ijpbs 2020; doi 10.22376/ijpbs.2020.11.1.b119-128

International Journal of Pharma and Bio Sciences

Review Article

Biomaterial Sciences for drug design

Antimicrobial Efficacy Of Photosensitizers

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Abstract: Inactivation of microorganisms using photodynamic therapy (PDT) has been defined as either antimicrobial photodynamic therapy (aPDT), Photodynamic antimicrobial chemotherapy (PACT) or photodynamic disinfection. Photodynamic therapy is a combination of nontoxic photosensitizer irradiated using visible light of appropriate wavelength, which in the presence of oxygen produces Reactive Oxygen Species (ROS). ROS is highly reactive and induces irreversible cytotoxic damage to the bacterial cell leading to the death of microorganisms. This review focuses on the various generations of photosensitizers and their antimicrobial effect against periodontal pathogens when used in periodontal and peri-implant disease management.

Keyword: photosensitizers, periodontitis, antimicrobial photosensitizers, toluidine blue, methylene blue.

Article History	Date of Receiving	10 September 2019	Date of Revision	27 January 2020
	Date of Acceptance	30 January 2020	Date of Publishing	06 February 2020

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Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

CItation Dr. Kanchana Sukumar, Dr. Anupama Tadepalli, Dr. Harinath.P and Dr. Deepa.P , Antimicrobial Efficacy Of Photosensitizers.(2020).Int J Pharm Sci.11(1), b119-128 http://dx.doi.org/10.22376/ijpbs.2020.11.1.b119-128

Int J Pharma Bio Sci., Volume11., No 1 (Jan) 2020, pp b119-128

1. INTRODUCTION

The success of periodontal therapy relies on complete elimination of periodontal pathogens and their endotoxins like lipopolysaccharides (LPS) from the root surface and soft tissues, as well as neutralization of host pro-inflammatory cytokines [1,2]. Conventional scaling and root planing techniques involve disruption of plaque biofilm through mechanical instrumentation and showed significant improvement in clinical parameters over time but it's a grim to eradicate the tissue invading microorganisms. Governing bacterial recolonization in pockets require regular periodontal maintenance and addition of co-adjuvant to mechanical debridement [3,4]. Commonly used adjuncts include, antimicrobial agents applied locally, systemically and supplementary irradiation using LASERs etc. Application of antibiotics has proven effective but it has limitations like,[3] as in with local and systemic administration of antibiotics to achieve therapeutic concentration of drug in gingival crevicular fluid (GCF) is challenging. More importantly, frequent administration of antibiotics resulted in development of resistant microorganisms. Thus, the development of alternative antimicrobial therapeutic strategies becomes important to control microbial growth in the oral cavity. One of such localized non-invasive therapeutic approach is photodynamic therapy (PDT). The aim of the present study is to systematically review the "physical and chemical structure of photosensitizers being used in periodontal therapy."

1.1 Photodynamic therapy

PDT requires three essential components namely, photosensitizer, oxygen and light. When a photosensitizer is administered locally, it binds to the target cell. On irradiation with light of suitable wavelength photosensitizer gets transformed from the ground state into a highly reactive state. PDT has been therapeutically applied in various fields of medicine since 1960s and has been defined as "the light induced inactivation of cells, microorganisms, or molecules." Photodynamic therapy in inactivation of microorganisms was first demonstrated by Oscar Raab, who had reported the lethal effect of acridine hydrochloride and visible light on Paramecium caudatum [5]. Jodlbaner and Von Tappeiner[6] coined the term 'photodynamic' in 1904, to describe the oxygen dependent chemical reactions induced by photosensitization which could inactivate the pathogens[7]. In 1913, Friedrich Meyer-Betz performed the pioneering study called photo radiation therapy (PRT) with porphyrins. It was John Toth, who acknowledged the "photodynamic chemical effect" of the therapy with early clinical argon dye lasers and renamed the therapy as "Photodynamic Therapy" (PDT)[8]. The first experiments in humans was performed by Kelly and Snell (1975)[9] using hematoporphyrin derivative photodynamic therapy in five patients with bladder carcinoma but it has few disadvantages which include skin photosensitivity, may last for few weeks or months and absorption spectrum is around 630 nm which can only be applied for treating surface tumours. Since then various attempts have been made to synthesize and discover new molecules to improvise the action of photosensitizers. Till date there are several hundred PS have been developed to treat cancer and other diseases.

