Antimicrobial Effect of 940 nm Diode Laser based on Photolysis of Hydrogen Peroxide in the Treatment of Periodontal Disease

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Moderate and severe periodontitis represents a challenge in the non-surgical periodontal therapy. Due to the lack of evidence regarding the antimicrobial effectiveness of 940 nm diode laser in periodontal treatment, this study aimed to evaluate the antimicrobial effect of hydrogen peroxide (H_2O_2) photolysis performed with 940 nm diode laser in the treatment of moderate and severe periodontitis. Twenty-five patients with 100 teeth were selected for this pilot study. The test teeth were randomly assigned to one of the four treatment groups: Group 1: scaling and root planning (SRP) (control group); and the following experimental groups: Group 2: H_2O_2; Group 3: 940 nm diode laser therapy; Group 4: 940 nm diode laser therapy and H_2O_2. Clinical examinations, like probing depth (PD), clinical attachment level (CAL) and bleeding on probing (BOP) were performed before and after the treatment. The microbiological evaluation, effectuated before and after the treatment, included nine periodontal bacteria species and investigated by means of real-time PCR assay. The clinical and bacterial differences in the tested groups, was assessed between control group and the other three experimental groups, as well as between the experimental groups. The total bacteria load was reduced for all four studied groups. Group 4 (diode laser + H_2O_2) showed significant bacterial reduction of the major periodontal bacteria like Pg., Tf., Td., Pi., Pm., Fn ($p<0.001$) than the other 3 groups ($p>0.001$). Also the periodontal clinical parameters, like PD, CAL and BOP showed a significant reduction after the photolysis of H_2O_2 with the 940 nm diode laser ($p<0.001$). Differences between tested groups showed a significant beneficial results in regard to Group 4.It is suggested that the photoactivation of H_2O_2 with the 940 nm diode laser can be used successfully in adjunctive to the non-surgical periodontal treatment as a bactericidal tool.

Key words: SRP, hydrogen peroxide, photoactivation, 940nm diode laser, antimicrobial effect in periodontal treatments

In the treatment of generalized chronic periodontitis and aggressive periodontitis, it is widely accepted that the use of scaling and root planning (SRP) alone cannot eliminate entirely periodontal bacteria, especially those belonging to red and orange complexes. This disadvantage was overcome by combining the use of local or/and systemic antibiotics with conventional periodontal therapy. Nevertheless, the worldwide increase in antibiotic-resistant Gram-positive and Gram-negative pathogens [1,2] has led in searching for alternative antimicrobial strategies. This alternative approaches are needed especially in dentistry in order to avoid the use of antibiotics in periodontal treatment, endodontic localized infections and other oral infections [3]. Besides the development of bacterial resistance to antibiotic, several drawbacks have been reported in the use of local and systemic antibiotics, such as gastrointestinal disorders, allergic reactions and problems with patient compliance [4]. Therefore, instead of using local antibiotic therapy, researchers and clinicians focused on the use of antimicrobial photodynamic therapy (aPDT) which is comparable to the broad spectrum antibiotics, and can eliminate a wide range of microorganisms in the oral cavity, without dealing with the antibiotic disadvantages [5-7].

Photodynamic therapy (PDT) is a minimally invasive procedure, which utilizes singlet oxygen and free radicals produced by a light-activated photosensitizer to eliminate bacteria. There are two mechanisms of photodynamic therapy, in which photosensitive substances can react with excited O_2 biomolecules [8, 9]. The type I photochemical mechanism, is resulting from the production of highly reactive oxygen species (ROS) (hydroxyl radicals, superoxide ions, hydrogen peroxide), which performs redox reactions with the environment. The ROS type II photochemical mechanism is represented by *singlet oxygen* species. The type II reaction is known as being an important pathway in bacterial cell destruction. These two mechanisms have the ability to kill microorganisms (bacteria, fungi, viruses) by damaging critical cellular molecules, including proteins, membrane lipids and nucleic acids (DNA, RNA) [10]. This technique has been successfully been used in oncology since 1975 [11].

Photodynamic inactivation of microorganisms (PDIM) is considered a new approach in periodontal therapy, where multi-resistant microorganisms can be destroyed efficiently, without affecting the surrounding tissue and without disturbing the nonpathogenic bacterial flora. Various chemical classes of photosensitizers have

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demonstrated their potential to inactivate Gram (+), Gram (-) and fungal cells [12].

Photosensitizers, molecules that are chemically excited by light of specific wavelengths, can sometimes limit the clinician due to the close link between wavelength and photosensitizer. Numerous compounds have been described as potential aPDT photosensitizers for the elimination of periodontal bacteria, however, contradictory results have been shown [13-16]. The use of 3% hydrogen peroxide is a cheap and available solution in every day practice. However, 3% hydrogen peroxide used as a single agent shows a weak antibacterial effect on biofilm associated with periodontitis and peri-implantitis. The photoactivated disinfection (PAD) in which ROS have the ability to affect bacteria components, such as cell membrane, nucleic acid and other cell components, can be achieved by photolysis of 3% hydrogen peroxide (H_2O_2) with light irradiation [17].

In periodontal therapy, studies suggest significant results by using hydrogen peroxide in combination with various devices and LED (Light Emitting Diode) or laser [18], but until now there were no results supporting the bactericidal effect of 3% hydrogen peroxide photoactivation with 940 nm diode laser in the treatment of periodontal disease.

The aim of the present study was to evaluate the bactericidal effect of 3% H_2O_2 photoactivation with 940 nm diode laser, and to present an alternative disinfection procedure for this wavelength in periodontal treatment.

