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Original Research

Effect of Hydrogen Peroxide Photoactivated Decontamination Using 940 nm Diode Laser in Periodontal Treatment: A Pilot Study

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Abstract

Objective: The aim of this study was to compare the antimicrobial effects of hydroxyl radical generation by photoactivation of hydrogen peroxide (H_2O_2) with diode laser ($\lambda = 940$ nm) in combination with conventional nonsurgical periodontal therapy.
Materials and methods: Thirty-eight patients and 114 teeth were included in this study. The test teeth were randomly assigned to one of the three treatment groups: Group 1 (control group): scaling and root planning (SRP); and the following experimental groups: Group 2: SRP +940 nm diode laser; Group 3: SRP+photoactivation of H_2O_2 with 940 nm diode laser. Clinical examinations, such as periodontal probing depth (PPD), clinical attachment level (CAL), and bleeding on probing (BoP) were performed before and after the treatment. The microbiological evaluation included nine periodontal bacterial species investigated by means of real-time polymerase chain reaction assay before and after the treatment. The clinical and bacterial differences were assessed between the investigated groups.
Results: The total bacteria load was reduced for all three studied groups and all periodontal indexes (PPD, CAL, and BoP) were improved after each treatment. Group 3 showed significant bacterial reduction of the major periodontal bacteria such as *Porphyromonas gingivalis*, *Tannerella forsythia*, *Treponema denticola*, *Prevotella intermedia*, *Peptostreptococcus micros*, *Fusobacterium nucleatum*, *Eubacterium nodatum* ($p < 0.001$) in contrast to the other two groups ($p > 0.001$). Differences between tested groups showed significant results with regard to Group 3.
Conclusions: The synergistic effect of SRP and photoactivation of H_2O_2 with 940 nm diode laser offers an efficient and reliable antimicrobial effect in the nonsurgical periodontal treatment approach.

Keywords: photoactivated disinfection, hydrogen peroxide, 940 nm diode laser, laser-assisted periodontal therapy

Introduction

Periodontal disease represents an important concern of public health worldwide and is probably the most common chronic infectious disease among human kind.[1] In chronic periodontal disease, the subgingival biofilm is responsible for hard and soft tissue loss. According to Socransky complexes, the most important pathogens in adult periodontal disease are represented by the red complex, which includes *Porphyromonas gingivalis*, *Treponema dentiticola*, and *Tannerella forsythia*. The orange complex bacteria are usually found together with the red complex and acts like a bridge between the primary and the secondary colonizers of the subgingival biofilm.

Beside *Aggregatibacter actinomycetemcomitans*, some species from red and orange complexes have the ability to

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invade the gingival epithelial and endothelial cells.[2–4] This virulence capability of the pathogenic bacteria may lead to reduced outcome of the scaling and root planning (SRP) technique when used as a sole therapy. Researchers suggested the use of antibiotics in conjunction with SRP but because of the high frequency of increase in antibiotic resistance, many periodontal pathogens cannot be removed from the periodontal structures. Furthermore, SRP fails to eliminate pathogenic bacteria in periodontal inaccessible areas such as deep periodontal pockets, root concavities, furcation involvement, and so on. To overcome these inconveniences, researchers proposed the use of lasers in periodontal therapy.[5]

The use of lasers as a complementary procedure to conventional therapy may facilitate treatment outcomes with improved periodontal regeneration potential.

From the near-infrared spectrum lasers, the Nd:YAG laser can remove periodontal pathogens because of its thermal effect. However, changes in the neighboring tissues can be attributed to these unwanted thermal effects. The diode lasers that belong to the 655–980 nm spectrum could represent a safer alternative.[6] Because of the transmission or scattering effect on hydroxyapatite, diode lasers have no effect on calculus. Anaerobic bacterial species such as *P. gingivalis* and *Prevotella intermedia* produce black pigments in *Brucella* media from blood agar. Hemoglobin in the soft periodontal tissues behaves like a chromophore, being absorbed by the diode laser. It acts as an endogenous dye, which can increase the laser effect at this level.[7] Therefore, the laser can be used as an adjuvant to SRP owing to its bactericidal and detoxifying effects. Dukic et al. have shown that clinical parameters in moderate periodontal pockets ranging from 4 to 6 mm depth can be improved by repeating the application of 980 nm laser in combination with conventional treatment.[8]

Other laser-assisted periodontal procedures use photosensitizers like dyes or different substances activated by different laser wavelengths to eliminate periodontal bacteria. Antimicrobial photodynamic therapy (aPDT) uses dyes like toluidine blue, methylene blue, rose Bengal, and indocyanine green photoactivated by laser wavelengths ranging from 630 to 810 nm.[9–11] These dyes, in the presence of light, produce reactive oxygen species (ROS) capable of damaging the biomolecules and cause oxidation of cellular structures leading to selective microorganism death.[12] Other photoactivated procedures use the photolysis of hydrogen peroxide (H_2O_2) with 405 nm wavelength.[13]

When consulting the literature, the ability of 940 nm laser wavelength to eliminate periodontal bacteria is lacking support. A more recent study investigated the use of H_2O_2 photoactivation with 940 nm diode laser without the use of the SRP.[14]

Based on these initial findings, we hypothesized that the photoactivation of H_2O_2 with 940 nm diode laser in the presence of conventional SRP will improve the periodontal condition more than SRP alone and in combination with 940 nm diode laser. Therefore, the aim of this study was to evaluate the bactericidal effect and the clinical outcomes of the photoactivation of H_2O_2 with 940 nm diode laser in combination with SRP in the nonsurgical periodontal treatment.