2. DISCUSSION

2.1 Photosensitizers

In 2004, Tim Maisch et al [10] stated that five important photochemical/photophysical properties are required for PS to induce cytotoxic effects, they were

(i) Quantum yield of triplet state formation
(ii) Redox potential of the excited state of photosensitisers, if the reaction follows type I mechanism.
(iii) The quantum yield of singlet oxygen generation, if the reaction follows type II mechanism.
(iv) The molecular extinction coefficient
(v) Lipophilicity and ionization of the photoreactive dyes.

Alexandra B. Ormond [11] proposed the ideal characteristics of photosensitizers

i. Should be available in pure form, of known chemical composition
ii. Should be easily synthesizable from available precursors and easily reproduced
iii. Should have a high singlet oxygen quantum yield
iv. Should have a strong absorption in the red region of the visible spectrum (680–800 nm)
v. effective accumulation in diseased tissue and possession of low dark toxicity for both photosensitizer and its metabolites
vi. It should be stable and soluble in the body's tissue fluids, and should be easily delivery to the body via injection or other methods
vii. Upon completion of treatment it should be excreted completely from the body.

Photosensitizers can be broadly classified as (a) Porphyrin photosensitizers (b) Non – porphyrin photosensitizers. (Table 1)

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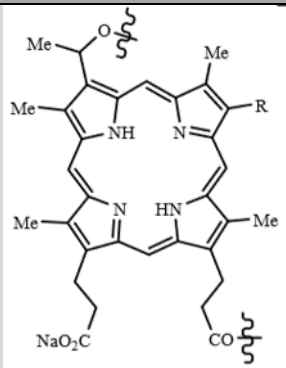
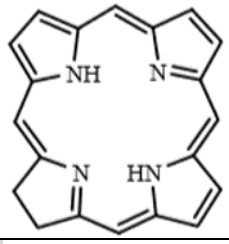
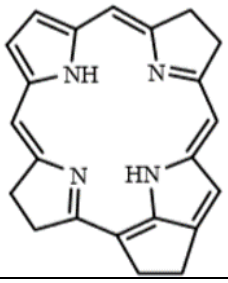
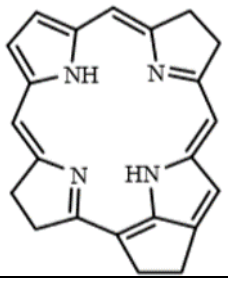
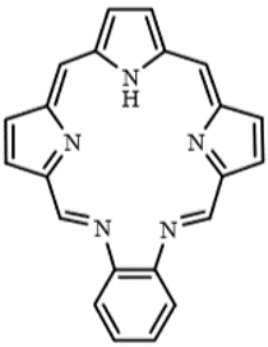
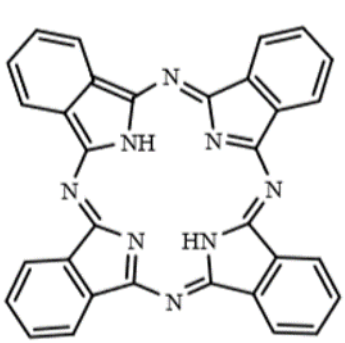
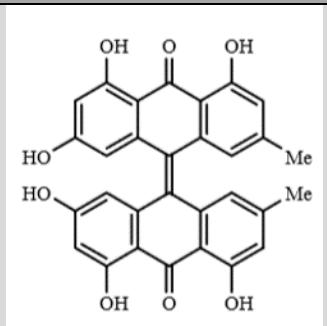
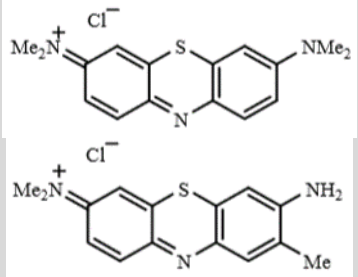
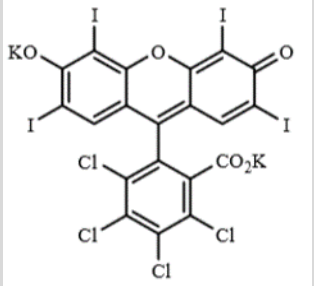
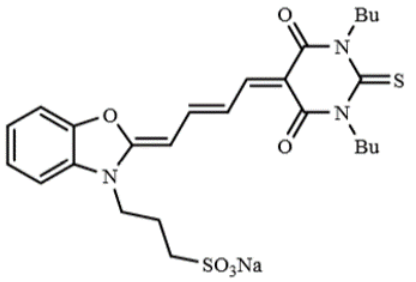
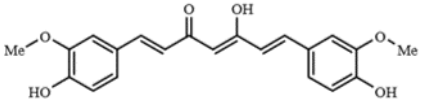
Table 1. Classification of photosensitizers