Experimental part

Materials and method

This study was designed as a randomized controlled, single-blind, multi-center trial with a split–mouth design to compare the antimicrobial effect of non-surgical periodontal therapy with scaling and root planning alone (SRP), H_2O_2 alone, 940 nm diode laser alone and 940 nm diode + H_2O_2. The study protocol was approved by Ethical Committee of Ovidius University of Constanta, Faculty of Dental Medicine, with No. 14533/22.09.2015, and conducted according to the Declaration of Helsinki (revised in 2013, Fortaleza, Brazil).

For this study, 30 patient with moderate to severe periodontal disease were selected from the Periodontology Department of Ovidius University of Constanta - Faculty of Dentistry (Constanta, Romania) and a private dental clinic (Dental Laser Center, Constanta, Romania). All subjects signed a written informed consent document prior to treatment. The inclusion criteria were as follows: at least 16 natural teeth present in the oral cavity distributed in four quadrants, a minimum 5 mm periodontal probing depth (PPD) per quadrant with bone resorption evidenced both clinically and radiologically, and bleeding on probing (BOP) in all four quadrants. The exclusion criteria were as follows: patients who are during active periodontal treatment or have had undergone periodontal treatment within 12 months, patients who have had antibiotic therapy (systemic or local) over the last 6 months, systemic conditions that may affect the therapeutic outcome (diabetes type I and II, immune deficiency, HBV, HCV, cancer, haematological disorders, epilepsy, etc.), pregnancy, breastfeeding, incapacity or refusal to follow the study protocol, severe co-morbid conditions that may affect life expectancy within 1 year (e.g. metastatic cancer). Five patients were excluded: two exclusion were due to previous periodontal treatment, one exclusion was due to pregnancy, one exclusion was due to antibiotic administration for acute sinusitis during the periodontal treatment and one exclusion due to interruption of the periodontal treatment. A total of twenty-five patients participated in the study until the end. The flow chart of the study is presented in figure 1.

Baseline examination was performed one week prior to periodontal treatment and included clinical and radiological analysis. The following clinical periodontal parameters were recorded: periodontal probing depth (PPD), clinical attachment level (CAL) and bleeding on probing (BoP). Probing was performed using a manual periodontal probe (CP15, Hu-Friedy Inc., Leimen, Germany)

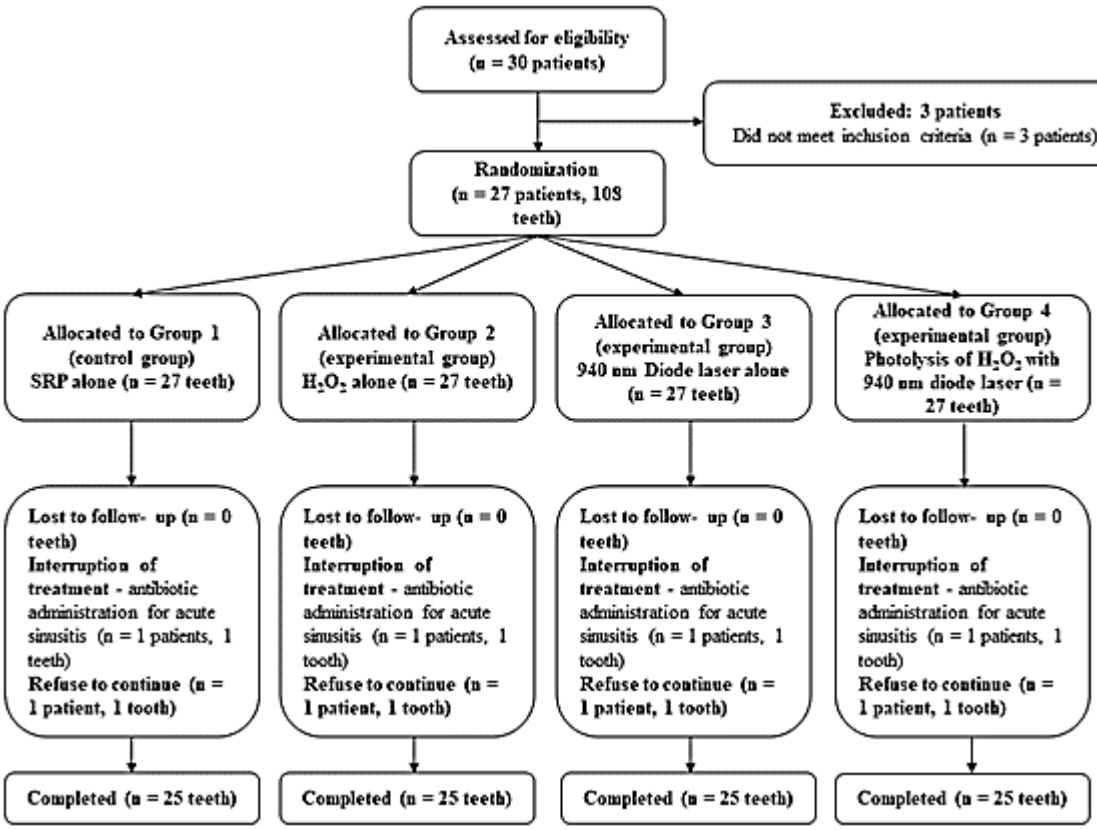

Fig. 1. Flow chart of the clinical study

	All Groups
No	25
Age (mean ± SD years)	47.8 ± 8
Gender M/F (%)	12/13 (48% / 52%)
Smokers/nonsmokers (%)	9/16 (36% / 64%)
PPD (mean ± SD mm)	3.8 ± 0.8
CAL (mean ± SD mm)	5.23 ± 1.1
BOP (mean ± SD %)	58.32 ± 24.61
Stage of periodontitis moderate/severe (%)	11/14 (44% / 56%)

Table 1
SAMPLE PATIENTS

at six sites per tooth by the same examiner in both facilities. Based on the initial findings, four test teeth (one in each quadrant) that exhibited ≥ 5mm PPD and (+) BoP were selected from each patient, resulting a total of 100 test teeth. The deepest PPD from each test tooth was selected as the test site. Teeth with fixed prosthesis (single crowns or bridges), furcation involvement, and second molars were excluded. The test sites were randomly allocated using Microsoft Excel (Microsoft Corporation, Washington, USA). The diagnosis for the subjects and the sample of patients are presented in table 1.

