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Photodynamic Therapy and its Role in Periodontitis Treatment

Terapia fotodynamiczna i jej znaczenie w leczeniu zapaleń przyzębia

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Summary

Photodynamic therapy is a novel therapeutic approach for eradicating pathogenic bacteria in periodontal disease. Inactivation of microorganisms using photodynamic therapy has been defined as either antimicrobial photodynamic therapy (aPDT), photodynamic antimicrobial chemotherapy (PACT) or photodynamic disinfection.
The use of aPDT requires a non-toxic photosensitizer, harmless visible light and oxygen. The photosensitizer binds to targeted bacteria and then can be activated by light of the appropriate wavelength in the presence of oxygen. Photoinactivation of bacteria is tightly restricted to the localization of the photosensitizer, ensuring the protection of distant cells from side-effects. Because of the fact that conventional treatment such as scaling and root planing (SRP) does not completely eliminate periodontal pathogens, especially in deep periodontal pockets, aPDT may be considered to be an alternative therapeutic strategy. This article describes the mechanism of aPDT and novel approaches such as nanoparticles. The aim of the study was to review the literature concerning the assessment of the effectiveness of aPDT in periodontitis treatment. Although studies have not indicated the superiority of aPDT compared to conventional periodontitis treatment, antimicrobial photodynamic treatment has been reported to be effective as an adjunct to conventional therapy to destroy bacteria in sites where there is limited access for mechanical instrumentation.

Keywords: antimicrobial photodynamic therapy • periodontal pockets • periodontitis • photosensitizer

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Introduction

The etiology of periodontitis is multifactorial, which results in therapeutic difficulties. Bacteria (periodontopathogens), considered to be one of the main factors of this disease thanks to their ability to grow in biofilms, are beyond the reach of antimicrobial chemical agents. Additionally, the anatomical complexity of tooth roots causes them to be predisposed to the development of many niches for bacterial deposits, making eradication of periodontopathogens more difficult both mechanically and chemically. Furthermore, some periodontopathogens (e.g. *Aggregatibacter actinomycetemcomitans*) can penetrate into and persist in epithelial cells of the periodontal pockets and outer gingiva [26,32,54], thus avoiding host immunity and conventional antimicrobial drugs [16]. In this case using systemic antibiotic therapy is limited by the MIC (minimal inhibitory concentration) of the drug, which is difficult to achieve in GCF (gingival crevicular fluid) and scarcely possible in bacteria biofilms. Moreover, there is also a problem of increasing bacterial resistance.

It is commonly known that the success of chronic periodontitis treatment depends on removal of periodontopathogens and their toxic products such as lipopolysaccharide from the dental root surface and periodontal soft tissues, as well as neutralization of host pro-inflammatory cytokines [3,17,33,53]. Conventional treatment such as scaling and root planing (SRP) does not completely eliminate periodontal pathogens, especially in deep periodontal pockets; moreover, it does not prevent this microorganism from penetrating into periodontal tissue. Finally, this predisposes the periodontal pockets to re-colonization, disease relapses and chronicization [1,16,21].

The above-mentioned issues justify the search for alternative antibacterial therapeutic strategies. One of them is a photodynamic therapy against microorganisms called antimicrobial photodynamic therapy.

The mechanism of action of aPDT

Photodynamic therapy was discovered at the beginning of the 20th century by accident and then implemented in medicine at the early stages of neoplasm treatment. It consists of three elements: harmless visible light, a non-toxic photosensitizer and oxygen [51]. It is based on the principle that the photosensitizer (or photo-activatable substance) binds to the targeted cells and then can be activated by light of the appropriate wavelength in the presence of oxygen. This results in the generation of singlet oxygen and free radicals, which are extremely toxic to certain cells and bacteria [27,28,44,56]. Fundamentally, neither photosensitizer nor light alone should induce a cytotoxic effect on the cells; however, some bacteria, called black-pigmented (e.g. *Prevotella* and *Porphyromonas spp.*), can be killed by light at a wavelength of 660 nm. They are related to inner porphyrins (photoactivatable substances), which are synthesized by bacteria themselves [51].