Porphyrin photosensitizers				
Generation	**Photosensitizer**	**Chemical structure**	**Absorption spectrum**	**Key applications**
First generation	Photofrin, hematoporphyrin derivatives		630 nm	Bladder cancer, oesophageal cancer, lung cancer, head & neck, abdominal, brain, skin, breast cancer
Second generation	Chlorins (FDA approved as Visudyne in 1999 [12]		640 to 700 nm	Used to treat lung, liver, brain and oral cancer
	Pheophorbide		665 nm & 408 nm	Phase I trial- oesophageal, head & neck and oropharynx.
	Bacterial pheophorbide		740 – 800 nm	Prostate cancer
	Texaphyrin		732 nm	Phase I trial-prostate and cervical cancer.

	Phthalocyanines		670-700 nm	Used in treatment of early stage or recurrent lip, pharynx, larynx, tongue lesions, lung and oesophageal tumour [13]
Third generation	Biologic conjugates (e.g. Antibody conjugate, liposome conjugate)			

Non-porphyrin photosensitizers				
	Photosensitizer	**Chemical structure**	**Absorption**	**Key applications**
Anthraquinones	Hypericin		600 nm	Under clinical trial to treat carcinomas
Phenothiazines	Methylene blue, Toluidine blue		666 nm 596 nm and 630 nm	Commonly used to treat cancer and endodontic infection and periodontitis.
Xanthenes	Rose Bengal		549 nm	Periodontitis
Cyanines	Merocyanine dyes, Methine cyanine dyes, Aza cyanine dyes Indocyanine Green	 **28** Merocyanine 540	556 nm 810 nm	leukaemia and neuroblastoma adjunctive use in wound healing or treating chronic infections of mucous membranes and skin. Also used to treat oral pathogens
Curcuminoids	Curcumin		420 nm	Used in dentistry to eradicate Oral pathogens

The interaction between the biomolecules and the excited photosensitizers are of two types

Type I

This mechanism involves transfer of hydrogen/electron between the excited photosensitizer and substrate of the cell, which results in the formation of free radicals. These formed free radicals rapidly react with oxygen and results in the production of highly reactive oxygen species, such as hydroxyl radicals, superoxide and hydrogen peroxide. This results in irreparable damage to the cell membrane integrity[14].