Based on the method of randomization, each patient quadrant was allocated to one of the four treatment groups as follows: Group 1: SRP as monotherapy; Group 2: H_2O_2 as monotherapy; Group 3: 940 nm diode laser as monotherapy; Group 4: 940 nm diode laser + H_2O_2 as associated therapy. Thus, the test sites in a patient were treated with different treatment procedures to compare their effects within the same individual (i.e., split-mouth study).

Treatment protocol

One week after the baseline periodontal examination and microbiological sampling, professional dental cleaning was performed using conventional ultrasonic scaler consisting supragingival calculus removal, polishing of the teeth surfaces with rotary brushes and prophylactic paste and Airflow (PROPHYflex 3 Kavo, Biberach, Germany). Every patient received oral hygiene instructions (OHI) that included mouth rinse without alcohol and chlorhexidine gluconate, twice a day after tooth brushing. A week later, non-surgical periodontal treatment was performed under local anesthesia by a periodontist who was not designated as examiner. The periodontal treatment was represented by a half-mouth protocol and divided in two sessions, with one day of resting between them: first session – Upper and Lower Right (UR/LR); second session: Upper and Lower Left (UL/LL). In each quadrant, the selected teeth and the adjacent mesial and distal surfaces of the neighboring teeth were treated with one of the four non-surgical periodontal therapies, while the remaining teeth were treated using the conventional periodontal treatment with manual Gracey curettes (Hu-Friedy Inc., Leimen, Germany) and ultrasonic scaler (Piezolux Kavo, Biberach, Germany). The periodontal protocol of control and experimental groups are presented in table 2.

In the control group (Group 1), SRP was performed using manual Gracey curettes and ultrasonic scaler until the operator judged sufficient (fig. 2 a,b). In Group 2, 3% H_2O_2 was inserted to the bottom of the periodontal pocket using a disposable plastic needle similar to the endodontic irrigation (fig. 2 c) and left in the periodontal pocket for 30 seconds. In Group 3, diode 940 nm laser (Epic 10, Biolase, USA) with 300 μm uninitiated fiber tip (fig. 3 d,e), 1.1 W, Continuous Wave (CW) was applied from the bottom to the free gingival margin of the periodontal pocket in parallel with the root surface, and side to side movements was performed for about 30 seconds per test surfaces. Group 4, received the photoactivation of 3% H_2O_2 with diode 940 nm laser with 300 μm uninitiated fiber tip, 1.1 W, Continuous

	Control	Test		
Groups	**Group 1**	**Group 2**	**Group 3**	**Group 4**
Baseline	Initial consult Diagnosis Informed consent	Initial consult Diagnosis Informed consent	Initial consult Diagnosis Informed consent	Initial consult Diagnosis Informed consent
Week 1	Periodontal indices recording (PPD, CAL, BoP) Microbiology sampling	Periodontal indices recording (PPD, CAL, BoP) Microbiology sampling	Periodontal indices recording (PPD, CAL, BoP) Microbiology sampling	Periodontal indices recording (PPD, CAL, BoP) Microbiology sampling
Week 2	Professional dental cleaning OHI	Professional dental cleaning OHI	Professional dental cleaning OHI	Professional dental cleaning OHI
Week 3	SRP	H_2O_2	940 nm diode laser, 1.1 W, CW	940 nm diode laser, 1.1 W, CW + H_2O_2
1 month	Microbiology sampling	Microbiology sampling	Microbiology sampling	Microbiology sampling
3 months	Periodontal indices recording (PPD, CAL, BoP)	Periodontal indices recordings (PPD, CAL, BoP)	Periodontal indices recording (PPD, CAL, BoP)	Periodontal indices recording (PPD, CAL, BoP)

Table 2
STUDY PROTOCOL FOR CONTROL AND TEST GROUPS

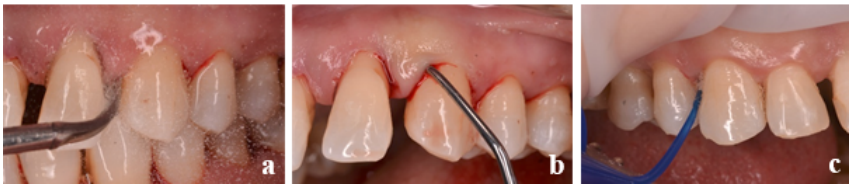

Fig. 2. Group 1 (SRP) was performed using ultrasonic scaler (*a*) and manual Gracey curette (*b*); Group 2 (only H_2O_2) (*c*)

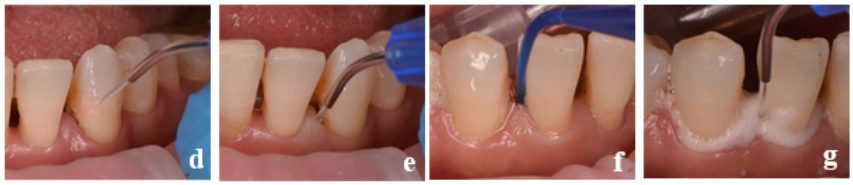

Fig. 3. Group 3 (only 940 nm Diode laser) – uninitiated tip (*d*) for decontamination only (*e*); Group 4 (photoactivation of 3% H_2O_2 using 940 nm diode laser) – insertion of H_2O_2 to the bottom of periodontal pocket (f) and activation with uninitiated tip (*g*)

Wave (CW), exposure time was for about 30 seconds per test surfaces (fig. 3 f,g). The 3% H_2O_2 and diode 940 nm laser was applied in the same manner like in Group 2 and respectively Group 3. Thus, the combination of 3% H_2O_2 and laser light, generated hydroxyl radicals as a result of photolysis (fig. 3 g).

