinfection system can be used safely in the oral cavity [44]. The hydroxyl radical is free from residual toxicity because of its extremely short-half life, approximately 10⁻⁹s [67,68]. Finally, antimicrobial effects can be confined by careful topical application of hydrogen peroxide and the area of irradiation can be restricted.

Visible light is preferable to UV light because UV irradiation might not only photolyze H₂O₂ but also damage normal tissues [44]. In addition, visible light is much safer than UV light for operators when the disinfection system is applied in a clinical setting [44].

The possible involvement of hydroxyl radicals in chemically induced carcinogenicity has been reported in several papers [69-73]. However, other studies [74-77] support the notion that long-term exposure of cells to heavy metals is required to cause carcinogenicity, even though the hydroxyl radical is a causative factor [78].

Prior to clinical testing, by Yamada [66], the safety of their system, the risks of mutagenicity and carcinogenicity were examined and reviewed in the literature [78]. It is concluded that there is little risk of carcinogenicity as long as the hydroxyl radical is for short term treatment of the oral cavity as reported in one of their previous studies [50,78].

Possible beneficial effects of the hydrogen peroxide and the hydroxyl radical

Possible advantages on the use of the hydroxyl radical have been reported [79-82]. A single treatment had no detrimental effects on the healing process histologically and there was an acceleration of the wound healing process observed [66]. Therefore, it is suggested that the disinfection technique does not have injurious effect on the healing process of the wounded skin and rather has a healing effect [66].

Conclusions

Oral plaque biofilm development consisting of prokaryotic cells presents a different substrate-environment than mammalian cell (eukaryotic cells) in tissues. PDT for the eradication of mammalian

cells and tissues, such as treatment for tumors presents a different paradigm.

Hydroxyl radicals generated by photolysis of H₂O₂ using LED could be adopted as a superior disinfection system. The advantages of the photolysis of H₂O₂ and LED light Methodology include:

- “Universal” killing of microorganisms in dental plaque biofilm may be utilized because of the unique and structured way pioneer microbes form and grow in oral dental plaque microenvironment.
- H₂O₂ easily penetrates deep layers of biofilm, providing ROS to the oral plaque microenvironment.
- It is unlikely that bacteria would develop resistance to the cytotoxic action of H₂O₂ or OH⁻ radical.
- Does away with the confusing array of Conventional PDT parameters presently being investigated
- “Self-shielding” phenomenon is not an issue
- Absence of genotoxic and mutagenic effect
- Possible beneficial effects in wound healing
- Practical, readily available, cost efficient Methodology for the practicing dentist

The production of the hydroxyl radical via the photolysis of H₂O₂ by LED light is possibly a “universal” effective methodology that warrants further investigation for the control and eradication of oral plaque biofilm in all dental microenvironments.

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