Association of oxygen-ozone therapy with high-power photodynamic therapy: a new protocol for the treatment of periodontopathies

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Abstract
In this study, we want to demonstrate that oxygen-ozone therapy together with laser therapy (high-power photodynamic therapy) leads to substantial and rapid benefits for periodontopathic patients.

Introduction
There are numerous studies that show that, in the comparison between surgical and non-surgical treatment of periodontal diseases, the results at ten years overlap: scaling and smoothing of the root, alone or in combination with flap procedures, are effective methods for the treatment of chronic periodontitis. However, it can be observed that in the treatment of deep pockets, the open flap debridement leads to a greater reduction in the depth of the pocket and a greater clinical attack gain compared to non-surgical modes. Non-surgical modes in shallow pockets always lead to less post-therapy recession and are clearly recognised as more conservative.1

The ideal treatment should therefore be non-surgical, which at the same time allows for less recession and greater reduction in the depth of the pocket and a greater clinical attack gain, possibly in the fastest possible time.

Laser therapy
In periodontology, today, in addition to mechanical treatment, lasers are used for photodynamic therapy (PDT): this therapy can be defined as the inactivation of cells, microorganisms or molecules, induced by light and not by heat; it is painless and after a few applications the bleeding, sensitivity and symptoms associated with periodontal disease disappear;2 furthermore the probing depth is reduced.

PDT requires a light source (laser), a photosensitizer (an oxygen-containing substance) and oxygenated tissues. Oxygen, in fact, is the crucial molecule for executing PDT. Photodynamic implies the application of luminous photonic dynamics on biological molecules.3,6 The action mechanism of PDT involves the interaction of light with the dye which the target tissues have soaked up. Dyed molecules attach to the bacterial membrane of microorganisms.7,8

The laser light activates the dye molecule or photosensitiser, while, at the same time, the resulting reaction with free oxygen triplet oxygen with 2 unpaired rotating electrons9,10 occurs. Given the coupling of two uninvited, unpaired electrons, the interaction between triplet oxygen and laser energy results in the formation of singlet oxygen, which determines the oxidation of the lipid membrane of bacteria and their cell death.2,11-14

To date, only high-penetration diode lasers (from 600 to 1100 nm) have been considered for PDT, in which photons are poorly absorbed by water and hydroxyapatite. Due to such a low level of absorption, the wavelengths included in this range can penetrate into the tissues up to 2 cm. This can be particularly suitable for the treatment of pathologies characterised by high bacterial dissemination, such as periodontal diseases, while mechanical treatment protocols can only act on directly treated surfaces, such as the hard tissues of the tooth (cement and dentin) and hard and soft tissues of the periodontium included in the treatment site. The possibility of deeper penetration could be useful to eradicate those bacteria that are involved in the pathology, but which are not necessarily adjacent to the sick tooth.

Low power LLLT (low level laser therapy) pulsed diode (LI) lasers are used in association with H2O2 at 3% at 10 volts; or high-frequency super-pulsed diode laser (LII) in association with hydrogen peroxide stabilised with a glycerol phosphate complex.

In normal setting conditions, diode lasers with power over 2 Watt (HLLT: high level laser therapy) demonstrate a high thermal effect;15 this is why research has fundamentally tested low power diode lasers (LLLT: low level laser therapy) with energy pulses within milliseconds (pulsed lasers) or continuous emission energy pulses that do not produce a significant temperature increase (not above 45°C). These types of lasers are normally used together with coloured photosensitisers, with typical absorption ranges in long wavelength bands.
However, it has been noted that classical PDT is only partially effective in diseases that show deep bacterial infiltration. This can be ascribed to the low peak power applied, below 2 Watts, as well as to the low penetration capacity of the laser light in the photosensitiser-impregnated tissues, with a biocidal effect that can be limited only to external and/or superficial areas.

The advantage of using LII over LI may be the greater efficacy in providing singlet oxygen when the laser meets hydrogen peroxide (more than 7000 times per second compared to 50/500 pulses). The high frequency could improve the activity of the impulses within the soft tissues and the effectiveness of the decontaminating effects of HLLT.

The comparative evaluation of bio stimulation data and biocidal efficiency data indicates that high frequency lasers (LII) are more efficient than diode lasers (LI). Experimental studies show the effects the stabilisers contribute to the properties of hydrogen peroxide. The use of hydrogen peroxide in a glycerol phosphate complex substantially decreases the bacterial load, compared to common hydrogen peroxide, and minimises the cytotoxic impact due to its particular physiological composition.

Oxygen-Ozone therapy

Background

In 1785, Van Marum noticed that the air near his electrostatic machine acquired a characteristic smell when electric sparks passed. In 1801, Cruickshank observed the same smell around the anode during the electrolysis of water. In 1840, Schonbein called the substance Ozein, from the Greek word which means to smell. In 1856, ozone was used to disinfect operating theatres. In 1857, Werner Von Siemens designed a cylindrical dielectric ozone generator, which represents most of the commercially available ozone generators, and called it a Siemens ozone generator. In 1860, Monaco first used the ozone generator in the water treatment plant.