Microbiological assessment was performed one week prior to periodontal treatment. Microbiological samples were obtained from the selected periodontal pockets in each quadrant at baseline (before treatment) and at 1 month after the periodontal treatment by the blinded examiner. The sampling sites were isolated, dried and the supragingival plaque was removed. Sterile paper points were inserted to the bottom of the test sites and held in place for 30 s, then removed by avoiding contact with saliva or epithelium of the oral cavity and placed into transfer tubes (individual sampling) provided by Pet Deluxe Diagnostic Set (MIP Pharma GmbH, Blieskastel-Niederwürzbach, Germany). One sterile paper point was used per site (25 patients, 4 tested teeth, 1 paper point/ site/ quadarant -individual sampling). All four transfer tubes were transported in the same box. The microbiological assays were performed by means of real time PCR (polymerase chain reaction) by the MIP Pharma Laboratory, in order to determine qualitatively and quantitatively nine periodontal pathogens: *Aggregatibacter actinomycetemcomitans (A.a.), Porphyromonas gingivalis (P.g.), Tannerella forsythia (T.f.), Treponema denticola (T.d.), Fusobacterium nucleatum (F.n.), Prevotella intermedia (P.i), Peptostreptococcus micros (P.m.), Eubacterium nodatum (E.n.), Capnocytophaga gingivalis (C.g.)*. The company stated that the detection limit for each bacterium was confirmed at 100 germs/mL.

Follow-up examination

Clinical periodontal parameters PPD, BoP and CAL were first assessed at 3 months after the periodontal treatment by the same blinded examiner. Only the test teeth were assessed. The quantitative and qualitative of total bacteria count and the 9 periodontal pathogenic bacteria were recorded after 1 month.

Statistical analysis

The experimental data were performed using the statistical processing program SPSS Statistics 23 (IBM Corp., Armonk, New York, USA). The following tests were used: Descriptive statistics (for characterization of discrete and continuous variables defined at the database level), Charts, Nonparametric statistical tests (the χ^2 test of the association between two class variables, McNemar test for change significance, Mann-Whitney test used for testing the difference between two independent groups and the Wilcoxon test was used to test the difference between two pair groups) and $p<0.05$ was considered significant.

Results and discussions

The postoperative healing was uneventful in all cases and no complications such as periodontal abscesses or infections were observed throughout the study.

Since the data distribution was nonparametric and considering the periodontal microbiota analysis in the literature, the median and percentiles are of relevance to analyse the variables. The microbiological variables at baseline and at 1 month postoperative are presented in table 3 and 4.

Table 3
INDEPENDENT SAMPLE OF MEDIAN AND PERCENTILES FOR MICROBIOLOGICAL VARIABLES (GROUP 1 AND 2)

Variables	Group 1 (SRP)			Group 2 (H_2O_2)		
	Median	25 - 75%	p value	Median	25 - 75%	p value
Total number of germs (NTG)						
Baseline	12 x 10^6	4.4 x 10^6 - 22 x 10^6	0.647	7.6 x 10^6	2.3 x 10^6 - 23 x 10^6	0.989
1 month postop	6.3 x 10^6	1.25 x 10^6 - 21 x 10^6		7 x 10^6	2.2 x 10^6 - 19 x 10^6	
Aggregatibacter actinomycetemcomitans (A.a.)						
Baseline	0 x 10^6	0 x 10^6 - 0 x 10^6	0.109	0 x 10^6	0 x 10^6 - 0 x 10^6	0.109
1 month postop	0 x 10^6	0 x 10^6 - 0 x 10^6		0 x 10^6	0 x 10^6 - 0 x 10^6	
Porphyromonas gingivalis (P.g.)						
Baseline	0.057 x 10^6	0.00215 x 10^6 - 0.51 x 10^6	0.01	0.054 x 10^6	0.0058 x 10^6 - 0.285 x 10^6	0.068
1 month postop	0.02 x 10^6	0.009 x 10^6 - 0.0585 x 10^6		0.015 x 10^6	0 x 10^6 - 0.29 x 10^6	
Treponema denticola (T.d.)						
Baseline	0.051 x 10^6	0.026 x 10^6 - 0.21 x 10^6	0.015	0.035 x 10^6	0.016 x 10^6 - 0.18 x 10^6	0.098
1 month postop	0.014 x 10^6	0.0012 x 10^6 - 0.0515 x 10^6		0.016 x 10^6	0.0058 x 10^6 - 0.062 x 10^6	
Tannerella forsythia (T.f.)						
Baseline	0.026 x 10^6	0.00305 x 10^6 - 0.117 x 10^6	0.000	0.011 x 10^6	0.00275 x 10^6 - 0.0825 x 10^6	0.054
1 month postop	0 x 10^6	0 x 10^6 - 0 x 10^6		0.009 x 10^6	0 x 10^6 - 0.0785 x 10^6	
Prevotella intermedia (P.i)						
Baseline	0.037 x 10^6	0 x 10^6 - 0.165 x 10^6	0.046	0.0058 x 10^6	0.000425 x 10^6 - 0.0535 x 10^6	0.296
1 month postop	0.0033 x 10^6	0 x 10^6 - 0.077 x 10^6		0 x 10^6	0 x 10^6 - 0.019 x 10^6	
Peptostreptococcus micros (P.m.)						
Baseline	0.0052 x 10^6	0.0027 x 10^6 - 0.021 x 10^6	0.052	0.0053 x 10^6	0.000895 x 10^6 - 0.0165 x 10^6	0.819
1 month postop	0.0021 x 10^6	0.0004 x 10^6 - 0.01155 x 10^6		0.0054 x 10^6	0.000065 x 10^6 - 0.035 x 10^6	
Fusobacterium nucleatum (F.n.)						
Baseline	0 x 10^6	0 x 10^6 - 0.005 x 10^6	0.199	0 x 10^6	0 x 10^6 - 0.002585 x 10^6	0.796
1 month postop	0.00013 x 10^6	0 x 106 - 0.0255 x 10^6		0 x 10^6	0 x 10^6 - 0.00215 x 10^6	
Eubacterium nodatum (E.n.)						
Baseline	0.00015 x 10^6	0 x 106 - 0.0011 x 10^6	0.001	0 x 10^6	0 x 10^6 - 0.000645 x 10^6	0.002
1 month postop	0 x 10^6	0 x 10^6 - 0 x 10^6		0 x 10^6	0 x 10^6 - 0 x 10^6	
Capnocytophaga gingivalis (C.g.)						
Baseline	0.00039 x 10^6	0 x 10^6 - 0.0046 x 10^6	0.012	0.00045 x 10^6	0 x 10^6 - 0.01125 x 10^6	0.073
1 month postop	0.008 x 10^6	0.001405 x 10^6 - 0.0255 x 10^6		0.015 x 10^6	0.00115 x 10^6 - 0.0435 x 10^6	

