PHOTODYNAMIC THERAPY IN THE TREATMENT OF PERIODONTAL DISEASE.

Maria-Alexandra Martu1, Irina-Georgeta Sufaru1*, Ionut Luchian1*, Anamaria Zaharescu2, Liviu Manole2, George-Alexandru Maftei2, Daniel Acatrinei2, Silvia Martu1

1“Grigore T. Popa” University of Medicine and Pharmacy, Depart. of Periodontology, Iasi
2Phd Student “Grigore T. Popa” University of Medicine and Pharmacy, Iasi

Corresponding author: Sufaru Irina Georgeta*: e-mail: irina_ursarescu@yahoo.com
Luchian Ionut* e-mail: ionut.luchian@yahoo.com

Abstract
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. Photodynamic therapy has been suggested as an alternative to chemical antimicrobial agents to eliminate subgingival species and treat periodontitis. Antimicrobial photodynamic therapy seems to be a unique and interesting therapeutic approach towards the treatment of periodontitis and peri-implantitis. The results of a number of in vitro studies clearly demonstrate the effective and efficient bactericidal effect of antimicrobial photodynamic therapy. Antimicrobial photodynamic therapy may hold promise as a substitute for currently available chemotherapy in the treatment of periodontal and peri-implant diseases.

Keywords: periodontal disease, periodontal treatment, photodynamic therapy

Introduction
In the twenty-first century, due to the discoveries made by Pasteur, Leeuwenhoek and Fauchard, periodontal disease is defined as an infectious and inflammatory disease, and the treatment of periodontal disease has evolved dramatically from ointments made of medicinal and mineral herbs combined with honey or gum, used by the Egyptians, therapeutic techniques and instruments developed by Abu al Quasi, then Pierre Fauchard, to the point where the periodontal therapy acquires a new meaning, therapy that emphasizes minimally invasive means, on eliminating the etiological factor, techniques that use local application of antibiotics, the use of lasers and small spectrum drugs.

Currently, one of the therapeutic innovations is Photoactivation, a therapy that was initially used in maxillofacial surgical treatment to delineate normal tissue from dysplastic tissue, and as it advanced in the development of this technique, it was integrated as a therapeutic method in several areas of dental medicine.

Photoactivation therapy is an efficient method based on a relatively simple concept, which requires three components: source light, photosynthesis and oxygen, the process itself involves the exposure of the photoinitiator to a light spectrum that causes a series of chemical reactions, resulting in the production of oxygen species, including singlet oxygen and free radicals, species that act on microbial cell membranes causing cell death.

The applications of this method in periodontology have been taken up since 2002, the method being adopted internationally due to the efficiency of removing perio-pathogenic microorganisms.

Antimicrobial photosensitizing agents and the wavelengths used in periodontal and peri-implant therapy
For the elimination of bacteria in supragingival and subgingival plaque, antimicrobial photodynamic therapy has been applied with various combinations of lasers and photosensitizing agents. In antimicrobial photodynamic therapy, the particular photosensitizers employed are toluidine blue O [tolonium chloride: (7-amino-8-methyl-phenothiazin-3-ylidene)- dimethyl-ammonium (C15H16N3S+)], methylene blue [3,7-
Gram-positive species have a relatively porous cytoplasmic membrane that permits entry of the photosensitizer into the cell. In gram-negative species, an additional outer membrane layer with a characteristic structure works as an effective permeability barrier that inhibits the penetration of host humoral and cellular defense factors and may lead to resistance against many antibiotics. Thus, the outer membrane may reduce or prevent photosensitizer uptake. However, it has been demonstrated that photosensitizers, such as toluidine blue O and methylene blue, which undergo a pronounced cationic charge, can bind to the outer membrane of gram-negative bacteria and penetrate bacterial cells [6], demonstrating a high degree of selectivity for killing microorganisms compared with host mammalian cells. Therefore, toluidine blue O and methylene blue have been the photosensitizers of choice in the treatment of periodontitis and peri-implantitis. However, toluidine blue O seems to exhibit a greater ability for killing gram-positive and gram-negative bacteria than methylene blue.

Elimination of A. actinomycetemcomitans, P. gingivalis and Fusobacterium nucleatum has been demonstrated to be more effectively achieved whilst using toluidine blue O than methylene blue [7]. It has been shown in vitro that toluidine blue O interacts with lipopolysaccharide more effectively than does methylene blue [6], thus a greater photobactericidal effect of toluidine blue O against gram-negative bacteria can be expected than for methylene blue. In the past, photosensitizer activation was achieved by a variety of light sources such as argon lasers, potassium titanyl phosphate or neodymium-doped: yttrium, aluminum and garnet (Nd:YAG) lasers [8].