Originally, the use of photodynamic therapy in medicine was focused on neoplasm cell inactivation. Because of Oskar Raab, who first demonstrated that the antimicrobial action of photodynamic therapy caused the lethal effect of acridine hydrochloride and visible light on *Paramecia caudatum*, photodynamic therapy was applied in medicine against bacteria [47]. Inactivation of microorganisms using photodynamic therapy has been defined as either antimicrobial photodynamic therapy (aPDT), photodynamic antimicrobial chemotherapy (PACT) or photodynamic disinfection.

The bactericidal effect of aPDT is achieved by bacteria DNA [12] or cytoplasmic membrane destruction [5]. The destruction of cytoplasmic membrane is the main mechanism of aPDT. The cytotoxic species generated by photodynamic therapy lead to inactivation of the membrane transport system and inhibition of plasma membrane enzyme activities.

The mechanism of the action of aPDT is as follows: initially, a photosensitizer at ground state is activated to a highly energized triplet state by irradiation with light of a certain wavelength. The excited photosensitizer has a longer lifetime, which results in interactions with the surrounding molecules, and it is generally assumed that at the triplet state the generation of cytotoxic species occurs. The triplet-state photosensitizer reacts with biomolecules using two different pathways (types of reactions) [14].

Type I reactions focus on hydrogen-atom abstraction or electron-transfer reactions between the excited state of the photosensitizer and an organic substrate molecule of the cells, which generates highly reactive free radicals and radical ions. These free-radical species interact with endogenous molecular oxygen to produce highly reactive oxygen species such as superoxide, hydroxyl radicals and hydrogen peroxide, which cause disintegration of the cell membrane resulting in irreversible biological damage [14].

The type II reaction involves direct interaction of the triplet-state photosensitizer with molecular oxygen. It leads to production of an electronically excited and highly reactive state of oxygen called singlet oxygen ($^1O_2$). It can react with a large number of biological structures because of its high chemical reactivity, causing oxidative damage and eventually lethal effects on the bacterial cell resulting from destruction of the cell membrane and wall [14].

Singlet oxygen can kill bacteria, viruses, protozoa and fungi. Its lifetime in biological systems is 0.04 μs and its radius of action is 0.02 μm. Due to its short lifetime, the migration of singlet oxygen from the site of its formation is limited, so initial cell damage is tightly restricted to the localization of the photosensitizer. Thus, local application of the photosensitizer leads to a localized response and ensures the protection of distant molecules, cells and organs from side-effects [51]. The type II reaction takes place in antimicrobial photodynamic therapy.

An important agent of aPDT is a photosensitizer, which should possess the following properties: a high binding affinity for the given microorganism, a broad spectrum of action, a low binding affinity for mammalian cells to avoid the risk of photodestruction of host tissues, a low propensity for selecting resistant bacterial strains, a minimal risk of promoting mutagenic processes, and low chemical toxicity [47].

Generally, gram-positive bacteria are susceptible to photoinactivation whereas gram-negative bacteria are often resistant to it, if the permeability of their outer membrane is not modified. This is connected with the difficulties encountered by a photosensitizer in penetrating into gram-negative bacterial cells. Antimicrobial photosensitizers such as porphyrins, phthalocyanines and phenothiazines (e.g. methylene blue and toluidine blue O) have been reported to penetrate into gram-positive and gram-negative bacteria. The positive charge seems to promote the binding of the photosensitizer to the gram-negative bacterial membrane and leads to its localized damage, resulting in an increase in its permeability. Hence, toluidine blue O and methylene blue are commonly used in aPDT. The hydrophilicity, low molecular weight and positive charge of methylene blue allow passage across the porin-protein channels in the gram-negative outer bacterial membrane. Methylene blue interaction with the anionic lipopolysaccharide macromolecule of gram-negative bacteria results in the generation of methylene blue dimers, which participate in the photosensitization process [47,55]

Effectiveness of PDT in periodontitis and perspectives

A lot of studies have shown that periodontal bacteria demonstrate susceptibility to photodynamic therapy in the planktonic phase [48,57,58], as well as in biofilms [59,60,61]. However, bacterial eradication from dental plaque-derived biofilms is still at a lower level compared to the planktonic condition. The study by Fontana et al. confirmed this fact. The aim of this study was to assess the effectiveness of methylene blue-mediated photodynamic therapy both in the planktonic and the biofilm phase. Photodynamic therapy eliminated approximately 63% of bacteria in the planktonic phase, whereas only 32% of bacteria in biofilms, which derived from the same plaque samples. Moreover, in both cases a lower percentage of persistent bacteria was noted when the photosensitizer concentration was 50 μg rather than 25 μg. Despite the lower effectiveness of photodynamic therapy in the reduction of biofilm bacteria as opposed to planktonic bacteria, the difference was only twofold, whereas antibiotics have been reported to be approximately 250-fold less effective under these conditions [13,35].