Variables	Group 3 (Diode 940 nm)			Group 4 (H_2O_2 + Diode 940 nm)		
	Median	25 - 75%	p value	Median	25 - 75%	p value
Total number of germs (NTG)						
Baseline	14 x 10^6	3.3 x 10^6 - 60.5 x 10^6	0.002	21 x 10^6	2.5 x 10^6 - 58 x 10^6	0.000
1 month postop	3 x 10^6	1.45 x 10^6 - 13 x 10^6		0.71 x 10^6	0.21 x 10^6 - 4.15 x 10^6	
Aggregatibacter actinomycetemcomitans (A.a.)						
Baseline	0 x 10^6	0 x 10^6 - 0 x 10^6	1.000	0 x 10^6	0 x 10^6 - 0 x 10^6	0.180
1 month postop	0 x 10^6	0 x 10^6 - 0 x 10^6		0 x 10^6	0 x 10^6 - 0 x 10^6	
Porphyromonas gingivalis (P.g.)						
Baseline	0.054 x 10^6	0.0058 x 10^6 - 0.285 x 10^6	0.033	0.02 x 10^6	0.014 x 10^6 - 0.365 x 10^6	0.000
1 month postop	0.029 x 10^6	0 x 10^6 - 0.08 x 10^6		0 x 10^6	0 x 10^6 - 0 x 10^6	
Treponema denticola (T.d.)						
Baseline	0.046 x 10^6	0.0195 x 10^6 - 0.205 x 10^6	0.041	0.11 x 10^6	0.0375 x 10^6 - 0.31 x 10^6	0.000
1 month postop	0.033 x 10^6	0.024 x 10^6 - 0.078 x 10^6		0 x 10^6	0 x 10^6 - 0 x 10^6	
Tannerella forsythia (T.f.)						
Baseline	0.019 x 10^6	0.00665 x 10^6 - 0.135 x 10^6	0.000	0.02 x 10^6	0.0051 x 10^6 - 0.205 x 10^6	0.000
1 month postop	0.004 x 10^6	0 x 10^6 - 0.0225 x 10^6		0 x 10^6	0 x 10^6 - 0 x 10^6	
Prevotella intermedia (P.i)						
Baseline	0.0091 x 10^6	0.00112 x 10^6 - 0.215 x 10^6	0.011	0.074 x 10^6	0.000305 x 10^6 - 0.28 x 10^6	0.000
1 month postop	0.0022 x 10^6	0 x 10^6 - 0.069 x 10^6		0 x 10^6	0 x 10^6 - 0.00006 x 10^6	
Peptostreptococcus micros (P.m.)						
Baseline	0.0068 x 10^6	0.00265 x 10^6 - 0.027 x10^6	0.065	0.0094 x 10^6	0.00165 x 10^6 - 0.035 x 10^6	0.000
1 month postop	0.005 x 10^6	0.005 x 10^6 - 0.03 x 10^6		0 x 10^6	0 x 10^6 - 0 x 10^6	
Fusobacterium nucleatum (F.n.)						
Baseline	0 x 10^6	0 x 10^6 - 0.00065 x 10^6	0.510	0.0006 x 10^6	0 x 10^6 - 0.037 x 10^6	0.001
1 month postop	0.00028 x 10^6	0 x 10^6 - 0.004350 x 10^6		0 x 10^6	0 x 10^6 - 0 x 10^6	
Eubacterium nodatum (E.n.)						
Baseline	0.00014 x 10^6	0 x 10^6 - 0.000775 x 10^6	0.001	0 x 10^6	0 x 10^6 - 0 x 10^6	0.003
1 month postop	0 x 10^6	0 x 10^6 - 0 x 10^6		0 x 10^6	0 x 10^6 - 0 x 10^6	
Capnocytophaga gingivalis (C.g.)						
Baseline	0.0024 x 10^6	0.0013 x 10^6 - 0.0175 x 10^6	0.581	0.0029 x 10^6	0 x 10^6 - 0.014 x 10^6	0.346
1 month postop	0.0041 x 10^6	0.00079 x 10^6 - 0.032 x 10^6		0.0022 x 10^6	0 x 10^6 - 0.00465 x 10^6	

Table 4
INDEPENDENT SAMPLE OF MEDIAN AND PERCENTILES FOR MICROBIOLOGICAL VARIABLES (GROUP 3 AND 4)

