Photodynamic Therapy – The Pragmatic Paradigm

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Abstracts: Photodynamic therapy (PDT), also known as photo-radiation therapy, phototherapy, or photo-chemotherapy, involves the use of a photoactive dye (photosensitizer) that is activated by exposure to light of a specific wavelength in the presence of oxygen. The transfer of energy from the activated photosensitizer to available oxygen results in the formation of toxic oxygen species, such as singlet oxygen and free radicals which damages proteins, lipids, nucleic acids and other cellular components.PDT has wide range of applications in Dentistry ranging from antimicrobial chemotherapy to the diagnosis & treatment of premalignant and malignant conditions. Its application in Periodontics represents a novel therapeutic approach in the management of oral biofilms with consequent alterations in plaque homeostasis. An improved post surgical healing with reduced periodontal inflammation and tissue damage are the hallmarks of PDT. Its scope has been extended in Implantology to promote osseointegration and to prevent peri-implantitis. With such myriad of applications PDT has a promising future depending on the interactions between clinical applications and technological innovations. The paper appraises the various scopes that PDT envisages beyond the horizon. [Shreedhar A NJIRM 2014; 5(4) :72-81]

Key Words: PDT, Singlet Oxygen, Photosensitizer, Implantology, Peri-implantitis, Osseointegration.

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Introduction: Man's eternal quest for an elixir as a remedy for rejuvenation has been in vogue since time immemorial. Photodynamic therapy (PDT) – a touchstone promising myriad of possibilities offers a non invasive and novel process in which light, after being absorbed by dyes, sensitizes organisms for visible light induced cell damage. PDT combines soft laser irradiation with the application of toluidine blue "O" dye (TBO). Photodynamic therapy (PDT) can be defined as eradication of target cells by reactive oxygen species produced by means of a photosensitizing compound and light of an appropriate wavelength.[1] It could provide an alternative for targeting microbes directly at the site of infection, thus overcoming the problems associated with antimicrobials.[2] Allison et al. described PDT as a therapy that ''is truly the marriage of a drug and a light''.[3]

Light has been employed in the treatment of disease since antiquity. In the later part of twentieth century it has been used in many different forms including phototherapy for neonatal jaundice, combination of psoralen molecules and ultraviolet molecules and ultraviolet A light(PUVA) in dermatology, photodynamic therapy and photo-detection. The use of photodynamic therapy for inactivating microorganisms was first demonstrated more than 100 years ago, when Oscar Raab reported the lethal effect of acridine hydrochloride and visible light on Paramecia caudatum. During his study he demonstrated that the effect was greater than that of either acridine alone light alone or acridine exposed to light and added to paramecium. He discovered the optical property of fluorescence and concluded that it was not the light but rather some products of fluorescence that induced in vitro toxicity. He postulated that this effect was caused by the transfer of energy from light to the chemical similar to that seen in plants after absorption of light by the chlorophyll.[1] Photodynamic therapy for human infections is based on the concept that an agent (a photosensitizer) which absorbs light can be preferentially taken up by bacteria and subsequently activated by light of the appropriate wavelength in the presence of oxygen to generate singlet oxygen and free radicals that are cytotoxic to microorganisms.[4] PDT has shown potential in the treatment of oral leukoplakia, oral lichen planus, and head and neck cancer.[8] Photodynamic antimicrobial chemotherapy (PACT) has been efficacious in the treatment of bacterial, fungal, parasitic, and viral infections.[9] The non-oncological applications of PDT include treatment of psoriasis

(Weinstein *et al.*, 1991), actinic keratosis (Itoh *et al.*, 2000), rheumatoid arthritis (Miyazawa *et al.*, 2006), and age-related macular degeneration (Kozak*et al.*, 2006).The absence of genotoxic and mutagenic effects of PDT is an important factor for long-term safety during treatment. PDT also represents a novel therapeutic approach in the management of oral bio-films. Disruption of plaque structure has important consequences for homeostasis within the biofilm.[10]

Phototargeting Oral Biofilms: Dental caries : Dental caries results from an ecological imbalance in the physiological equilibrium between tooth minerals and oral microbial biofilms, mainly supragingival plaque[11]. Biofilm bacteria, such as mutans streptococci (Streptococcus mutans and Streptococcus sobrinus) and Lactobacillus species, secrete organic acids as a by-product of the metabolism of fermentable carbohydrates. This process leads to the demineralization of tooth hard-tissue cavitation in its advanced stages[12].