The use of another photosensitizer in aPDT such as toluidine blue O, chlorin e6 or poly-L-lysine also failed to eradicate microorganisms in dental biofilms completely [13,20,41]. The probable explanations for the lower effectiveness of photodynamic therapy in dental plaque-derived biofilms are as follows:

- the reduced susceptibility to aPDT may be related to the distinct and protected phenotypes expressed by dental plaque microorganisms once they attach to the tooth [9,13]. These phenotypic changes, which are critical for the development of dental biofilm resistance, are still retained by bacteria in suspension [13],
- the effects of methylene blue-mediated aPDT may be related to the inactivation of the photosensitizer [12] and its reduced penetration may result from the presence of proteins derived from both saliva and gingival crevicular fluid [13],
- it has been shown that phenothiazine-based photosensitizers, including methylene blue and toluidine blue O, are substrates of multidrug resistance pumps in bacteria [52],
- biofilm bacteria can exist in a slow-growing or starved state [6].

In the studies by Fontana et al., the reduced susceptibility of biofilms was caused by reduced penetration of methylene blue into a biofilm and its retention in the outer layers of biofilm clusters as revealed by confocal scanning laser microscopy [13]. Similar findings were obtained by O'Neill et al. [38], who studied toluidine blue-mediated aPDT. It has been suggested 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 [49], the diameter of which may range from 20 to 600 μm [40].

The role of photodynamic therapy in periodontitis treatment is growing. However, the issue of the reduced susceptibility of complex oral biofilms to aPDT requires the development of novel delivery and targeting approaches [47].

Recently, attention has been paid to substances designed to target the biofilm matrix or non-growing bacteria (persistent cells) within biofilms. Among these one can find bacteriophages and naturally occurring or synthetic antimicrobial peptides, which act against bacteria without the emergence of resistance. Targeted therapy using light alone, antibody-photosensitizer and bacteriophage-photosensitizer conjugates or nanoparticles has gained increasing attention [47].

Phototherapy operates via killing bacteria, especially those having their own natural photosensitizer. It is particularly concerned with the oral black-pigmented periodontopathogens. Species such as *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, and *Prevotella melaninogenica* account for the increased bleeding tendency of long-standing gingivitis and the development of periodontitis [34,45]. In addition, they are associated with the pathogenesis of cardiovascular disease [31].

Prevotella spp. have also been recognized as potential producers of volatile sulfur compounds responsible for oral malodor (halitosis) [25]. The Soukos and Goodson studies have shown that broadband light ranging from 380 to

520 nm was able to achieve a threefold reduction in the growth of *P. gingivalis* and *Prevotella* spp. [47]. In another study a reduction in the levels of volatile sulfur compounds was found after human salivary microflora was exposed to blue light of 400-500 nm, suggesting that this kind of light may be applicable in halitosis treatment [49].

In healthy subjects dental plaque remains stable for prolonged periods of time because of a dynamic balance among the resident members of its microbial community [30]. A breakdown of the microbial homeostasis leads to an increase in the number of pathogens [29]. So, in this case specific suppression of key pathogens may result in an increase in the microbial flora associated with oral health [47]. Based on these issues, visible light could be used prophylactically to reduce the levels of black-pigmented bacteria associated with gingivitis, periodontitis and halitosis. Daily and very short exposures of periodontal pockets to visible blue light in human subjects with gingivitis, periodontitis and malodor may have an impact on the reduction of bleeding in gingivitis and of inflammation in periodontitis, and cure oral malodor [47]. The advantages of this novel technique are as follows:

- rapid and painless application of light,
- selectivity in its effect,
- full penetration of dental plaque by light,
- absence of phototoxicity to human cells,
- no effects on taste,
- possible clinical and microbiological benefit with minimal impact on natural microbiota [47].