Variables	**Group 1 SRP**		**Group 2 H_2O_2**		**Group 3 diode 940 nm**		**Group 4: H_2O_2 + Diode 940 nm**	
	Bacteria count (%)	*p value*	*Bacteria count (%)*	*p value*	*Bacteria count (%)*	*p value*	*Bacteria count (%)*	*p value*
Total number of germs (NTG)								
Baseline	25 (100%)	-	25 (100%)	-	25 (100%)	-	25 (100%)	-
1 month postop	25 (100%)		25 (100%)		25 (100%)		25 (100%)	
Aggregatibacter actinomycetemcomitans (A.a.)								
Baseline	0 (0%)	<0.05	0 (0%)	<0.05	0 (0%)	-	2 (8%)	<0.05
1 month postop	3 (12%)		3 (12%)		0 (0%)		0 (0%)	
Porphyromonas gingivalis (P.g.)								
Baseline	22 (88%)	0.10	23 (92%)	0.063	22 (88%)	0.061	20 (80%)	0.001
1 month postop	21 (84%)		18 (72%)		17 (68%)		3 (12%)	
Treponema denticola (T.d.)								
Baseline	25 (100%)	>0.05	25 (100%)	>0.05	25 (100%)	>0.05	25 (100%)	<0.05
1 month postop	19 (76%)		23 (92%)		24 (96%)		2 (8%)	
Tannerella forsythia (T.f.)								
Baseline	25 (100%)	<0.05	24 (96%)	0.021	24 (96%)	0.031	24 (96%)	<0.05
1 month postop	3 (12%)		16 (64%)		18 (72%)		0 (0%)	
Prevotella intermedia (P.i)								
Baseline	18 (72%)	0.754	20 (80%)	0.008	21 (84%)	0.375	20 (80%)	<0.001
1 month postop	16 (64%)		12 (48%)		18 (72%)		6 (24%)	
Peptostreptococcus micros (P.m.)								
Baseline	22 (88%)	0.687	24 (96%)	0.063	25 (100%)	>0.05	24 (96%)	<0.001
1 month postop	20 (80%)		19 (76%)		23 (92%)		4 (16%)	
Fusobacterium nucleatum (F.n.)								
Baseline	10 (40%)	0.581	8 (32%)	0.791	6 (24%)	0.039	13 (52%)	0.001
1 month postop	13 (52%)		10 (40%)		13 (52%)		2 (8%)	
Eubacterium nodatum (E.n.)								
Baseline	13 (52%)	<0.001	12 (48%)	0.003	13 (52%)	0.002	11 (44%)	<0.05
1 month postop	1 (4%)		1 (4%)		3 (12%)		0 (0%)	
Capnocytophaga gingivalis (C.g.)								
Baseline	14 (56%)	0.012	18 (72%)	0.219	22 (88%)	0.996	17 (68%)	1
1 month postop	23 (92%)		22 (88%)		21 (84%)		17 (68%)	

Table 5
QUALITATIVE ANALYSIS FOR MICROBIOLOGICAL VARIABLES

All nine bacterial species evaluated in this pilot study were etected in different levels before the treatment. Microbiological analysis showed that total bacterial counts decreased after treatment. As shown in table 3 and 4, *A. actinomycetemcomitans* had a low frequency among the tested sites.

Regarding the bacteria count (table 5), *A.a* was present postoperative in Group 1 (SRP) and Group 2 (H_2O_2), while in Group 4 (H_2O_2 + Diode laser) experienced a total elimination.

The red complex, *Pg., Td., Tf.* in the control group (SRP) experienced a significant reduction ($p<0.05$) at 1 month

Variables		PD (mm)		CAL (mm)	
		Baseline	*3 months postop*	*Baseline*	*3 months postop*
Group 1 SRP	Median	6	4	8	6
	25 - 75%	5.5 – 7	3 – 5	7 – 9	5.5 - 7
	p value	0.000		0.000	
Group 2 H_2O_2	Median	6	4	8	8
	25 - 75%	5 – 7	4 - 6	7 – 9.5	7 - 9
	p value	0.001		0.384	
Group 3 diode 940 nm	Median	6	4	7	6
	25 - 75%	5 – 7	4 – 7	7 – 9.5	5.5 – 8.5
	p value	0.002		0.006	
Group 4 H_2O_2 + Diode 940 nm	Median	6	3	8	5
	25 - 75%	5 – 7	3 – 4	6.5 – 9	4 – 7
	p value	0.000		0.000	

Table 6
CLINICAL ASSESSMENT RESULTS OF PD AND CAL

Variables		BoP positive count (%)	
		Baseline	*3 months postop*
Group 1 (SRP)	+ Count (%)	24 (96%)	18 (52%)
	p value	0.003	
Group 2 (H_2O_2)	+ Count (%)	23 (92%)	18 (72%)
	p value	0.063	
Group 3 (diode 940 nm)	+ Count (%)	24 (96%)	15 (60%)
	p value	0.012	
Group 4 (H_2O_2 + Diode 940 nm)	+ Count (%)	24 (96%)	2 (8%)
	p value	< 0.001	

Table 7
CLINICAL ASSESSMENT OF BoP

after the treatment, while in Group 2 (only H_2O_2) the reduction was not significant ($p>0.5$). The laser treated groups (Groups 3 and 4) showed a significant reduction of the most aggressive periodontal pathogens, but a clear outcome can be noticed in Group 4 (H_2O_2 + Diode 940 nm laser) with a total reduction of the red complex bacteria ($p=0.000$).

The orange complex (*Pi., Pm., Fn.*) showed different results among the study groups. *Pi.* presented a significant reduction in Group 1 ($p = 0.046$), Group 3 ($p = 0.011$) and Group 4 ($p=0.000$) after 1 month, while in Group 2 the outcome was not significant ($p=0.296$). *Pm.* and *Fn.* showed a significant reduction only in Group 4 ($p < 0.001$) in contrast with Group 1, 2 and 3. *En.* presented statistically significant reduction in all groups.

In what *Cg.* is concerned, only Group 1 ($p = 0.012$) experienced significant outcome, while in Group 2, Group 3 and 4 the p values were 0.073, 0.581 and 0.346 respectively.

