# Photo-activated Toluidine Blue O as Adjunctive Periodontal Treatment

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*The periodontal disease represents an inflammatory disease with an infectious etiology; the photo-activated disinfection of the periodontal pockets was proposed as an adjunctive form of treatment to the classic mechanical scaling and root planning. The purpose of our study is to assess the efficiency of the photo-activated toluidine blue on the clinical parameters of the patient with chronic periodontitis versus classic mechanical treatment alone.*

*Keywords: toluidine blue O, photo-activated disinfection, periodontal bacteria, clinical parameters.*

The periodontal disease is an inflammatory disease with a complex etiology, among which the bacteria play an essential role. The breakdown of the periodontal tissue is initiated by the host response to the bacterial activity and virulence factors. There are various parameters to quantify the inflammation and the tissue lost, such as: bleeding on probing, clinical attachment loss, probing depth [1], tooth mobility.

The specific periodontal therapy includes three major steps: the etiologic therapy, the corrective therapy and the maintaining therapy, individualized for each clinical case. The etiologic therapy combines different methods for the bacterial plaque removal (supra-gingival and sub-gingival calculus removal, root planning, periodontal curettage) considering also the anatomo-clinical features of cervical area [2]. Adjunctive therapy methods can be added to the standard measures of etiologic therapy, including the antibiotic therapy or the host defense modulators. In the last decade a new method was emphasized: the photodynamic therapy.

The photodynamic therapy involves three major components: the visible light, the oxygen and a nontoxic photosensitizer (a photo-activated substance). The photosensitizer binds to the target cells and is activated by the light source, producing singlet oxygen and other reactive agents, highly toxic to bacteria [3, 4].

The excited singlet oxygen can oxidize many biological molecules (proteins, nucleic acids and lipids), leading to its cytotoxicity. Singlet oxygen has a diffusion distance of approximately 100nm and a half-life of <0.04 ls [5]. The photodynamic activity is influenced by the type, the dose, the incubation time and the localization of the photosensitizer, the wavelength of the light source (nm), the light power density (mW/cm$^2$) and the light energy fluence (J/cm$^2$). In this type of therapy the toxic effect is mainly due to the damage of the cytoplasmic membrane and of the DNA [6].

The ideal photosensitizer should present the following properties: a high quantum yield of triplet state to obtain large concentrations of the activated drug; a high singlet oxygen quantum yield; high binding affinity for microorganisms; a broad spectrum of action; low binding affinity for mammalian cells to avoid the risk of photo-destruction of host tissues; a low propensity for selecting resistant bacterial strains; a minimal risk of promoting mutagenic processes; and low chemical toxicity [7-10].

Toluidine blue O (tolonium chloride) is a vital dye used for detecting the mucosal abnormalities of the uterine cervix and oral cavity and for demarcating the extent of the lesion before surgery [5] (Fig.1). Toluidine blue O, which undergoes a pronounced cationic charge, can bind to the outer membrane of G- bacteria, penetrating the bacterial cells [11].

It has been demonstrated in vitro that toluidine blue O interacts with bacterial lipopolysaccharides, with a great photo-bactericidal effect [12].

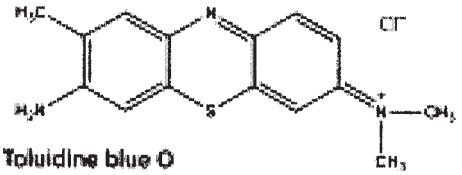


Fig. 1. The chemical structure of the phenotiazine photosensitizer toluidine blue O: $C_{15}H_{16}N_3SCl$ (tolonium chloride, basic blue 17, blutene chloride and methylene blue T50 or T extra)

The purpose of our study was to assess the clinical efficiency of the adjunctive photodynamic therapy in the etiologic periodontal treatment, associated to classic mechanical bacterial plaque removal.

## Experimental part

The present study was conducted in the Periodontology Clinic of "Grigore T. Popa" University of Medicine and Pharmacy, in Iaşi, in collaboration with the Faculty of Medicine of the "Lucian Blaga" University of Sibiu, between May 2013 and February 2014.

The research methodology respected the international standard. The experiments were conducted according to the ethical directives of the Helsinki Declaration and the methods were the ones certified for clinical and para clinical use.

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The information and confirmation principles for research purposes were strictly respected; the signed informed consent for study inclusion was obtained from each patient.

We recruited a number of 72 patients with chronic periodontitis, divided in two groups: the study group and the control group. The patients with periodontal therapy in the last 12 months or with antibiotic therapy in the last 6 months, patients with inflammatory or infectious systemic diseases, patients taking various types of drugs which can affect the periodontal status and smokers were excluded from the study.

Each subject received a rigorous clinical examination; the periodontal clinical indexes (bleeding on probing, periodontal pocket depth, clinical attachment loss) were recorded; all the measurements were conducted with the aid of periodontal probes (Williams). The bleeding on probing was quantified as follows: 0 (no gingival bleeding), 1 (point of bleeding), 2 (linear bleeding), 3 (triangular bleeding) and 4 (drop of blood).

The probing depth was registered for all the teeth present on the dental arch, measured in six points per tooth (mesial-facial, middle-facial, distal-facial, mesial-oral, middle-oral, distal-oral), from the free gingival margin to the base of the pocket; the measurements higher than 3mm per site were considered as pathological.

The clinical attachment loss was measured from the cementum-enamel junction to the base of the periodontal pocket. The periodontal diagnosis was set after the completion of the clinical examination.

The method for dividing the groups was randomized. The study group received etiologic therapy (supra- and sub-gingival scaling, root planning, professional brushing), followed by photo-activated disinfection of the periodontal pockets (LED PAD therapy). The control group received only etiological standard therapy (supra- and sub-gingival scaling, root planning, professional brushing), without photo-activated disinfection.