Materials and Methods

This study was designed as a randomized controlled, single-blind, multicenter trial with a split-mouth design to compare the antimicrobial effect of nonsurgical periodontal therapy with SRP alone, 940 nm diode laser in combination with SRP, and photoactivation of H_2O_2 with 940 nm diode in combination with SRP. The study protocol was approved by the 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, 40 patients 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 before treatment. The inclusion criteria were as follows: at least 16 natural teeth present in the oral cavity distributed in 4 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 4 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 past 6 months, smokers, systemic conditions that may affect the therapeutic outcome (diabetes type 1 and 2, immune deficiency, hepatitis B virus, hepatitis C virus, cancer, hematological disorders, epilepsy, etc.), pregnancy, breastfeeding, incapacity or refusal to follow the study protocol, and severe comorbid conditions that may affect life expectancy within 1 year (e.g., metastatic cancer). Two patients did not meet the inclusion criteria and were excluded: one exclusion was because of previous periodontal treatment and another was owing to antibiotic administration within the last 6 months for periodontal abscess. A total of 38 patients participated in the study until the end. The flow chart of the study is given in Fig. 1.

Baseline examination was performed 1 week before periodontal treatment and included clinical and radiological analysis. The following clinical periodontal parameters were recorded: PPD, clinical attachment level (CAL), and BoP. Probing was performed using a manual periodontal probe (CP15; Hu-Friedy, Inc., Leimen, Germany) at six sites per tooth by an experienced periodontist in both facilities. Based on the initial findings, three test teeth (one in each quadrant) that exhibited ≥5 mm PPD and (+) BoP were selected from each patient, resulting in a total of 114 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, second and third molars were excluded. The test sites were randomly allocated using Microsoft Excel (Microsoft Corporation, WA). The diagnosis for the subjects and the sample of patients are given in Table 1.

Based on the method of randomization, each patient quadrant was allocated to one of the three treatment groups as follows: Group 1: SRP as monotherapy; Group 2: SRP and 940 nm diode laser decontamination; and Group 3: SRP + H_2O_2 photoactivation with 940 nm diode laser. 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). The remaining quadrant was treated with SRP and it was not microbiologically assessed.

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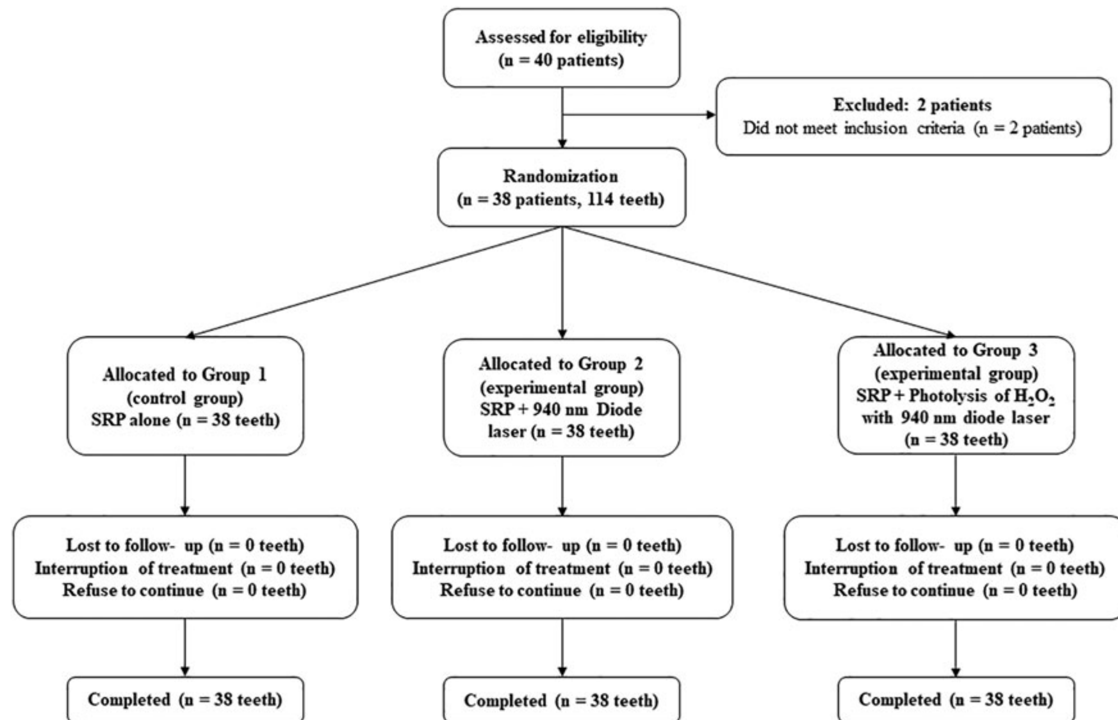

FIG. 1. Flow chart of the clinical study.

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Treatment protocol

One week after the baseline periodontal examination and microbiological sampling, professional dental cleaning was performed using conventional ultrasonic scaler consisting of 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 that included modified BASS brushing technique and mouth rinse without alcohol and chlorhexidine gluconate, twice a day after tooth brushing. A week later, nonsurgical periodontal treatment was performed under local anesthesia by another experienced periodontist who was not designated as examiner. The periodontal treatment was represented by a half-mouth protocol and divided into two sessions, with 1 day of resting between them: first session—upper and lower right; second session: upper and lower left. In each quadrant, the selected teeth and the adjacent mesial and distal surfaces of the neighboring teeth were treated with one of the three nonsurgical periodontal therapies, whereas the remaining teeth were treated using the conventional periodontal treatment with manual Gracey curettes (Hu-Friedy, Inc.) and ultrasonic scaler (Piezolux; Kavo).