Currently, however, the light sources of a specific wavelength mostly applied in photodynamic therapy are those of helium–neon lasers (633 nm), gallium-aluminum-arsenide diode lasers (630–690, 830 or 906 nm) and argon lasers (488–514 nm), the wavelengths of which range from visible light to the blue of argon lasers, or from the red of helium–neon and gallium–aluminum–arsenide lasers to the infrared area of some diode lasers.
High-level-energy laser irradiation is not used to activate the photosactive dye because relatively low-level exposure produces a high bactericidal effect. Several types of laser devices have been applied during in vitro research studies. However, in the case of in vivo and clinical investigations, the diode lasers are the light source predominantly applied. Although toluidine blue O was generally selected as the photosensitizer of choice in previous in vitro studies, methylene blue has been used mainly in clinical studies because clinical photodynamic therapy kits that include methylene blue are already commercially available (PeriowaveTM; Ondine Biopharma Corporation, Vancouver, Canada) (Helbo; Photodynamic Systems GmbH & Co. KG, Grieskirchen, Austria). Nonlaser light sources, such as light-emitting diodes (LED), have been suggested as new light activators in photodynamic therapy as LED devices are more compact and portable and the cost is much lower compared with that of traditional lasers.

![Diagram showing the steps of application of antimicrobial photodynamic therapy in the treatment of periodontitis.](image)

Based on the advantages and characteristics of antimicrobial photodynamic therapy, it has been proposed that periodontal and peri-implant diseases are potential targets of this novel antimicrobial photochemotherapy. Antimicrobial photodynamic therapy is expected to resolve the difficulties and problems of conventional antimicrobial therapy and can work as an adjunctive to conventional mechanical treatments.

The photosensitizer is placed directly in the periodontal and peri-implant pocket and the liquid agent can easily access the whole root or implant surface before activation by the laser light through placement of the optical fiber directly in the pocket (Fig. 1). As a result of the technical simplicity of the method and the high effectiveness of bacterial killing, the application of antimicrobial photodynamic therapy in the treatment of periodontal and peri-implant diseases has been studied extensively.

**In vivo studies of the antimicrobial effects of photodynamic therapy in periodontal therapy**

Animal studies have been performed to help clarify the clinical response to antimicrobial photodynamic therapy application in periodontal therapy. Some animal studies have reported a reduction in the microbial load in ligature-induced periodontitis following the application of photodynamic therapy. Komerik et al. [9] demonstrated that a significant reduction in the *P. gingivalis* count was detected after the treatment of experimentally induced...
periodontitis in rats using toluidine blue O in combination with a diode laser. Sigusch et al. [10] showed that the chlorin-e6 plus diode laser also achieved a reduction in the P. gingivalis count in dogs, but failed to reduce the number of F. nucleatum.

Following a reduction in the microbial load in periodontal diseases, improvements in signs of clinical inflammation, such as redness and bleeding on probing, were also demonstrated. Both toluidine blue O-mediated photodynamic therapy used in rats and chlorin-e6-mediated photodynamic therapy applied in dogs [10], resulted in improvement of the periodontal symptoms. Qin et al. [11] reported a significant reduction in the total bacterial flora and, histologically, a large reduction in inflammatory cell infiltration after application of antimicrobial photodynamic therapy (toluidine blue O + diode laser) in the treatment of experimentally induced periodontitis in rats. Comparing the photosensitization of periodontal bacteria with scaling and root planing, the clinical and histological improvements, as well as bacterial elimination, following photodynamic therapy gave results similar to those of conventional scaling. Sigusch et al. [10] demonstrated that antimicrobial photodynamic therapy (chlorin-e6 and BLC1010 + diode laser) was distinctly more advantageous than laser treatment alone or no treatment in reducing the periodontal signs of redness and bleeding on probing in dogs, and resulted in significant suppression of P. gingivalis.

Regarding the effect of antimicrobial photodynamic therapy on bone levels, Komerik et al. [9] demonstrated, in a histological examination using rats, that, 90 days post-treatment, toluidine blue O-mediated photodynamic therapy had induced a decrease in alveolar bone loss around teeth with experimentally induced periodontitis. de Almeida et al. [12] compared, histologically and radiographically, the progression of experimentally induced periodontitis after treatment with methylene blue alone, low-level laser therapy alone, or with methylene blue followed by low-level laser therapy (photodynamic therapy). The results of radiographic evaluation demonstrated that photodynamic therapy had a short-term effect (up to 15 days) upon the reduction of periodontal tissue destruction. However, at 30 days there were no significant differences between the groups. de Almeida et al. [12] also compared the effect of methylene blue, low-level laser therapy and photodynamic therapy treatments on the bone loss of periodontally affected furcations in rats.