Targeted therapy using antibodies conjugated with photosensitizers is useful mainly in the treatment of malignant diseases. Selective killing of *Porphyromonas gingivalis* was achieved in human gingival fibroblasts using a murine monoclonal antibody against *Porphyromonas gingivalis* lipopolysaccharide conjugated with toluidine blue O [5].

The therapeutic potential of these approaches for bacterial targeting is connected with a minimal risk of host cell damage. Thus, these approaches are a reason for further exploration via in vitro and in animal studies [47].

One goal of the introduction of nanoparticles to antimicrobial photodynamic therapy was an increase in the effectiveness of photodynamic therapy via greater penetration of photosensitizers and a reduction of their pump back out by multi-drug resistant pumps. Recently, PLGA (poly-lactide-co-glycolide) nanoparticles (150-200 nm in diameter) have been used for various photosensitizers [19,23,24,39,42]. The nanoparticle matrix PLGA is a polyester co-polymer of polylactide and polyglycolide that has received approval by the US Food and Drug Administration as a result of its biocompatibility and its ability to degrade in the body through natural pathways [62]. Photosensitizers encapsulated with PLGA lose their phototoxicity because of excited state quenching. Phototoxicity returns when a photosensitizer is released from nanoparticles.

The PLGA nanoparticles are attractive products for clinical use due to their large critical mass (concentrated package of photosensitizer) for the production of reactive oxygen species that destroy cells. These limit the cell's ability to pump the drug molecule back out and reduce the risk of multiple drug resistance. In addition, the nanoparticle matrix is non-immunogenic [47].

The use of PLGA nanoparticles as carriers of various photosensitizers has not yet been sufficiently explored in aPDT. Klepac-Ceraj et al. investigated the effect of aPDT on human dental plaque bacteria in suspension and the biofilm phase in vitro using methylene blue (MB)-loaded PLGA nanoparticles with a positive or negative charge and red light at 665 nm [22]. Antimicrobial photodynamic therapy with MB-loaded PLGA nanoparticles resulted in the reduction of bacterial viability in a biofilm by 48% for cationic nanoparticles and by 40% for anionic nanoparticles. The results are only 10% better than aPDT without using nanoparticles. The cationic MB-loaded PLGA nanoparticles exhibited higher phototoxicity towards bacteria in a biofilm than anionic ones. This may be related to the faster release of MB by cationic nanoparticles, whereas the lower reduction of bacterial viability in a biofilm compared to in suspension should not be surprising because the biofilm bacteria showed resistance to aPDT. The negatively charged biofilm matrix that hinders penetration of a positively charged agent because of its strong ionic interactions may be the reason for the reduced bacterial susceptibility in biofilms. However, it has been reported that even when there is strong ionic interaction between a negatively charged matrix and a positively charged antimicrobial agent, diffusion of the agent is not hindered to a great extent and, once the binding sites have been filled, the matrix would not present any further barrier to diffusion [36].

Another reason for the lower photodestruction of biofilm bacteria in MB-loaded PLGA mediated aPDT may be related to the failure of nanoparticles to penetrate into the interior of cell clusters by forming aggregates with other nanoparticles as well as sticking to the biofilm surface. An aggregation of nanoparticles can form a mass larger than the size of the biofilm channel and therefore completely block or hinder the entrance of released MB. Moreover, the increased density of bacterial clusters within biofilms results in a micro-environment with a low pO_2, causing a reduced PDT effect [22].

Finally, it has been suggested that the positively charged PLGA nanoparticles have the potential to be used as carriers of MB for photodestruction of oral biofilms [22].

Antimicrobial photodynamic therapy is commonly used alone or as an adjunct to scaling and root planing in periodontitis treatment. Methylene blue-mediated aPDT using the Periowave™ (Ondine Biopharma, Vancouver, Canada) or phenothiazine chloride-mediated aPDT using a HELBO Photodynamic System (Grieskirchen, Austria) are usually applied in clinical studies in the following way: the

photosensitizer is applied directly in the dental pockets for 60 s followed by exposure to red light with a 670 nm wavelength via a fiberoptic probe for 60 s per pocket or per tooth (10 s per site, six sites in total). Output power is 140-150 mW and energy density 10-20 J/cm^2 using the Periowave system, whereas average output power in HELBO Photodynamic Systems is 75 mW [43,47]. However, it is worth emphasizing that optimal conditions for efficient aPDT, such as photosensitizer type and its concentration, application time of photosensitizer, exposure time, type of light and its intensity and exposure frequency, have not been established yet.