Table 1. Sample Patients

	All groups
N	38
Age, years, mean ± SD	47.45 ± 7.82
Gender, M/F, *n* (%)	21/17 (55.3/44.7)
PPD, mm, mean ± SD	3.85 ± 0.74
CAL, mm, mean ± SD	5.41 ± 1.04
BoP, %, mean ± SD	58.08 ± 24.06
Stage of periodontitis moderate/severe, *n*(%)	13/25 (34.2/65.8)

BoP, bleeding on probing; CAL, clinical attachment level; PPD, periodontal probing depth; SD, standard deviation.

In the control group (Group 1), SRP was performed using manual Gracey curettes and ultrasonic scaler until the operator judged sufficient (Fig. 2a, b). In Group 2, SRP was performed in the same manner as in Group 1, followed by decontamination with diode 940 nm laser (Epic 10; Biolase) with 7 mm length and 300 μm in diameter uninitiated fiber tip (Fig. 3a), 1.1 W, continuous wave (CW). The activated fiber 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 were performed for ~30 sec per test surface (Fig. 3b). Group 3 received the SRP procedure followed by photoactivation of 3% H_2O_2 with 940 nm diode laser with 300 μm uninitiated fiber tip, 1.1 W, CW, exposure time for ~30 sec per test surface (Fig. 4). The 3% H_2O_2 solution was inserted to the bottom of the periodontal pocket using a disposable plastic needle similar to the endodontic irrigation (Fig. 4a) and photoactivated by 940 nm laser, which was applied in the same manner as in Group 2 (Fig. 4b, c). Thus the combination of 3% H_2O_2 and laser light generated hydroxyl radicals as a result of photoactivation (Fig. 4d). The laser parameters for Groups 2 and 3 are summarized in Table 2.

Microbiological assessment was performed 1 week before 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 and dried while the supragingival plaque was removed. Sterile paper points were inserted to the bottom of the test sites and held in place for 30 sec, then removed by avoiding contact with saliva or

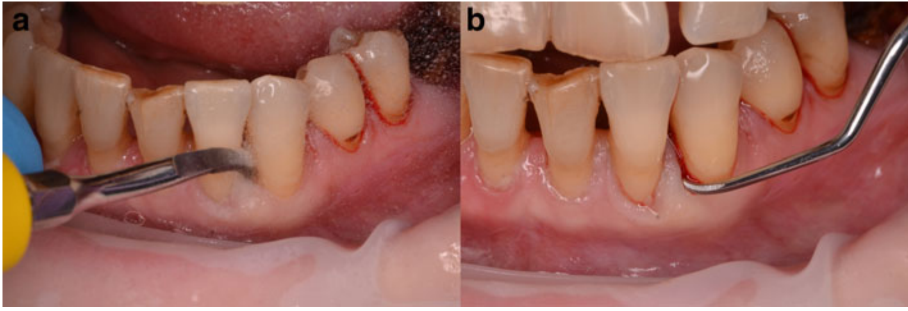

FIG. 2. Group 1 (SRP) was performed using ultrasonic scaler **(a)** and manual Gracey curette **(b)**. SRP, scaling and root planning.

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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 (38 patients, 3 tested teeth, 1 paper point/site/quadrant = 114 individual samplings). All three transfer tubes were transported in the same box. The microbiological assays were performed by means of real-time polymerase chain reaction (PCR) by the MIP Pharma Laboratory, to determine qualitatively and quantitatively nine periodontal pathogens: *A. actinomycetemcomitans, P. gingivalis, T. forsythia, Treponema denticola, Fusobacterium nucleatum, P. intermedia, Peptostreptococcus micros, Eubacterium nodatum, Capnocytophaga gingivalis*. In addition, the total bacteria count (TBC) was assessed per sample. The company stated that the detection limit for each bacterium was confirmed at 100 germs per milliliter.

Follow-up examination

Clinical periodontal parameters PPD, BoP, and CAL were first assessed 3 months after the periodontal treatment by the same blinded examiner. Only the test teeth were assessed. To eliminate any possible bias, intra-examiner calibration was performed both initially and at 3 months after examination. Three repeated measurements were performed and had to show a >90% agreement for ±1 mm between initial and repeated probes. The quantitative and qualitative of TBC and the nine 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, NY). 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 significance change, 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.

The periodontal treatment protocol of the control and experimental groups are given in Table 3.

Results

The postoperative healing was uneventful in all cases and no complications such as periodontal abscesses or infections were observed throughout the study. During the study, no antibiotic therapy or other medication were administrated for all participating subjects. Table 4 provides the clinical characteristics of each quadrant assigned for the control and experimental groups. From the total number of teeth investigated in this study, 80 teeth were single rooted and 34 multirooted.

Since the data distribution was nonparametric and considering the periodontal microbiota analysis in the literature, the median and percentiles are of relevance to analyze the variables. The microbiological variables at baseline and at 1 month postoperative are given in Table 5.

All nine bacterial species evaluated in this pilot study were detected in different levels before the treatment. Microbiological analysis showed a decrease in TBC postoperative in all investigated groups.