The photodynamic therapy showed a short-term effect (up to 15 days) upon decreasing bone loss, but no significant differences between groups were observed at 30 days post-therapy. In addition, de Almeida et al. [12] confirmed that adjunctive antimicrobial photodynamic therapy led to significant reductions in periodontal bone loss in diabetic rats, suggesting that antimicrobial photodynamic therapy might also be an effective adjunctive to conventional mechanical treatment in diabetic patients. Generally, antimicrobial photodynamic therapy appears to suppress periodontal pathogens and to reduce signs of inflammation effectively and safely in periodontitis in vivo. However, there is a lack of evidence to prove that antimicrobial photodynamic therapy is capable of suppressing periodontopathogens in a single dose or course. Further in vivo studies investigating the antimicrobial effects on different periodontal pathogens need to be performed.

The use of antimicrobial photodynamic therapy may reduce signs of periodontal inflammation and alveolar bone loss in experimentally induced periodontitis. However, two studies have shown a tendency for regression within 30 days after treatment in the effects on bone levels. Consequently, the long-term therapeutic outcomes should be further evaluated in animal models. The limited number of in vivo studies available indicates that antimicrobial treatment to scaling.

Clinical studies of application of antimicrobial photodynamic therapy in the treatment of periodontal disease

One study has reported on the use of nonsurgical therapy in aggressive periodontal disease [13] (Fig. 2).
Yilmaz et al. [14] randomly assigned a total of ten patients to receive repeated application of scaling and root planing + photodynamic therapy (methylene blue + 30 mW diode laser), scaling and root planing alone, photodynamic therapy alone or supragingival oral hygiene instructions. Methylene blue served as the photosensitizer and was used as a mouth rinse. Scaling and root planing was performed on days 1 and 7, while the laser was repeatedly applied over each papillary region (not into periodontal pockets) on days 1, 2, 4, 7, 9 and 11. After 32 days of healing, significant clinical and microbiological improvements were only observed in the scaling and root planing + photodynamic therapy and scaling and root planing alone groups.

By contrast, improvements following photodynamic therapy treatment alone, as well in those receiving oral hygiene instructions, did not reach statistical significance. Regarding laser treatment, there were no complaints (such as discomfort, sensitivity or pain) from subjects immediately after therapy or at 3 weeks post-therapy.

The authors concluded that antimicrobial photodynamic therapy provided no additional microbiological and clinical benefits over conventional mechanical debridement. The reduced effectiveness of photodynamic therapy in this study may be a result of the indirect application of photodynamic therapy from the external surface of the gingiva.

Fig. 2 Clinical application of antimicrobial photodynamic therapy in the treatment of periodontitis.

Two randomized controlled clinical studies have evaluated the short-term clinical effects (up to a period of 3 months) of adjunctive antimicrobial photodynamic therapy to scaling and root planing in patients with chronic periodontitis [15].

Andersen et al. [16], using a parallel three-arm design, compared the effectiveness of antimicrobial photodynamic therapy with that of scaling and root planing for nonsurgical treatment of moderate to advanced periodontal disease. A total of 33 patients were assigned to photodynamic therapy alone (methylene blue + 50 mW diode laser), scaling and root planing alone or scaling and root planing + photodynamic therapy.

Clinical assessments of bleeding on probing, probing pocket depth and clinical attachment level were made. After three months of healing it was observed that a combination of scaling and root planing + photodynamic therapy resulted in significant improvements in the investigated parameters over the use of scaling and root planing alone at all evaluation time points.

Braun et al. [15] evaluated the effect of adjunctive antimicrobial photodynamic therapy (methylene blue + 100 mW diode laser) in chronic periodontitis using a split-mouth design. A total of twenty patients received a scaling and root planing procedure and the quadrants were randomly assigned to
an additional treatment with photodynamic therapy. Following irrigation after a residence time of 3 mins, the remaining photosensitizer was activated for 10 s per site (six sites in total).

After 3 months of healing, the adjunctive use of photodynamic therapy resulted in a significantly higher change in mean relative attachment level, probing pocket depth, sulcus fluid flow rate and bleeding on probing at the sites receiving photodynamic therapy than at the sites receiving scaling and root planing alone.