From the periodontologist's perspective, aPDT clinical safety is very important. The risk and side effects of antimicrobial photodynamic therapy should be considered. Basically, they are classified into two categories: one relates to the effect of light energy itself; and the other is related to the photosensitizer and the photochemical reaction.

Currently, diode lasers are mostly used as the light source. When using lasers safety rules relating to eye protection must be followed. The most important precaution in laser surgery is the use of protective glasses by the patient, the operator and the assistants [41]. An additional problem is thermogenesis occurring as a result of interaction of the laser with the tissue. This problem mainly concerns high level lasers but also using a diode laser for an extended period of irradiation must be avoided to prevent any thermal accumulation or injury to deeper tissues such as bone or dental pulp [51].

The side effects relating to photosensitizer and photochemical reaction concern the probable toxicity of the photosensitizer to periodontal tissues because of the fact that the photosensitizer alone can exhibit bactericidal action [10]. Moreover, most of the dyes used in aPDT adhere strongly to the soft tissue surface of the periodontium, causing retention of the dyes in the pocket. Their presence, even for a short time, can negatively affect periodontal tissue attachment healing. It should be pointed out that the dye solution is not routinely removed clinically after a completed aPDT application, which causes temporary pigmentation of the periodontal tissue. It is unfavorable for the patient's aesthetics. Thus, the use of photosensitizers with a paste base instead of liquids has been suggested, because pastes can be easily removed after the treatment [41,51].

Several studies assessing the effectiveness of photodynamic therapy in periodontitis treatment have not so far indicated the superiority of antimicrobial photodynamic therapy over conventional treatment. A systemic review and meta-analysis concerning the effect of photodynamic therapy on periodontitis was performed by Azarpazhooh et al. in 2009. Conclusions were as follows: Photodynamic therapy as an independent therapy or as an adjunct to SRP was not superior to control treatment than SRP. Combined therapy of PDT + SRP indicated a probable efficacy in clinical attachment level (CAL) gain of 0.34 mm and probing depth (PD) reduction of 0.25 mm [2]. Results were compared to SRP alone. Another meta-analysis was performed by Sgolastra et al. in 2011. The authors suggested that the use of aPDT as an adjunct to conventional treatment provides short-term benefits in terms of CAL gain (at 3 months after treatment CAL +0.23 mm) and PD reduction (at 3 months after treatment PD -0.21 mm). There were no significant changes after 6 months, but this may be related to the small number of studies that report results at 6 months. The safety of aPDT was confirmed in this meta-analysis. The authors emphasized that there is not enough evidence concerning the effectiveness of the use of antimicrobial photodynamic therapy as an alternative to scaling/root planing in chronic periodontitis treatment and they suggested that more well-designed, long-term, randomized clinical trials are needed before adjunctive aPDT can be considered a reliable, routine and predictable treatment [43].

Recently, Novaes et al. investigated changes occurring in the subgingival microbiological composition of subjects with aggressive periodontitis treated with antimicrobial photodynamic therapy in a single episode or SRP. This trial indicated that aPDT is more efficient in reducing the presence of *Aggregatibacter actinomycetemcomitans* than SRP. On the other hand, SRP limited the number of periodontal pathogens of the Red Complex more effectively than aPDT. Because of the fact that aPDT and SRP affect different species, it is suggested that both methods be combined to gain better results in non-surgical treatment of aggressive periodontitis [37].

In order to obtain optimal conditions for effective antimicrobial photodynamic therapy, an attempt was made to change the photosensitizer and use Radachlorin in ligature-induced periodontitis in dogs, which resulted in no additional benefits for either clinical parameters (PPD – pocket probing depth, CAL – clinical attachment level, BoP – bleeding on probing) or cytokine profile in GCF (gingival crevicular fluid) [46]. In other studies, the influence of repeated adjunctive antimicrobial photodynamic therapy on bone loss (BL) in furcation areas in rats with experimental periodontitis was also evaluated. This trial showed that repeated aPDT did not improve BL reduction when compared to a single episode of aPDT [15].