The red virulence complex represented by *P. gingivalis*, *T. denticola*, and *T. forsythia* recorded highly significant results ($p = 0.000$) in all three investigated groups.

Regarding the orange virulence complex, the postoperative results showed significantly reduced values ($p < 0.05$), but with some particularities. Pathogen *P. intermedia* in

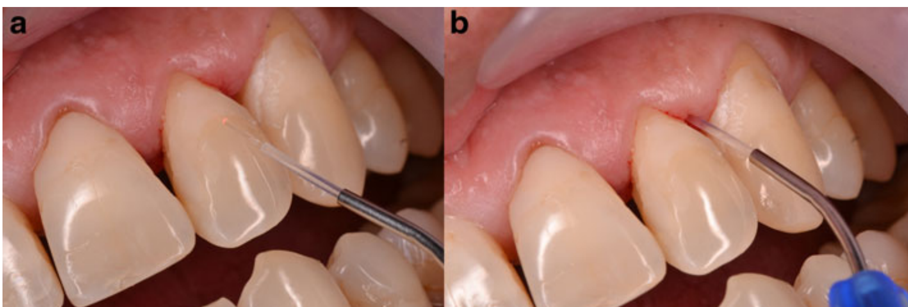

FIG. 3. Group 2 (SRP +940 nm diode laser)—uninitiated tip **(a)** for decontamination only **(b)**.

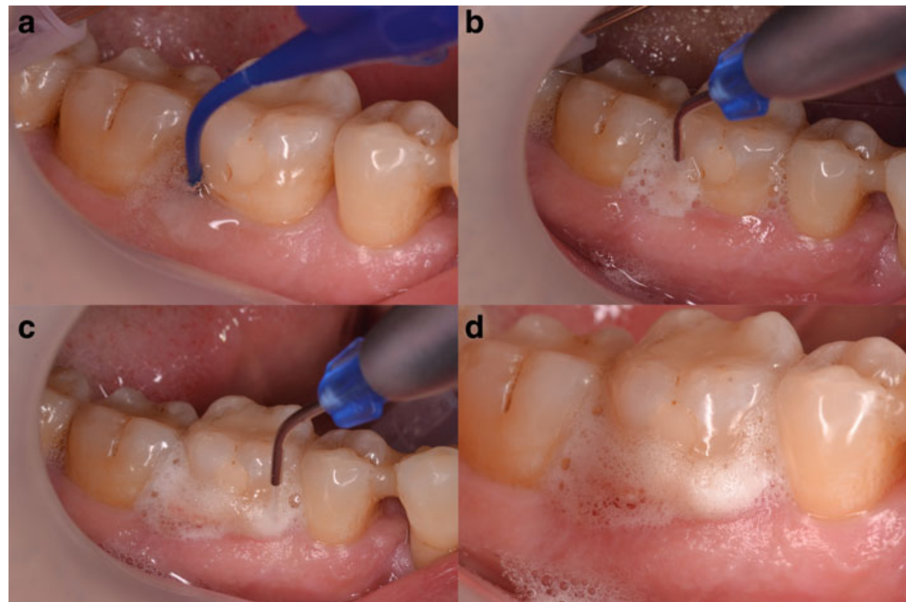

FIG. 4. Group 3 (SRP+photoactivation of 3% H_2O_2 using 940 nm diode laser)—insertion of H_2O_2 to the bottom of periodontal pocket **(a)** and activation with uninitiated tip **(b, c)**. Generation of hydroxyl radicals **(d)**. H_2O_2, hydrogen peroxide.

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Group 1 recorded a p-value = 0.03, p = 0.008 in Group 2, and p = 0.000 in Group 3. *P. micros* showed values for Group 1 with p = 0.04, for Group 2 p = 0.029, and for Group 3 p = 0.000.

F. nucleatum failed to show a significant reduction in Group 1 (p = 0.220) and Group 2 (p = 0.872), in contrast to Group 3 results (p = 0.000).

The associated orange complex, represented by *E. nodatum*, registered a significant reduction in all three investigated groups (p = 0.000).

The green complex, being a health-compatible complex, expressed different values for *C. gingivalis.* In Group 1, significant differences (p = 0.031) were observed, unlike the groups treated with laser (Group 2, p = 0.275; Group 3, p = 0.858).

Table 2. The Parameters of the Light Used for Laser-Assisted Periodontal Treatment (Groups 2 and 3)

Device	
Name, manufacturer	Epic X, Biolase
Power output	10 W
Wavelength of light source	940 ± 10 nm
Type of laser	Diode laser light
Power mode	CW
Shape, size, length of the fiber tip	Flat tip of 300 μm in diameter, 7 mm length
Treatment	
Power	1.1 W
Exposure time	30 sec
Frequency of treatment	Once
Beam area	0.00071 cm^2
Power density	1549 W/cm^2
Energy density	33 J/cm^2

CW, continuous wave.

To have a complete image of all the processed variables, we also considered performing the qualitative assessment of the bacteria distribution in all tested groups. Table 6 provides the qualitative analysis of the microbial species assessed by means of independent median sample and percentiles.

A. actinomycetemcomitans showed a low frequency among the tested groups. Regarding the bacteria count, *A. actinomycetemcomitans* was recorded after the Group 1 (SRP) treatment and before Group 3 (SRP + H_2O_2+diode laser), whereas Group 2 (SRP+diode laser 940 nm) could not be detected (Table 6).