From the quantitative point of view, these bacteria among the treated groups responded differently. With the exception of *Aa.* (in Group 1 and 2), *Fn.* and *Cg.* (in Group 1, 2, 3), almost all the evaluated bacteria showed a statistically significant reduction (table 5). *Aa.* was present after the treatment in Group 1 and 2, while in Group 4 was no longer detected. *Pg.* and *Td.* was detected in all four groups, but significant outcome was observed only in Group 4. With regard to *Pg.* in Group 4, from a total of 20 cases, only in 2 was present in comparison to control group. *Tf.* recorded a statistically significant reduction in all four groups, while the bacterial count of *Pi.* was more significant in Group 2 and 4. Similar results were noticed for *Pm., Fn., En.,* and *Cg.*

Clinical examination showed at 3 months a significant improvement of all investigated periodontal indexes (*Table 6* and *Table 7*). PPD showed a significant reduction in all investigated groups ($p<0.002$), while CAL and BoP showed a statistically significant reduction in Group 1, 3 and 4 with exception to group 2 (CAL $p=0.384$, BoP $p=0.063$).

Regarding the clinical and bacterial differences between tested groups, it was decided to assess the control group with the other experimental groups and also the experimental groups between them (*Table 8*). There was no significant differences between all groups at baseline. Between Group 1 (SRP) and Group 2 (H_2O_2) postoperative there was a very strong significance for *Tf.*, PPD and CAL ($p=0.000$). Between Group 1 and Group 3 (only Diode laser), there was a significant reduction for *Td.*, *Tf.*, and PPD, while

Variables	P Values											
	Group 1 vs. Group 2		Group 1 vs. Group 3		Group 1 vs. Group 4		Group 2 vs. Group 3		Group 2 vs. Group 4		Group 3 vs. Group 4	
	Baseline	*Postop*	*Baseline*	*Postop*	*Baseline*	*Postop*	*Baseline*	*Postop*	*Baseline*	*Postop*	*Baseline*	*Postop*
NTG	0.485	0.554	0.691	0.771	0.372	0.002	0.221	0.229	0.168	0.000	0.854	0.005
A.a.	1.000	0.932	1.000	0.077	0.153	0.077	1.000	0.077	0.153	0.077	0.153	1.000
P.g.	0.861	0.992	0.749	0.992	0.923	0.000	0.985	0.658	0.478	0.000	0.382	0.000
T.d.	0.248	0.472	0.684	0.035	0.362	0.000	0.461	0.123	0.048	0.000	0.218	0.000
T.f.	0.388	0.000	0.923	0.000	0.641	0.077	0.221	0.484	0.168	0.000	0.684	0.000
P.i.	0.300	0.252	0.915	0.906	0.674	0.001	0.350	0.192	0.280	0.034	0.733	0.000
P.m.	0.541	0.453	0.461	0.177	0.607	0.000	0.177	0.992	0.295	0.000	0.907	0.000
F.n.	0.668	0.245	0.337	0.673	0.258	0.000	0.524	0.428	0.150	0.005	0.047	0.000
E.n.	0.597	0.977	0.943	0.312	0.859	0.317	0.568	0.274	0.816	0.317	0.794	0.077
C.g.	0.389	0.992	0.024	0.683	0.209	0.006	0.152	0.734	0.595	0.031	0.696	0.084
PPD	0.731	0.000	0.606	0.040	0.686	0.328	0.903	0.372	0.943	0.000	0.976	0.005
CAL	0.641	0.000	0.350	0.478	0.401	0.700	0.594	0.026	0.607	0.000	0.889	0.008

Table 8
CLINICAL AND BACTERIAL DIFFERENCES BETWEEN TEST AND EXPERIMENTAL GROUPS

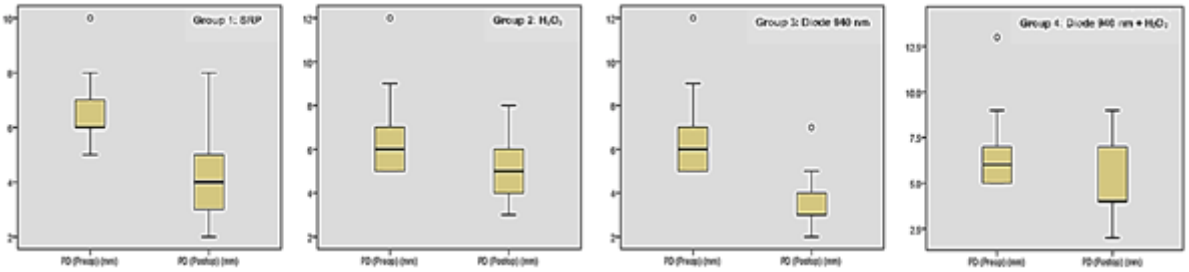

Fig. 4. Changes in PPD before and after the treatment for each tested group based on median and percentiles 25-75% values

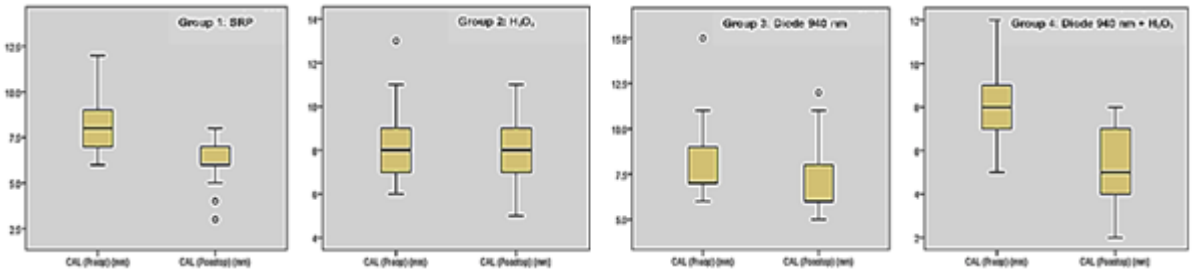

Fig. 5. Changes in BoP before and after the treatment for each tested group based on median and percentiles 25-75% values