The LED source used in this study was in the red spectrum (wavelength of 635nm, Denfotex UK) and a viscous solution of tolonium chloride 0.01mg/mL provided by the manufacturer served as a photo-sensitizer (Denfotex UK) (fig.2). We followed all the steps from the operatory protocol, according to the manufacturer's re-commendations.

After the tooth isolation, the photo-sensitizer was meticulously placed in the periodontal pockets, followed by a LED irradiation for 60 s (fig. 3).

For the periodontal pockets with a depth higher than 5 mm we used a special Perio-tip for the light source.

During the photo-activation we used protection goggles for the protection of the patient and of the medic.

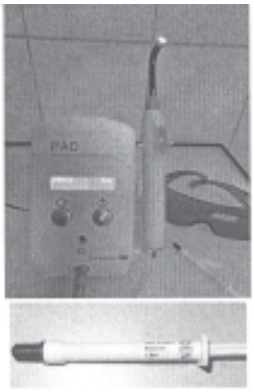

Fig. 2. The PAD device and the syringe with viscous solution of toluidine blue O

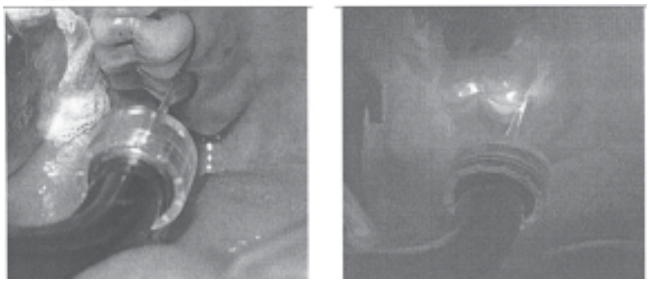

Fig.3. The light source with the Perio-tip placed in the periodontal pocket, before and after activation

The PAD therapy was repeated at 7, 14 and 21 days from the first session. The patients were recalled after 2 months for re-assessment. The periodontal clinical parameters were also re-assessed.

The baseline and after two months data were registered and statistically analyzed; for the statistical analysis we used the Microsoft Excel 2010 and PASW 18 Statistics soft-wares.

## Results and discussions

The 72 subjects were divided in two groups: the study group (n = 35) and the control group (n = 37). The subjects included 48 males and 24 females.

The age of the subjects in the study group ranged between 31 and 75 years old (with a mean value of 47.3 ± 3.9 years old) and in the control group, between 36 and 68 years old (with a mean value of 49.6 ± 2.8 years old). The demographic data are summarized in table 1.

After the 2 months examinations we observed a significant improvement of the periodontal parameters. Decreased values for the probing depth and for the BOP were noticed for both groups, with higher differences for the study group than the control group. We also remarked a gain of periodontal clinical attachment, more significant for the PAD group. The clinical statistic results are presented in table 2.

| Parameter | | Study group | Control group |
|---|---|---|---|
| Age (years) | Interval | 31 - 75 | 36 - 68 |
| | Mean | 47.3 ± 3.9 | 49.6 ± 2.8 |
| Gender | Male | 22 | 26 |
| | Female | 14 | 10 |
| Provenience environment | Urban | 26 | 23 |
| | Rural | 9 | 14 |
| Number of sites with classic therapy | | 521 | 552 |
| Number of sites with adjunctive therapy | | 521 | 0 |

**Table 1**
DEMOGRAPHIC DATA OF THE STUDY AND THE CONTROL GROUP

| Periodontal parameter | Study group | Control group | *p* value |
|---|---|---|---|
| Bleeding on probing | 68% | 53% | $p < 0.03$ |
| Pocket depth | 1.24 mm | 0.57 mm | $p < 0.05$ |
| Gain of clinical attachment | 0.37 mm | 0.14 mm | $p < 0.04$ |

**Table 2**
CHANGES OF CLINICAL PARAMETERS OF THE STUDY AND THE CONTROL GROUP

The results of our study support a series of published data reporting positive results of the photo-activation therapy. Improved results were observed in a study using PAD with methylene blue on chronic periodontitis patients [13]. The same favorable results were obtained in another study with LED PAD and phenothiazine chloride [14].

Our re-evaluation results contradict other studies which did not observe a clear advantage of laser PAD in chronic periodontitis patients. A study conducted on patients with chronic periodontitis who received only one session of LED PAD and toluidine blue therapy did not reveal significant changes of the clinical parameters [15]. Another study demonstrated that PAD therapy reduces a series of clinical parameters but not the bleeding on probing, demonstrating a non-significant diminishing of the inflammation degree [16-19].

The optimal parameters required for effective antimicrobial photodynamic therapy-induced killing of supra gingival periodontal pathogens using the combination of different toluidine blue O concentrations and laser-irradiation energies were investigated and reported that diode laser irradiation at 12 JD $cm^2$ with 1 mgD mL of toluidine blue O was the most effective option [20].

The differences between the various studies results can be explained by the different study designs, by the different types of activation sources and by the high variety of photo-sensitizers.

It was also demonstrated in vitro that lipopolysaccharide treated by photodynamic therapy did not stimulate the production of pro-inflammatory cytokines by mononuclear cells [21]; thus, photodynamic therapy may be inactivating endotoxins such as lipopolysaccharide, by decreasing their biological activity.

## Conclusions

The photo-activated disinfection therapy of the periodontal pockets proves itself as a viable adjunctive method to the classical mechanical plaque removal in the chronic periodontitis patients; the LED source is also less aggressive than the usual laser ones, providing a safer and more accessible method and the association of the toluidine blue O determines significant improvement of the periodontal clinical parameters (bleeding on probing, clinical attachment loss and pocket depths).

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