From a qualitative point of view, Groups 1 and 2 failed to show significant postoperative outcomes ($p > 0.05$) for orange complex (*P. intermedia*, *P. micros*, *F. nucleatum*), in contrast to Group 3 which showed significant reduction ($p < 0.001$) for *P. gingivalis*, *T. denticola*, *T. forsythia*, *P. intermedia*, *P. micros*, *F. nucleatum*, and *E. nodatum*.

For periodontal index examination at baseline and postoperative, the result of intra-examiner reproducibility of >90% for ±1 mm between initial and repeated probes was 100%. Tables 7 and 8 show the outcome of the clinical periodontal indices represented by the PPD, CAL, and BoP. All the tested groups showed a significant postoperative reduction (p = 0.000).

To have a better understanding with regard to our proposed therapy, we decided to compare the outcome of the test group with the experimental groups and the experimental groups between them. As Table 9 shows, Group 3 exhibited a statistically highly significant outcome in contrast to Groups 1 and 2.

Discussions

To date, there was no report that evaluated the bactericidal effect of 3% H_2O_2 photoactivation with 940 nm diode laser in conjunction with SRP procedure in treating periodontal disease.

Since it is an in vivo pilot study, the rational of performing the microbial sampling at 1 month after therapy was to

Table 3. Study Protocol for Control and Test Groups

	Control	*Test*	
Groups	*Group 1*	*Group 2*	*Group 3*
Baseline	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
Week 2	Professional dental cleaning OHI	Professional dental cleaning OHI	Professional dental cleaning OHI
Week 3	SRP	SRP +940 nm diode laser, 1.1 W, CW	SRP + H_2O_2+940 nm diode laser, 1.1 W, CW
1 Month	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)

H_2O_2, hydrogen peroxide; OHI, oral hygiene instructions; SRP, scaling and root planning.

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eliminate any potential risk that could influence the bactericidal outcomes related to speed and degree of further biofilm recolonization.[15] Thus, for the periodontal examination a minimum of 3 months was necessary to assess the first relevant clinical signs of periodontal status.

This study is the first report regarding the possible antimicrobial effect of the H_2O_2 photoactivation with 940 nm diode laser. Although this wavelength is lacking support when it comes to bactericidal effect in the treatment of periodontal pockets, in this study we showed that in combination with SRP a strong bactericidal effect can be achieved. Nevertheless, there is no report about photoactivation of any solutions with this wavelength to increase their antimicrobial effect against periodontal microorganisms.

However, some limitation should be noted. The first limitation of this study was based on the small sample size of only 38 patients. However, a pilot study was necessary to investigate the bactericidal effect of H_2O_2 photoactivation with 940 nm diode laser. Second limitation was with regard to 1-month microbiological examination after periodontal treatments. Because of the high cost of real-time PCR analysis of three individual samples per patient, we decide not to increase the medical costs for further investigation such as 3 and 6 months. The third limitation was represented by the short-term clinical outcomes of only 3 months, which may lead to an underestimation of the periodontal pocket regeneration. The last limitation of this pilot study was based on the impossibility to assess different laser parameters such as exposure time, power settings, and number of sessions. Owing to the study design (split-mouth study) we were not able to investigate different combinations of laser parameters at the same individual because the sole bactericidal effect of H_2O_2 photoactivation with 940 nm diode laser after SRP was not reported yet. We know from the literature that multiple sessions of laser decontamination could improve the bactericidal effect within the periodontal pockets.[16] For future research, we suggest to extend the clinical and microbiological examination at 3, 6, and 12 months after this procedure. In addition, the use of different laser parameters such as longer exposure time and a higher number of laser decontamination sessions at different intervals should be investigated.

Table 4. Clinical Characteristics of Each Quadrants

Tested groups assigned to quadrants	*Number of teeth in each quadrant (median)*	*Number of teeth that exhibited PD ≥5 mm/quadrant (median)*	*Single/ multirooted teeth investigated*
Group 1	6	4	27/11
Group 2	7	5	25/13
Group 3	7	5	28/10

This trial evaluated the antimicrobial effect of a new nonsurgical laser-assisted periodontal protocol for moderate to severe periodontitis with the 940 nm wavelength. The rationale of using 1.1 W in continuous mode (CW) is represented by our previous clinical findings. By increasing the power above this setting, the fiber optic tip will start to initiate in the presence of blood from the bleeding pockets or right after the bleeding from SRP resulting in removal of the inner epithelium of the pocket and losing the photoactivation of H_2O_2 effect.

The present results demonstrated that every investigated procedure (all groups) showed a statistically significant outcome in terms of bacteria elimination at 4 weeks postoperative (Table 5). Nevertheless, Group 3 presented statistically highly significant outcomes ($p < 0.001$) for all the investigated parameters leading to the premise that this procedure can enhance the antimicrobial effect of SRP and diode laser decontamination.

SRP alone failed to decrease TBC ($p = 0.124$) in contrast to Group 2 and Group 3 that successfully decreased TBC ($p < 0.001$), although Group 3 exerted a better outcome than Group 2 (Table 5).

A. actinomycetemcomitans was present in only two patients; therefore, it was not reasonable to calculate percentiles and median values, but to discuss them individually. In one patient, 1 month after SRP (Group 1), *A. actinomycetemcomitans* was present. The other patient presented *A. actinomycetemcomitans* at baseline in Group 3 and 1 month after the proposed treatment it showed complete elimination.