Type II

In this mechanism the triplet state photosensitizer reacts with oxygen to produce highly reactive state of oxygen known as singlet oxygen. Due to its high chemical reactivity, singlet oxygen can interact with a large number of biological substrates and can induce oxidative damage, thus causing lethal effects on the bacterial cell. Therefore, the antibacterial effect of photodynamic therapy is mainly mediated by the type II reaction, which is the most accepted pathway involved in microbial cell damage[15].The effect of PDT depends upon the type and concentration of PS used and the type of microorganism it acts upon. The important parameters for interaction of PS with microorganisms are its relative solubility in water and lipids, ionization constant and other more specific factors, such as, light absorption characteristics and the efficiency of formation of the excited state triplet and singlet oxygen production [16,17]. PDT is more effective in killing Gram positive bacteria because its cell wall is composed of peptidoglycan and lipoteichoic acid which is relatively more porous, allowing PS to reach the cytoplasmic membrane[16]. PS including porphyrin derivatives, Rose Bengal, erythrosine and eosin bind efficiently with Gram positive microorganisms and inactivate them. However,hese PS are not effective against gram negative organisms as they contain negatively charged lipopolysaccharide, lipoproteins and proteins with a porin function, in addition to peptidoglycan in the outer cell wall. This complex cell wall structure hinders the penetration of PS and makes it difficult for photoinactivation of gram-negative bacteria[18]. In order to gain access to the internal targets, PS could be combined with polycationic molecule or membrane active agents, which weakens the intermolecular interactions of LPS constituents and render it more permeable to PS by enabling them to cross the outer membrane[19]. In periodontal therapy mainstream organisms targeted were gram negative species. So, it is essential to understand the effectiveness of various photosensitizers in eliminating periodontal pathogens.

2.2 *Application of various photosensitizers in periodontal and peri-implant diseases*

Periodontal and peri-implant diseases are primarily biofilm induced diseases and PDT is extensively studied in periodontics because of its ease of application, localized site of action by inactivating bacteria and their virulence factors[20] (Table 2 and 3)

Table 2. *In vitro* and animal studies using various photosensitizers and their effect on pathogenic microorganisms

Author name	Photosensitizer	Light source	Susceptible bacteria
Dobson et al (1992) [21]	Toluidine blue O (TBO), methylene blue (MB), azure B chloride - concentrations of 0.005% (wt/vol)	HeNe gas laser, power output of 7.3 mW, 632.8 nm with 30 secs exposure time.	Effective against Porphyromonas gingivalis (P.g), Fusobacterium nucleatum, Aggregatibactor actinomycetemcomitans(A.a)
Wilson et al (1995) [22]	Toluidine blue O or Aluminium disulfonated phthalocyanine (AlPcS2)	HeNe 632 nm, 7.3 mW and Diode laser 660 nm, 11 mW	HeNe/TBO combination – reduction in total anaerobic count, streptococci and actinomyces
Chan Y et al (2003) [23]	Methylene blue (MB)	He-Ne laser - 632.8 nm,30 mW power output, a 100 mW Diode laser - 665 nm, or a 100 mW diode laser at 830 nm for 60s	MB + 665 nm diode laser showed 90% reduction of A.a and F. nucleatum 99–100% reduction of P.g and P. intermedia and S. sanguis
Matevski D et al (2003) [24]	Toluidine blue O (50 µg/ml)	Helium–Neon laser as control and Xenon lamp as test	10 J/cm2 100 mW/cm2 and 12.5 lg/ml TBO and xenon lamp - Significant reduction in P. intermedia, F. nucleatum, Aa and B. forsythus
Zanin et al (2005)[25]	TBO	HeNe 632 nm and LED 620-660 nm	TBO+HeNe and TBO+ LED = 99.99% reduction in viability of Streptococcus mutans reductions
Zanin et al (2006)[26]	TBO	Red LED 620–660nm	significant reduction (̃95%) in viability for S. mutans and S. sobrinus
Qin Y et al (2008) [27]	Toluidine blue O (1 mg⁄ml)	Diode laser 635 nm 12 J/cm2 at 159 mW/cm2	Fusobacterium, Actinomycetes, Streptococcus, Veillonella, etc.
Martin Schneider (2011) [28]	Phenothiazine chloride (PTC)	Diode laser 660 nm and 100mW	Streptococcus Mutans Application of PS+ Diode laser showed significant reduction in the fluorescence values compared to baseline ($p<0.05$)
Teixeira et al (2012) [29]	TBO	RedLED620–660nm	Significant reduction in the viability of Streptococcus mutans biofilms.
Pereira et al (2013) [30]	erythrosine (ER) and Rose Bengal (RB)	blue light-emitting diode (LED), 455±20 nm for	Significant reduction of Streptococcus mutans and Streptococcus sanguinis biofilms on using