Several studies have compared the effectiveness of treatment of residual pockets with photodynamic therapy, diode laser or deep scaling [7,8,18]. The use of photodynamic therapy, deep scaling and diode laser for the treatment of residual pockets in the trial of Giannopoulou et al. resulted in a significant clinical improvement for all three treatments and led to significant changes in several cytokines and acute phase proteins after treatment irrespective of treatment modality. It was indicated that aPDT and SRP suppressed *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* more strongly, and resulted in fewer persisting pockets after 6 months than diode soft laser therapy [8,18].

Treatment of residual pockets usually involves repeated mechanical cleaning of the tooth surface to remove mineralized and non-mineralized bacterial deposits. This procedure leads to irreversible hard tissue damage and gum recession, causing increased sensitivity of the treated teeth to various stimuli [18]. Thus, the use of an alternative treatment modality, such as aPDT, may be beneficial for clinical practice.

Giannelli et al., in one of their more recent studies, compared the efficacy of photoablative and photodynamic diode lasers in adjunct to SRP and SRP alone for the treatment of chronic periodontitis. Initially, an 810 nm diode laser was used in photoablative (Pa) mode for removal of junctional, sulcular and outer gingival epithelium. Photoablative intra/extra-pocket de-epithelization with a diode laser was followed by single SRP and multiple photodynamic treatments (once weekly, 4-10 applications) using the 635 nm diode laser and 0.3% methylene blue as photosensitizer. The therapy effects were evaluated at the beginning and one year after treatment. The laser and SRP therapy enabled a significant reduction of PD (-1.9 mm) and BoP (-33.2% bleeding sites) and gain CAL (1.7 mm) to be achieved. A reduction in the level of bacterial contamination, especially spirochetes [16], was also observed.

Thanks to photoablative (Pa) and photodynamic (Pd) diode laser treatment adjunctive to conventional SRP there was improved healing in a chronic periodontitis patient. The authors suggested that de-epithelization by Pa irradiation and further periodontal decontamination by repeated Pd applications could be considered as synergistic treatments and may both be required for optimum clinical results [16].

Conclusions

Antimicrobial photodynamic therapy seems to be an attractive option as a non-invasive and low-cost treatment approach in the field of periodontology, with confirmed clinical safety [51].

Because antimicrobial photodynamic therapy can be administered locally, a high concentration of the chemical agent can be achieved at the locus of infection, enabling efficient bacterial elimination without inducing bacterial resistance [13,16,51].

Although many studies assessing the effectiveness of antimicrobial photodynamic therapy have not so far indicated superiority of aPDT compared to conventional periodontitis treatment, aPDT adjunctive to SRP improves clinical and microbiological parameters. Furthermore, using aPDT can achieve the same clinical outcomes compared to nonsurgical treatment, whereas antimicrobial photodynamic therapy is a non-invasive modality that allows the prevention of damage to hard and soft periodontal tissues. aPDT may be especially useful as an alternative therapeutic strategy for residual pocket treatment in supportive periodontal maintenance. Finally, the use of low-level energy lasers in aPDT can exert an additional positive influence on the healing of periodontal tissues as a result of the potential biomodulatory effects, such as the stimulation and proliferation of cells [20].

Currently, nonsurgical treatment is still the gold standard of chronic periodontitis treatment. Antimicrobial photodynamic treatment has been reported to be effective as an adjunct to conventional therapy to destroy bacteria in sites where there is limited access for mechanical instrumentation as a result of the anatomical complexity of the roots [51].

The use of aPDT in residual pocket treatment may be an alternative therapeutic strategy because of the additional benefits that can be achieved. There is the prevention of hard and soft tissue damage and the minimizing of the risk of hypersensitivity.

Biofilm resistance to antimicrobial photodynamic therapy still remains the challenge for medical researchers. Development of novel delivery and targeting approaches may help to overcome the low biofilm susceptibility to aPDT and allow aPDT to become a new, efficient modality of periodontitis treatment.

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