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Table 5. Independent Sample Test of Median and Percentiles for Microbiological Variables

	Group 1 (SRP)			Group 2 (SRP+diode 940 nm)			Group 3 ($SRP + H_2O_2$+Diode 940 nm)		
Variables	*Median*	*25–75%*	p	*Median*	*25–75%*	p	*Median*	*25–75%*	p
TBC									
Baseline	13.5×10^6	6.5×10^6–28×10^6	0.124	7.15×10^6	2.95×10^6–29.25×10^6	0.001	18.5×10^6	3.625×10^6–58×10^6	0.000
1 Month postop	5.85×10^6	1.275×10^6–17.25×10^6		3.15×10^6	1.475×10^6–12×10^6		0.71×10^6	0.09525×10^6–3.525×10^6	
Aggregatibacter actinomycetemcomitans									
Baseline	0×10^6	0×10^6–0×10^6	—	0×10^6	0×10^6–0×10^6	—	0×10^6	0×10^6–0×10^6	—
1 Month postop	0×10^6	0×10^6–0×10^6		0×10^6	0×10^6–0×10^6		0×10^6	0×10^6–0×10^6	
Porphyromonas gingivalis									
Baseline	0.082×10^6	0.009875×10^6–0.57×10^6	0.000	0.125×10^6	0.0265×10^6–0.36×10^6	0.000	0.155×10^6	0.0455×10^6–0.5125×10^6	0.000
1 Month postop	0×10^6	0×10^6–0.00595×10^6		0.00023×10^6	0×10^6–0.023×10^6		0×10^6	0×10^6–0×10^6	
Treponema denticola									
Baseline	0.051×10^6	0.031×10^6–0.1625×10^6	0.000	0.0565×10^6	0.022×10^6–0.21×10^6	0.000	0.102×10^6	0.033×10^6–0.285×10^6	0.000
1 Month postop	0×10^6	0×10^6–0.00885×10^6		0.0005×10^6	0×10^6–0.007075×10^6		0×10^6	0×10^6–0×10^6	
Tannerella forsythia									
Baseline	0.033×10^6	0.0066×10^6–0.155×10^6	0.000	0.039×10^6	0.009375×10^6–0.13×10^6	0.000	0.0395×10^6	0.016×10^6–0.2025×10^6	0.000
1 Month postop	0×10^6	0×10^6–0×10^6		0×10^6	0×10^6–0.002575×10^6		0×10^6	0×10^6–0×10^6	
Prevotella intermedia									
Baseline	0.024×10^6	0×10^6–0.1175×10^6	0.030	0.00775×10^6	0.000357×10^6–0.17×10^6	0.008	0.057×10^6	0×10^6–0.245×10^6	0.000
1 Month postop	0.00012×10^6	0×10^6–0.01125×10^6		0.056×10^6	0×10^6–0.02975×10^6		0×10^6	0×10^6–0×10^6	
Peptostreptococcus micros									
Baseline	0.0135×10^6	0.00315×10^6–0.03325×10^6	0.040	0.006050×10^6	0.0016×10^6–0.0185×10^6	0.029	0.00915×10^6	0.002775×10^6–0.0335×10^6	0.000
1 Month postop	0.0053×10^6	0.0004325×10^6–0.014×10^6		0.00425×10^6	0.0004875×10^6–0.0098×10^6		0×10^6	0×10^6–0×10^6	
Fusobacterium nucleatum									
Baseline	0×10^6	0×10^6–0.01875×10^6	0.220	0×10^6	0×10^6–0.00595×10^6	0.872	0×10^6	0×10^6–0.00885×10^6	0.000
1 Month postop	0.000065×10^6	0×10^6–0.007225×10^6		0×10^6	0×10^6–0.003625×10^6		0×10^6	0×10^6–0×10^6	
Eubacterium nodatum									
Baseline	0.00017×10^6	0×10^6–0.00155×10^6	0.000	0×10^6	0×10^6–0.0006825×10^6	0.000	0×10^6	0×10^6–0.00165×10^6	0.000
1 Month postop	0×10^6	0×10^6–0×10^6		0×10^6	0×10^6–0×10^6		0×10^6	0×10^6–0×10^6	
Capnocytophaga gingivalis									
Baseline	0.000505×10^6	0×10^6–0.00485×10^6	0.031	0.0015×10^6	0×10^6–0.009875×10^6	0.275	0.00115×10^6	0×10^6–0.006675×10^6	0.858
1 Month postop	0.00555×10^6	0.000335×10^6–0.02525×10^6		0.0028×10^6	0.0001575×10^6–0.02425×10^6		0.0015×10^6	0×10^6–0.006025×10^6	

postop, postoperative; TBC, total bacteria count.