	5 µM for 5 min	180 s	ER and RB (p=0.001),
Araujo et al (2014) [31]	Curcumin Used at concentrations- 0.75,1.5,3.0, 4.0, and 5.0 g/L	LED - 5.7 J/cm2	LED + curcumin combination showed reduction in Streptococcus mutans and L.acidophilus counts in a biofilm phase.
Sigusch BW et al (2005) [32]	Chlorine e6 and BLC 1010	662 Diode laser, 0.5 W power, 20 seconds	Conducted in dog model Outcome: PDT + chlorine e6 = significant reduction in P. gingivalis but not with BLC 1010 treated sites

Toluidine blue O (TBO), methylene blue (MB), Aluminium disulfonated phthalocyanine (AlPcS2), Phenothiazine chloride (PTC), erythrosine (ER), Rose Bengal (RB), Helium–Neon laser (He-Ne), Light Emitting Diode (LED), Porphyromonas gingivalis (P.g), Fusobacterium nucleatum (F.n), Actinobacillus actinomycetemcomitans (A.a), Streptococcus mutans (S.mutans), Prevotella intermedia (P.i).

Table 3. Human studies using various photosensitizers for treating periodontitis and their outcomes

Author name	Photosensitizer	Light source	Susceptible bacterias
Christodoulides et al (2008) [33]	TBO (concentration not reported)	Diode Laser 670 nm, 75 mw/cm2, 1 min, single application	at 6 months follow up - Aa, Pg, Tf counts were comparable among both the groups
Polansky et al (2009) [34]	PTC (concentration not reported)	Diode laser 680 nm, 60 seconds – exposure time, single application	at 3 months follow-up - P.g, T.f, and T.d counts were comparable among both groups
Chondros et al. (2009) [35]	PTC (10 mg/ml)	Diode laser of 670 nm, 75 mw/cm2, 1 min- exposure time and single application	24 weeks post application T.denticola counts increased in the PDT sites. Aa, Pg, and Tf counts were comparable among both groups
Ruhling et al. (2010) [36]	Tolonium chloride (concentration not reported)	Diode Laser of 635 nm, 100 mw/cm2, 1 min- exposure time and single application	30-40% reduction of microbial count was noted immediately following application but relapsed to baseline values at 3 months follow up.
Cappuyns et al. (2012) [37]	PTC (0.1 mg/ml)	Diode Laser of 660 nm, 40 mw/cm2, 1 min- exposure time, single application	6 months postoperatively there was reduction in P.g, T.f, T.d counts at the PDT treated sites.
Novaes Jr et al (2012) [38]	PTC (concentration not reported)	Diode laser 660 nm, 0.06 W/ cm2, 10 s – exposure time, single application	PDT group showed significant reduction in Aa counts at 3 months follow up
Theodoro et al (2012) [39]	TBO (100 µg/ml)	Diode laser 660 nm, 400 mw/cm2, 150 s – exposure time, single application	PDT+SRP= significant reduction in Aa, P.g, and T.f counts at 24 weeks follow up
Chitsazi et al (2014) [40]	TBO (concentration not reported)	Diode laser of 690nm, 75mW, 120 secs-exposure time, single application	At 12 weeks follow up A.a counts were comparable among both groups
Kolbe et al (2014) [41]	MB (10 mg/ml)	Diode laser of 660 nm, 60 mw/cm2,129 J, 1minute – exposure time, single application	3 months post application A.a, P.g, and T.f counts were comparable among both groups whereas at 6 months follow up recolonization was apparent.
Petelin et al (2014) [42]	PTC (concentration not reported)	Diode Laser – 660 nm, 60 mW/cm2, 1 minute – exposure time, PDT application- 3 (1st, 3rd and 7th day after ultrasonic debridement)	Multiple application of PDT showed significant reduction in A.a, P.g, T.f, and T.d counts at 6 months follow up.
Moreira et al (2014) [43]	PTC (10 mg/ml)	Diode laser of 670 nm 75mW, 10 secs – exposure time, PDT application done at Baseline, 2, 7 and 14 days	12 weeks post application there was significant reduction in A.a, P.g, T.f, and T.d counts compared to SRP alone
Birang et al (2015) [44]	Indocyanine Green (1 mg/ml) (ICG)	Diode laser 810 nm, 0.5W/cm2, 10 secs –exposure time, PDT application done at baseline and 2 weeks later	at 3 months follow-up A.a, P.g, and T.d counts were comparable among all the groups.
Carvalho et al (2015) [45]	MB (concentration not reported)	Diode laser of 660 nm, 40 mw/cm2, 90 J 1-minute- exposure time, PDT application done at baseline, 3, 6	at 12 months follow-up A.a, P.g, T.f, and T.d counts were comparable among both groups