This technique could offer the following benefits: rapid non invasive topical in vivo application of the drug to the carious lesion; rapid bacterial killing after a short exposure to light; unlikely development of resistance considering the widespread generic toxicity of reactive oxygen species; and confined killing by restricting the field of irradiation and the inherently short diffusion radius of reactive oxygen species. Several laboratory studies have demonstrated (using toluidine blue O) the susceptibility of cariogenic bacteria, either in the planktonic phase [13,14,15,16 or] in the biofilm phase [17, 18, 19] to photodynamic therapy. Rose Bengal, a fluorescent dye that is used to study liver function, has been employed to target S. Mutans species in suspension[20], and disulfonated aluminium phthalocyanine (AlPcS2) has been shown to be effective against suspensions [21] and biofilms of cariogenic bacteria[22] as well as against humansupragingival dental plaque microbes in the planktonic phase[23,24]. The synergistic effect of erythrosine, a dental plaque-disclosing agent currently in clinical use, and photodynamic therapy, induced bacterial cell killing of >1.5 log10 in S. mutans biofilms in vitro[25, 26].

Oral candidiasis: Candida albicans becomes a serious opportunistic infectious agent in immunocompromised patients[27]. C. albicans can grow as biofilms on oral mucosal surfaces[28] and prosthetic devices[29]. Antifungal treatment with agents such as nistatin and miconazole often induce resistance, severely limiting their ability to eradicate fungal biofilms, so that recurrent infection occurs[30]. Numerous in vitro studies by Souza SC et al, So CW et al, Munin E et al etc have shown that photodynamic therapy is effective in killing Candida in planktonic[31-38] and biofilm[39,40] phases using methylene blue,[33,34, 36-38] toluidine blue O[36,38,40] photofrin[39] , tionin[38], porphyrins[32] , phthalocyanine[35,38] and malachite green[36]. Topical treatment of oral candidiasis by photodynamic therapy may be an alternative to traditional antifungal drug therapy, especially in patients with human immunodeficiency virus (HIV) for whom persistent infection is a major problem[41]. Further animal studies should establish a protocol for successful targeting of candidiasis lesions, which will then be tested in human studies. Recently, it has been shown that laser irradiation alone exerted antifungal effects in vitro[36, 37]. These data are supported by a human study, in which a reduction of inflammation was observed on the palate of subjects with denture stomatitis after five consecutive treatments with laser irradiation[42]. The presence of endogenous chromophores within C. albicans that may contribute to photosensitization requires further investigation.

Periodontal Diseases: Biofilms that colonize tooth surfaces and epithelial cells lining the periodontal pocket ⁄ gingival sulcus (subgingival dental plaques) are among the most complex biofilms that exist in nature. These biofilms include a subset of selected species from more than 700 bacterial species or phylotypes[43-45] that can lead to periodontal diseases (gingivitis or periodontitis). Mechanical removal of the periodontal biofilms is currently the most frequently used method of periodontal disease treatment. Antimicrobial agents are also used, but biofilm species exhibit several resistance mechanisms[46-48] and maintaining therapeutic concentrations of antimicrobials in the oral cavity can be difficult[49]. Photodynamic therapy has been suggested as an alternative to chemical antimicrobial agents to eliminate subgingival

species and treat periodontitis[50]. The application of methylene blue-mediated photodynamic therapy in clinical studies using either the Periowave Treatment kit or the Helbo Blue treatment kit is as follows: methylene blue is applied directly in the dental pockets for 60 s followed by exposure to red light via a fiberoptic probe for 60 s per pocket or per tooth (10 s per site, six sites in total). In the majority of these studies, photodynamic therapy as an adjunct to scaling and root planing did not show any beneficial effects over scaling and root planing alone. It is possible that short exposures to light may be responsible for the lack of clinical benefits. Several studies have shown that periodontal bacteria are susceptible to photodynamic therapy in planktonic cultures[38,51-54] plaque scrapings[55,56] and biofilms[57,58] using methylene blue[53,55,57] toluidine blue O,[51,52,55-57,59] phthalocyanine,[56,57] hematoporphyrin HCl,[57] hematoporphyrin ester[57] and a conjugate between poly-L-lysine and the photosensitizer chlorin e6[54]. Biofilms were also exposed to methylene blue (25 or 50 lg ⁄ ml) and the same light conditions as their planktonic counterparts.