Table 6. Qualitative Analysis for Microbiological Variables

	Group 1 (SRP)		Group 2 (SRP +940 nm)		Group 3 (SRP + H_2O_2+940 nm)	
Variables	*Bacteria count, n (%)*	p	*Bacteria count, n (%)*	p	*Bacteria count, n (%)*	p
TBC						
Baseline	38 (100)	—	38 (100)	—	38 (100)	—
1 Month postop	38 (100)		38 (100)		38 (100)	
A. actinomycetemcomitans						
Baseline	0 (0)	—	0 (0)	—	1 (2.7)	—
1 Month postop	1 (2.7)		0 (0)		0 (0)	
P. gingivalis						
Baseline	35 (92.1)	<0.001	35 (92.1)	<0.001	33 (86.8)	<0.001
1 Month postop	17 (44.7)		20 (52.6)		5 (13.2)	
T. denticola						
Baseline	35 (92.1)	<0.001	36 (94.7)	<0.001	36 (94.7)	<0.001
1 Month postop	18 (47.4)		20 (52.6)		3 (7.9)	
T. forsythia						
Baseline	37 (97.4)	<0.001	38 (100)	<0.001	37 (97.4)	<0.001
1 Month postop	8 (21.1)		15 (39.5)		0 (0)	
P. intermedia						
Baseline	27 (71.1)	0.118	30 (78.9)	0.727	28 (73.7)	<0.001
1 Month postop	20 (52.6)		28 (73.7)		6 (15.8)	
P. micros						
Baseline	36 (94.7)	0.125	36 (94.7)	0.065	38 (100)	<0.001
1 Month postop	31 (81.6)		29 (76.3)		7 (18.4)	
F. nucleatum						
Baseline	17 (44.7)	0.815	12 (31.6)	0.549	17 (44.7)	<0.001
1 Month postop	19 (50)		15 (39.5)		5 (13.2)	
E. nodatum						
Baseline	22 (57.9)	<0.001	18 (47.4)	<0.001	16 (42.1)	<0.001
1 Month postop	1 (2.6)		3 (7.9)		38 (100)	
C. gingivalis						
Baseline	25 (65.8)	0.057	28 (73.7)	>0.1	24 (63.1)	>0.1
1 Month postop	33 (86.8)		29 (76.3)		25 (65.7)	

Although in our study the presence of *A. actinomycetemcomitans* was inconstant, there are other reports suggesting that conventional SRP alone is inefficient in eliminating aerobe facultative anaerobe species as *A. actinomycetemcomitans* and it is appropriate to combine other therapies with SRP to remove it from the periodontal pockets.[17–19] Based on this observation there is a possibility for the photoactivation of H_2O_2 with 940 nm diode laser to eliminate aerobe and anaerobe species like *A. actinomycetemcomitans* and maybe others that could not be investigated in this study.

Regarding the keystone periodontal bacteria from the red complex (*P. gingivalis*, *T.* denticola, and *T. forsythia*), all three groups showed high statistical results ($p = 0.000$) at 1 month postoperative as given in Table 5.

Table 7. Clinical Assessment of Periodontal Probing Depth and Clinical Attachment Level

	PPD (mm)		CAL (mm)	
Variables	*Baseline*	*3 Months postop*	*Baseline*	*3 Months postop*
Group 1 (SRP)				
Median	6.5	4	8	6
25–75%	6–7	3–5	7–9	5.75–7
p	0.000		0.000	
Group 2 (SRP +940 nm)				
Median	6	4	8	6.5
25–75%	5–7	3–5.25	7–10	5–8.25
p	0.000		0.000	
Group 3 (SRP + H_2O_2+940 nm)				
Median	6	3	8	5
25–75%	5–8	2–4	7–10	4–6.25
p	0.000		0.000	

Table 8. Clinical Assessment of Bleeding on Probing

	BoP positive, n (%)	
Groups	*Baseline*	*3 Months postop*
Group 1 (SRP)		
(+) Present	37 (97.4)	21 (55.3)
p	<0.001	
Group 2 (SRP+laser)		
(+) Present	36 (94.7)	19 (50)
p	<0.001	
Group 3 (SRP + H_2O_2+laser)		
(+) Present	37 (97.4)	3 (7.9)
p	<0.001	

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Table 9. Clinical and Bacterial Differences Between Test and Experimental Groups

	p					
	Group 1 vs. Group 2		*Group 1 vs. Group 3*		*Group 2 vs. Group 3*	
Bacteria	*Preop*	*Postop*	*Preop*	*Postop*	*Preop*	*Postop*
TBC	0.226	0.540	0.880	0.000	0.448	0.000
A. actinomycetemcomitans	—	—	—	—	—	—
P. gingivalis	0.823	0.415	0.526	0.001	0.282	0.000
T. denticola	0.827	0.743	0.352	0.000	0.451	0.000
T. forsythia	0.629	0.095	0.747	0.003	0.336	0.000
P. intermedia	0.679	0.034	0.282	0.001	0.352	0.000
P. micros	0.294	0.437	0.771	0.000	0.483	0.000
F. nucleatum	0.164	0.432	0.918	0.000	0.237	0.003
E. nodatum	0.333	0.314	0.477	0.317	0.964	0.079
C. gingivalis	0.346	0.595	0.750	0.015	0.544	0.086
PPD	0.262	0.201	0.915	0.023	0.462	0.001
CAL	0.800	0.257	0.799	0.002	0.577	0.000

It is well known that from the orange complex, *F. nucleatum and P. intermedia* have a particular role as a bridging organism between early and late colonizers.[20–22] As shown in Table 5, Groups 1 and 2 manage to reduce statistically only *P. intermedia* and *P. micros*, whereas photoactivation of H_2O_2 successfully eliminated all three species ($p = 0.000$), especially the bridge bacteria *F.* nucleatum, which is the most important bacteria from the orange complex.

C. gingivalis belonging to the green complex presented a significant decrease in only Group 1, whereas Group 2 and Group 3 exhibited an increase in *C. gingivalis* counts, $p = 0.275$ and $p = 0.858$, respectively. These results support the hypothesis that increased green complex bacteria are correlated to better periodontal health.[23]

From a qualitative point of view (Table 6), all three groups managed to eliminate significantly ($p < 0.001$) *P. gingivalis*, *T.* denticola, and *T. forsythia.* Group 3 was the only one capable of successfully eliminating ($p < 0.001$) the orange complex bacteria (*P. intermedia*, *P. micros*, and *F. nucleatum*) responsible for creating the living conditions for the strictly anaerobic bacteria of the red complex and their colonization of the periodontal pocket.[24]

All investigated groups demonstrate a statistical improvement ($p < 0.001$) of all investigated clinical parameters like PPD, CAL (Table 7), and BoP (Table 8).