Accordingly, it was concluded that the clinical outcomes of conventional scaling and root planing may be improved by adjunctive antimicrobial photodynamic therapy in patients with chronic periodontitis.

Christodoulides et al. [17] evaluated the clinical and microbiological effects of the adjunctive use of antimicrobial photodynamic therapy (methylene blue + 75 mW diode laser) to nonsurgical periodontal treatment. A total of twenty-four patients suffering from chronic periodontitis were randomly assigned to either scaling and root planing followed by a single application of photodynamic therapy, or scaling and root planing alone.

The photosensitizer was applied to the instrumented sites and thoroughly rinsed with sterile saline after 3 mins. The fibre tip was moved circumferentially around the tooth for 1 min, as recommended by the manufacturer. After 3 and 6 months of healing, both treatment procedures resulted in statistically and clinically significant reductions in mean probing pocket depth and clinical attachment level. However, no statistically significant differences in terms of clinical attachment level and probing pocket depth changes were found between the two groups. Similarly, both treatment procedures revealed comparable microbiological changes in common periodontal pathogens.

However, at 3 and 6 months, the test group exhibited a significantly higher improvement in mean full-mouth bleeding scores, which might be partly attributed to the additional photo-biomodulation effect mediated by the low-level laser irradiation during photodynamic therapy [18]. Based on these findings, it was concluded that a single episode of photodynamic therapy, as an adjunct to scaling and root planing, failed to result in an additional improvement in terms of probing pocket depth reduction and clinical attachment level gain. However, it resulted in a significantly higher reduction in bleeding scores, which should be taken into consideration under clinical conditions [17].

Similar results were also observed when the same device was used as an adjunct to nonsurgical periodontal treatment in patients on periodontal maintenance in a study reported by Chondros et al. [19].

Only one study, by de Oliveira et al. [13], reported on the outcome of antimicrobial photodynamic therapy monotherapy for the treatment of aggressive periodontitis. A total of 10 patients were randomly assigned, according to a split-mouth design, to either photodynamic therapy (methylene blue + 60 mW diode laser) or scaling and root planing. Laser application was performed for 10s per site after 3 mins of residence time of the photosensitizer. Three months later, both treatment procedures gave comparable clinical outcomes, as evidenced by probing pocket depth reductions and clinical attachment level gains, suggesting a potential clinical effect of photodynamic therapy as an alternative to scaling and root planing. In both groups, the beneficial effects were more pronounced at initially moderate and shallow pockets.

Taken together, the data available from controlled clinical studies indicate that in patients with chronic periodontitis, the adjunctive use of antimicrobial photodynamic therapy to scaling and root planing may result, on a short-term basis (up to 3 or 6 months), in (i) higher reductions in bleeding on probing compared with scaling and root planing (as observed in three studies) and (ii) higher probing pocket depth reductions and clinical attachment level gains compared with scaling and root planing alone (in two studies).

When interpreting the available data, it should be kept in mind that the evidence from randomized controlled clinical studies, evaluating the potential clinical benefit of photodynamic therapy in the treatment of periodontitis, is still limited.

The main drawbacks may be related to the rather limited number of patients, the
short-term duration of studies (i.e. 3 or 6 months) and the fact that the most effective protocol of antimicrobial photodynamic therapy has not been established. The available data seem to indicate that the adjunctive use of antimicrobial photodynamic therapy in nonsurgical periodontal therapy may improve the clinical outcome, but further studies are warranted before definitive conclusions can be drawn on the clinical relevance of antimicrobial photodynamic therapy in periodontal therapy.

Furthermore, Brink and Romanos compared the clinical and microbiological effects of scaling and root planing + Nd:YAG laser (2W), scaling and root planing + 980 nm diode laser (2W), and scaling and root planing + antimicrobial photodynamic therapy [methylene blue + 670 nm diode laser (75 mW)] and scaling and root planing alone in patients with chronic periodontitis [20]. The authors reported that in the group treated with antimicrobial photodynamic therapy + scaling and root planing, bleeding on probing was reduced significantly more, one to three months following treatment, than in the other groups. In addition, the bactericidal effects of scaling and root planing + antimicrobial photodynamic therapy appeared to be greater than those of the scaling and root planing + Nd:YAG laser, scaling and root planing + diode laser, or scaling and root planing alone treatments.

Conclusions
Antimicrobial photodynamic therapy may hold promise as a substitute for currently available chemotherapy in the treatment of periodontal and peri-implant diseases. 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.

References