Photodynamic therapy produced approximately 63% killing of bacteria in the planktonic phase, whereas in biofilms derived from the same plaque samples the effect of light was reduced (31% killing). The reduced susceptibility of bacteria to photodynamic therapy in the biofilm may be related to the distinct and protected phenotypes expressed by them once they attach to the tooth, which are still carried by dental plaque bacteria in suspension. The reduced susceptibility of biofilms to photodynamic therapy may be related to the inactivation of methylene blue[68], the existence of biofilm bacteria in a slow growing or starved state[69] and to certain phenotypes expressed by biofilm species when they attach to the agar surface. The reduced susceptibility of biofilms to photodynamic therapy may also be attributed to the reduced penetration of methylene blue, an explanation that has been introduced previously[70]. It has been suggested, in studies of model systems, that water channels can carry solutes into or out of the depths of a biofilm, but they do not guarantee access to the interior of the cell clusters[71] whose diameter may range from 20 to 600 lm[72]. Biophysical means, such as ultrasonic irradiation[73] and electric fields[74], known as the bioacoustic effect and the bioelectric effect, respectively, have been employed to enhance the efficacy of various agents in killing biofilm microorganisms. These methodologies, however, require an application time of up to 48 h in order to achieve significant bacterial killing[75,76], which would preclude their clinical use. Recently, it has been showed that the application of photomechanical waves also enhances the methylene blue concentration and the penetration depth into multispecies biofilms evolved from human saliva in vitro.[77] The hypothesis was that photomechanical waves enhance fluid forces at the biofilm–bulk water interface that deform the microcolonies of bacteria and the matrix, so that fluid movement occurs. The synergistic action of photomechanical waves and photodynamic therapy has the potential to contribute to the development of a new system for the topical, rapid and non-invasive treatment of periodontitis. In vivo studies with experimentally induced periodontits in rats have shown suppression of periodontal pathogens and a reduction of periodontitis following photodynamic therapy with toluidine blue O.[60,80] The authors also found significant reductions of periodontal bone loss in diabetic[83] and immunosuppressed[84] rats using toluidine blue O. Several clinical studies have been carried out to investigate the effects of adjunctive photodynamic therapy in human periodontitis. In all of these studies, methylene blue was the photosensitizer. Two of these studies reported significant clinical improvement (reduced probing pocket depth and bleeding on probing, increased clinical attachment level) when photodynamic therapy was used along with scaling and root planing.[85-6]

New Frontiers In Oral Antimicrobial Photodynamic Therapy: The role of photodynamic therapy as a local treatment of oral infection, either in combination with traditional methods of oral care, or alone, arises as a simple, nontoxic and inexpensive modality with little risk of microbial resistance. Lack of reliable clinical evidence, however, has not allowed the effectiveness of photodynamic therapy to be confirmed. Studies have been performed using different treatment conditions and parameters with insufficient clinical and microbiological findings. The reduced susceptibility of complex oral biofilms to

antimicrobial photodynamic therapy may require the development of novel delivery and targeting approaches. Evolving therapeutic strategies for biofilm-related infections include the use of substances designed to target the biofilm matrix, non growing bacteria (persister cells) within biofilms and ⁄ or quorum sensing.[47] The use of bacteriophages[87] and naturally occurring orsynthetic antimicrobial peptides[88] may offer the possibility of bacterial targeting without the emergence of resistance. Recently, the advantages of targeted therapy become more apparent, and the use of light alone, antibody–photosensitizer and bacteriophage–photosensitizer conjugates or non antibody based targeting moieties, such as nanoparticles, are gaining increasing attention.