By comparing the outcomes between the investigated groups, the results demonstrated that SRP (Group 1) and SRP +940 nm diode laser (Group 2) failed to offer significant differences in contrast to photoactivation of H_2O_2 with 940 nm diode laser (Group 3). As given in Table 9, the results between Group 1 and Group 2 are comparable; the only significant difference can be seen in *P. intermedia.* On the contrary, superior differences in both antimicrobial and periodontal status improvement were seen in favor of Group 3. SRP and photoactivation of H_2O_2 with 940 nm wavelength was able to provide a significant elimination of the most aggressive periodontal pathogens, both in comparison with classical therapy and laser therapy proposed by other authors.[25]

Thus, the hypothesis is accepted, suggesting that photoactivation of H_2O_2 with 940 nm diode laser can be beneficial when used as an adjunctive antimicrobial therapy during nonsurgical periodontal treatment.

There is a concern that the ROS, including hydroxyl radicals, cause oxidative damage to cellular and oral tissues, but the use of 3% H_2O_2 is a safe disinfection procedure because of the rapid decomposition into water and oxygen. Hydroxyl radical, one of the ROS, has a single unprotected electron in its structure, being capable of easily oxidizing other substances.[13] When it comes to laser photoactivation of 3% H_2O_2, it was demonstrated that the generation of ROS stopped immediately after the cessation of laser irradiation,[26] providing a controllable disinfection system that can be used safely in periodontal treatments.

By consulting the literature, there are few studies that have demonstrated the synergistic effect of photolysis of H_2O_2 with different light sources, used both in periodontal and endodontic pathology.[26–30] The most frequently used wavelength for the photolysis of H_2O_2 is the 405 nm LED (light-emitting diode).

The first report about the photoactivation of 3% H_2O_2 with the laser wavelength of 940 nm in the treatment of chronic periodontal disease was first introduced by Odor et al.[14]

Odor showed that the photoactivation of 3% H_2O_2 with 940 nm diode laser without combining it with SRP, can efficiently eliminate ($p < 0.001$) periodontal bacteria like *P. gingivalis*, *T. denticola*, *T. forsythia*, *P. intermedia*, *P. micros*, *F. nucleatum*, and *E. nodatum* and decrease the TBC. In addition, they compare the outcomes between each investigated group (SRP, H_2O_2 alone, 940 nm laser alone, and photoactivation of H_2O_2 with 940 nm) and the study showed great significant results ($p < 0.001$), eliminating the major periodontal pathogenic species like *P. gingivalis*, *T. denticola*, *T. forsythia*, *P. intermedia*, *P. micros* and also for the TBC ($p < 0.005$) when using the photoactivation of H_2O_2 with 940 nm. Although their trial study was investigating the periodontal clinical indexes for a short period of time (3 months), PPD and CAL showed great improvement without using the mandatory SRP with $p < 0.005$ and $p < 0.008$, respectively.[14]

In a systematic review, Akram et al.[31] evaluated the bactericidal outcomes of aPDT as an adjunct to SRP based on 17 in vivo studies. The wavelengths used by the investigated studies were ranging from 470 to 810 nm. In

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addition, a variety of photosensitizers were used in different concentrations. The authors underlined the importance of standardized wavelength related to photosensitizer and power densities in nonsurgical periodontal treatments. Furthermore, they observed a significant reduction of *A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola,* and *T. forsythia* when aPDT+SRP was used with higher power densities (60–400 mW/cm^2) than SRP alone. In contrast, lower power densities (13–75 mW/cm^2) offer a comparable periodontal bacteria reduction for aPDT and SRP applications. In our study the power density for each laser group was 1549 W/cm^2, therefore we can confirm their statement. Although the outcomes of the investigated articles indicate a satisfying bactericidal effect against the major periodontal pathogens (*A. actinomycetemcomitans*, *P. gingivalis*, *T. denticola*, and *T. forsythia*), the authors concluded that the bactericidal efficacy of aPDT as an adjunct to SRP in periodontal disease remains unclear.

In an in vitro study, Eick et al.[32] evaluated the response of photoactivated disinfection (PAD) in 6 mm artificial pockets by using 630 nm LED wavelength, concerning 16 microbial species. The authors reported that after preexposure to 0.25% of H_2O_2 the PAD was efficiently eliminating most of the periodontal species. Nevertheless, the preexposure to H_2O_2 before the PAD was the only procedure that eliminated the *A. actinomycetemcomitans* within the biofilm significantly.

P. gingivalis, one of the most important species in periodontal disease, is successfully eliminated when using H_2O_2 and PAD,[27,32] which correspond to our findings, although we used the 940 nm diode laser wavelength.

Conclusions

Within the limitations of this study we can conclude the synergistic effect of SRP and photoactivation of H_2O_2 with 940 nm diode laser offers an efficient and reliable antimicrobial effect in the nonsurgical periodontal treatment approach.

The suggested protocol may represent a new alternative for the 940 nm diode users in the treatment of periodontal disease, eliminating the need for local or general antibiotic administration and their side effects. Further investigation is needed for long-term results.

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