		and 9 months	
Sreedhar et al (2016) [46]	TBO (1mg/ml)	Diode laser 810 nm, continuous mode, 30 secs – exposure time, PDT application done at 0, 7th and 21st day	Multiple applications of PDT showed reduction in level of A.a, P.g, P.i compared to single application and SRP group at 3 months follow up.

Toluidine blue O (TBO), methylene blue (MB), Aluminium disulfonated phthalocyanine (AlPcS2), Phenothiazine chloride (PTC), erythrosine (ER), Rose Bengal (RB), Indocyanine green (ICG), Helium–Neon laser (He-Ne), Light Emitting Diode (LED), Porphyromonas gingivalis (P.g), Fusobacterium nucleatum (F.n), Actinobacillus actinomycetemcomitans (A.a), Streptococcus mutans (S.mutans), Prevotella intermedia (P.i), Tannerella forsythia (T.f), Treponema denticola (T.d), Scaling and Root planing (SRP)

2.3 Effect of PDT on biofilm

Invitro and animal studies (Table 2 and 3) reported that toluidine blue and methylene blue photosensitizers showed effective results in reduction of common periodontal pathogens like P.gingivalis, T. denticola, T. forsythia, A. actinomycetemcomitans and P.intermedia. TBO and MB selectively damage gram positive and gram-negative organisms, because of the interaction between the positive charge of the dye and negative charge of the outer surface of the microbial cell [47]. Invitro study by Dobson and Wilson [48] demonstrated that TBO and MB in combination with Helium-Neon laser as light source elicited detectable killing of P.gingivalis, F.nucleatum, A.actinomycetemcomitans and S.sanguis, indicating that lethal photosensitization may be an effective method of eliminating periodontal pathogens. Martin Schneider [28], assessed the efficacy of PDT on Streptococcus mutans, using phenothiazine chloride as a photosensitizer with diode laser of 660 nm. Author concluded that PDT was effective in reducing bacteria within a 10µm thickness of biofilm.