Group 1 vs. 4 (H_2O_2 + Diode laser) showed a statistically high significance for NTG., *Pg., Td., Pi., Pm., Fn.*, and *Cg.*

In what the differences between the experimental groups concerned (table 8), Group 2 vs. Group 3 showed only a significant p value only for CAL (p=0.026), in contrast to Group 2 vs. Group 4 which showed a high significant differences (p=0.000) for NTG, Pg., Td., Tf., Pm., PPD, CAL and a p value <0.05 for Pi., Fn., and Cg. With regard to the differences between the diode laser groups (Group 3 vs. Group 4), there was a considerable outcome with reduction of the major periodontal pathogens like Pg., Td., Tf., Pi., Pm., Fn. with p=0.000 and also for NTG (p=0.005) and clinical periodontal parameters like PPD (p=0.005) and CAL (p=0.008).

Although, this study did not include in the experimental groups the conventional therapy – scaling and root planning (SRP), the outcome of the laser treated groups as a monotherapy (Group 3 and 4) showed a significand reduction of periodontal bacteria. With regard to Group 4 (H_2O_2 + Diode laser) as a single therapy, the photoactivation of 3% hydroxide peroxide with 940 nm diode laser achieved greater results than the SRP alone, although the subgingival calculus was not removed throughout the study. Based on this fact, we can confirm that the subgingival calculus can be decontaminated and can interrupt, for a short period of time, the anaerobic bacteria growth within the periodontal pocket [19]. We strongly recommend the SRP as a primary therapy in order to eliminate the contributing factor such as the porous surface of subgingival calculus for bacteria overgrowth, which acts like a receptor for primary and secondary colonizers. In laser groups, 3 and 4, the red complex bacteria such as *Pg., Td., Tf.* showed a significant reduction, especially in group 4 (p value = 0.000) due to high absorption of 940 nm wavelength in black pigmented bacteria such as *Pg., Td., Tf.* and *Pi.*

In Fig. 4 are presented the changes in PPD before and after the treatment for each tested group based on median and percentiles 25-75% values, and in *Fig. 5* the changes in BoP before and after the treatment for each tested group based on median and percentiles 25-75% values.

The study demonstrated that the photoactivation of 3% H_2O_2 with 940 nm diode laser has an increased bactericidal effect and can achieve biofilm disruption due to the acceleration of hydroxyl radical generation by thermal energy. There are previous studies that demonstrated the synergistic effect of photolysis of H_2O_2 with different light sources in periodontal disease and also in endodontic pathology [20-24].

Resent clinical trial published by Kanno T. et al. [20], presented a new device equipped with an ultrasonic scaler and a laser unit that emits light at a wavelength of 405 nm. The steel scaler is hallow and is hosting the optical fiber. The cooling system is based on 3% hydrogen peroxide, which is released from the end of the scaler tip. This newly developed device combines three function: one which acts like a subgingival ultrasonic calculus removal, second one the insertion of 3% hydrogen peroxide in periodontal pockets and the third one is the emission of laser light which produces photolysis of hydrogen peroxide, resulting hydrogen radicals generation in the periodontal pocket during root debridement. Their study assessed total bacterial counts and only one specific anaerobic periodontal bacteria - *Pg.* Also clinical periodontal parameters like PPD and BoP were included in the study. Their study included three groups one control group (root debridement only), and two experimental groups (root debridement + H_2O_2 photolysis; root debridement + local drug delivery system). The study outcomes showed a significant reduction of *Pg.* in the H_2O_2 photolysis group. Also the clinical parameters were significantly improved.

In an in vitro study, Ikai H. et al. [24], showed that the photolysis of hydrogen peroxide with 405 nm diode laser can effectively eliminate all the tested bacteria within 3 min. The most sensitive bacteria to photodesinfection was *A.actinomycetemcomitans* followed by *S. aureus* and *S. mutans*, while in contrast, *E.faecalis* showed high resistance to this method. From this findings we can state that photolysis of hydrogen peroxide with diode laser source has a synergistic effect on bactericidal action of oral pathogenic bacteria, while in endodontic treatments the outcomes are relatively insignificant.

Although the photolysis of H_2O_2 is achieved using UV-light sources as an effective disinfection system, the microbiological and clinical outcomes of this study shows that photolysis of H_2O_2 can be achieved also with 940 nm diode laser, despite that in the literature there is no research that supports this theory. Future experimental chemical research should be performed regarding the photolysis of H_2O_2 with a near infra-red source like 810 nm, 940 nm and 980 nm diode lasers in periodontal treatment, in order to

analyse the amount and the efficacy of generated hydroxyl radicals.

Hydroxyl radicals generated by photolysis of H_2O_2 with diode lasers has a series of advantages in periodontal treatment. In contrast to systemic or local administration of antibiotics, it can eliminate the bacterial resistance development due to the cytotoxic action of H_2O_2 or OH^- radical formation. Furthermore, H_2O_2 can penetrates the deep layers of bacterial biofilm. It may have a beneficial effect on wound healing without genotoxic or mutagenic effects [25].

Conclusions

Although the limitations of this study, the following conclusions can be drawn:

The clinical efficacy of non-surgical periodontal therapy with H_2O_2 photolysis-based antimicrobial chemotherapy was demonstrated and offer an alternative to antibiotic prescriptions in periodontitis;

Microbial and inflammatory modifications were significantly reduced compared to SRP as monotherapy;

Associated therapy with H_2O_2 photolysis and diode laser therapy presented the most beneficial results, and after six months of monitoring we observed that this therapy maintained the obtained clinical parameters.

A longer follow-up study should be conducted to verify the obtained results.

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