Phototherapy: In some instances, application of a photosensitizer may not be required because photosensitizers occur naturally within some microbial species. This is particularly true of the oral black-pigmented species. According to Soukos NS et al it has been shown that broadband light ranging from 380 to520 nm was able to achieve a threefold reduction in the growth of P. gingivalis, P. intermedia, Prevotella nigrescens and Prevotella melaninogenica in dental plaque samples obtained from human subjects with chronic periodontitis.[89] In this study, the presence and amounts of endogenous porphyrins in black-pigmented bacteria were estimated and analysis of bacteria in dental plaque samples was performed by DNA–DNA hybridization for 40 taxa before and after phototherapy. Inactivation of black-pigmented bacteria by visible light has also been reported by investigators like Feuerstein O et al, Fux CA et al, Henry CA et al,etc.[89-95] Black-pigmented bacteria, such as P. intermedia, P. nigrescens and P. melaninogenica, are associated with gingivitis as reported by Danielsen B et al, Goodson JM, Tanner[96-8] and may be responsible for the increased bleeding tendency of long-standinggingivitis.[97] Prevotella species have also been recognized as potent producers of volatile sulfur compounds on the dorsum of the tongue[99] and were detected at high numbers in tongue samples obtained from subjects with oral malodour.[100-01] In another study by Sterer N et al, human salivary microflora was exposed to blue light of 400–500 nm and a reduction in the levels of volatile sulfide compounds was found, together with a selective inhibitory effect on the gram-negative bacteria, suggesting that it may be possible to use light to treat oral malodour.[200]Additionally, Moore WE et al have reported that black-pigmented bacteria, such as P. gingivalis and P. intermedia, are associated with the development of periodontitis[102-3] and Meurman JH et al have reported it to be involved in the pathogenesis of cardiovasculardisease.[104] Studies by Chiu B et al, Haraszthy VI et al, Taylor-Robinson D have reported black-pigmented bacteria to be detected in atheroma plaques[105-7] and this may have an impact on the reduction of bleeding in gingivitis, the reduction of inflammation in periodontitis and the cure of oral malodor. In all of the cases, exposure to visible light may result in the gradual suppression of black-pigmented bacteria that will lead to a shift of the microbial composition towards a new one associated with health. This novel technique may offer the following advantages compared with other forms of periodontal therapy (scaling, mouth washes and surgery): (i) rapid and painless application of light; (ii) selectivity in its effect; (iii) full penetration of dental plaque by light; (iv) limited penetration of light into gum tissue; (v) absence of phototoxicity to human cells; (vi) no effects on taste; and (vii) possible clinical and microbiological benefit with minimal impact on natural microbiota.

Antibody-Targeted Antibacterial Approaches Using Photodynamic Therapy: Antibodies conjugated with photosensitizers have been used to target Staphylococcus aureus.[108-9] Selective killing of P. gingivalis was achieved in the presence of Streptococcus sanguinis (previously S. sanguis) or in human gingival fibroblasts using amurine monoclonal antibody against P. gingivalis lipopolysaccharide conjugated with toluidine blue O.[110] In two studies by Embleton ML et al and Hope CK et al bacteriophages were used as vehicles to deliver the photosensitizer tin(IV) chlorinee6 to the surface of S. aureus strains.[111-2] This led to approximately 99.7% killing of microorganisms[112] The combination of pulsed laser energy and absorbing gold nano particles selectively attached to the bacterium for killing of microorganisms is a new technology that was introduced recently as suggested in studies by Zharov VP et al,[113] Gold

nanoparticles are promising candidates for application as photothermal sensitizers and can easily be conjugated to antibodies. The surface of S. aureus was targeted using 10- to 40-nm gold nano particles conjugated with anti-protein antibodies.[113] The energy that was absorbed by nano particles during irradiation was quickly transferred through non radiative relaxation in to heat accompanied by bubble-formation phenomena around clustered nano particles, leading to irreparable bacterial damage. Antibody-targeted approaches using photodynamic therapy have been most frequently focused on the treatment of malignant diseases. The therapeutic potential of these approaches for bacterial targeting is based on their ability to demonstrate minimal damage to host cells. Therefore, these approaches should be further explored in vitro and in animal studies.

Conclusion: The potential applications of photodynamic therapy to treat oral conditions seem limited only by our imagination. Applications appear not only the common oral diseases of dental caries and periodontal disease but also the conditions of oral cancer, periimplantitis, endodontic therapy, candidiasis and halitosis. Low toxicity and rapidity of effect are qualities of photodynamic therapy that are enviable. It is now the time to demonstrate clear evidence of clinical efficacy and applicability. At this time in history, it is difficult to know where light will lead us in the oral cavity but the promise is clear and the opportunities are visible.

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