2.4 PDT in the treatment of Periodontitis and Peri-implantitis

Chambrone et al[49], in a systematic review, reported that PDT and SRP provided similar outcomes in terms of probing depth reduction and clinical attachment level gain. In terms of alteration in the microbial profile, several studies have reported that PDT in combination with TBO was effective in reducing the periodontal pathogens [37,39,42,43,46]. Methylene Blue is a prototype of phenothiazine derivative and relatively its low toxicity to humans[50]. Toluidine blue and Methylene Blue have been most commonly studied photosensitisers against peri-implantitis pathogens. Other photosensitizers that are effective in treating peri-implantitis include porphyrins, chlorins, and phthalocyanines[51]. Haas et al, 1997[52], conducted an in vitro study using titanium platelets incubated with P.gingivalis, P.intermedia and A.actinomycetemcomitans to evaluate the antibacterial efficacy of PDT. Platelets received PDT (TBO+Diode laser), revealed destruction of bacterial cells, suggesting that PDT can be used as an adjunctive treatment modality for peri-implantitis. Similar results were reported by Marotti et al, 2013[53]. In clinical studies (Table: 4) application of TBO using diode laser of 690 nm wavelength irradiation for 1 minute reported significant reduction in A.a, P.g and P.i in peri-implantitis sites. Bombeccari et al[55], stated that though application of TBO resulted in decrease of potential pathogens, absolute eradication of bacteria from the peri-implant site cannot be achieved. Though there are conflicting results reported on using PDT as an adjunct for peri-implantitis management, a recent systematic review by Renato Silva Fraga (2018)[57] had concluded that aPDT appears to be an effective treatment option to reduce bacterial load in peri-implant disease and it also has a positive potential as an alternative therapy for peri-implantitis. However, additional clinical trials need to be conducted to evaluate the efficacy. As an adjunct to mechanical debridement in treating peri-implantitis, Dortbudak et al, Gian Paolo Bombeccari et al, Bassetti et al,[54-56] reported that TBO in combination with diode laser was effective in reducing bacterial count for an extent of 6 months whereas it failed to show significant reduction at longer duration of 12 months follow up. Factors such as, presence of local factors which may limit the penetration of photosensitizers, thickness of biofilm on the implant surface and the peri-implant defect anatomy would have resulted in incomplete elimination of pathogens and led to recolonization.

Table 4. Effect of PDT in treating Peri – implantitis

Author	Photosensitizer	Laser source	outcomes
Dortbudak et al(2001)[54]	TBO (100 µg/ml)	Diode laser of 690 nm and exposure time 60s	TBO + laser resulted in significant reduction in P.g, A.a and P. intermedia (P,0.0001). whereas complete elimination of bacteria cannot be achieved
Gian Paolo Bombeccari et al (2013)[55]	TBO (100 µg/ml) applied for 1 min	Diode laser of 810 nm and a continuous wave mode of 1 W, exposed for 20 secs. The same procedure was repeated 5 times with a total exposure time of about 100 secs.	Compared to the surgical treatment group, PDT group showed significant reduction in A.a levels (P=0.01), whereas at 12 and 24 weeks there was an increase in the anaerobic bacterial count. The proinflammatory index of peri-implantitis was significantly lower at 24 weeks follow up in PDT group.
Bassetti et al, 2013 [56]	PTC	Diode laser of 660 nm, power density of 100 mW	PDT group showed significant reduction in P. gingivalis and T.forsythia counts at 6 months follow up (P<0.05) but not statistically significant at 12 months follow up when compared to local drug delivery (LDD) group.

Toluidine blue O (TBO), methylene blue (MB), Phenothiazine chloride (PTC), Porphyromonas gingivalis (P.g), Fusobacterium nucleatum (F.n), Actinobacillus actinomycetemcomitans (A.a), Streptococcus mutans (S.mutans), Prevotella intermedia (P.i), Tannerella forsythia (T.f), Treponema denticola (T.d), local drug delivery (LDD).

3. CONCLUSION

The concept of photodynamic therapy is plausible and could foster new therapeutic avenues for periodontal and peri implant diseases as it is minimally invasive and has property of selective killing of microorganisms. Effective concentration of photosensitizers could be applied in the infected sites to enable bacterial elimination without inducing side effects on the host tissues. The development of resistance to PDT appears to be unlikely even after multiple applications. The knowledge of characteristics, efficacy of various photosensitizers enable clinician to appropriately select the therapeutic regimen in different clinical scenarios.

4. AUTHORS CONTRIBUTION STATEMENT

Dr Kanchana.S and Dr Anupama.T conceptualized and gathered the data with regard to this work. Dr Anupama.T and Dr Kanchana.S analysed these data and necessary inputs were given towards the designing of the manuscript. All authors discussed the results and commented on the manuscript and contributed to the final manuscript.

5. CONFLICT OF INTEREST

Conflict of interest declared none.

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