In 1870, ozone was used for the first time in a medical application, when Dr. C. Lender purified blood in test tubes. In 1901, Wiesbaden used ozone in a municipal water supply. In 1931, a dentist, Dr. E.A. Fisch, used ozonated water for dental procedures and was a pioneer in its use in medicine.

Forms of administration

- **Ozone gas**: ozone can be used in gas form using an open system or a sealed suction system to avoid inhalation and its adverse effects.
- **Ozonated water**: ozonated water has proven to be very effective against bacteria, fungi and viruses.
- **Ozonated oil**: in addition to gas ozone and ozonated water, ozonated oils are also very much recommended from the clinical point of view.
- Administration routes used in medicine include: i) large auto emo infusion; ii) small auto emo infusion; iii) subcutaneous and submucosal; iv) intramuscular; v) intra-articular; vi) insufflation (anal, vaginal, uterine, urethral, articular); vii) topical route; viii) hydropinic treatment.
- Advantages: i) disinfectant; ii) anti-inflammatory; iii) activation of intracellular metabolism of oral mucosa and dental wounds; iv) improved regional circulation; v) stimulation of regenerative processes; vi) haemostasis in capillary haemorrhage.
- Disadvantages: i) toxic if inhaled; ii) instable; iii) it requires certified medical equipment to produce it.

Oxygen-ozone therapy in dentistry

Ozone is a chemical compound consisting of three oxygen atoms (O3), with a molecular weight of 41.98 g/mol. It has a higher energy form than normal atmospheric oxygen (O3), which makes it highly instable and, for this reason, it is a powerful oxidiser.

Its effectiveness in killing bacteria, fungi, viruses and parasites has been demonstrated.

Over the past 50 years, extensive research has been conducted on the use of ozonated fluids for infection control and wound management. Ozone has been proposed as an alternative oral antiseptic in dentistry. This has given rise to a series of procedures that aim to eliminate only the infected and demineralised dental tissue, while preserving and protecting the tooth structure.

Other actions that ozone is known to have on the human body include its immunostimulant, analgesic, anti-hypoxic, detoxifying and antimicrobial properties.

Numerous authors recommend ozone for its positive effects on bone and soft tissue. In oral surgery, ozonated water is suitable for prophylactic applications against infections after osteotomies. In a prospective study, which involved 250 patients, the use of ozonated water during surgery, as a cooling and rinsing medium in the osteotomy of the third molars, reduced the onset of complications due to infectious diseases after the operation.

In another prospective study, the positive effect of ozonated water on oral soft tissue healing was clinically and histologically demonstrated.

In addition to its microbiological effect, ozone also has a therapeutic effect. Several experimental studies have shown that the treating blood with ozone leads to a modulation in the release of interferons (IFN-α, IFN-β, IFNγ), interleukins (IL-1β, IL-2, IL-6, IL-8) and tumour necrosis factor (TNF-α), in addition to growth factor transformation (TGF-β1). Furthermore, ozone improves the rheological properties of erythrocytes and facilitates the release of oxygen in the tissues, which can be attributed to the stimulation of 2,3-diphosphoglycerate and production of adenosine triphosphate (ATP) in erythrocyte metabolism.

Indications in dentistry

- Chronic or recurrent infections in the oral cavity
- Prophylaxis and prevention of dental caries
- Mineralisation of furrow and pit caries, roots and smooth surface caries
- Sterilisation of cavities and root canals
- Hygiene
- Stomatitis and candida albicans
- Mouth ulcers
- Herpes
- Pre- and post-surgery
- Dental extractions
- Alveolitis
- Periodontology
- Implantology: increase in implant hydrophilicity, treatment of peri-implantitis
- TMJ disorders
- Bisphosphonate osteonecrosis
- Full mouth disinfection
- Regenerative surgery
- Cervico-cranio-mandibular disorders
- Preservation of the alveolus

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- Bone grafts
- Abscesses
- Revascularized flaps
- Healing delays in oral surgery
- Preparation and management of complications of the large and small maxillary sinus
- Apical reactions
- Osteomyelitis

Use of oxygen-ozone in periodontal diseases

As we have said, gingivitis and periodontitis are the most common inflammatory diseases of teeth support tissues. The role of microbial etiology and host response in the progression of gingival and periodontal diseases has been scientifically confirmed.21

As a result of its beneficial biological effects and thanks to its antimicrobial and immunostimulatory effect, oxygen-ozone is well indicated in the treatment of gum and periodontal diseases.

To date, for periodontal diseases, ozone has been exclusively administered using the topical route, in all three forms, namely ozone gas, ozonated water and ozonated oil. However, given the close correlation between the oral cavity and the upper respiratory tract, special precautions must be taken when ozone is administered as a gas.

The topical application of ozone in all its forms inside the periodontal pockets does not give rise to particular concerns because it is sufficient to bring a high-speed aspirator close to the insufflation area to guarantee wide safety margins. On the other hand, the use of large quantities of ozone gas on dental arches must include the construction, by the prosthodontist, of special customised masks on the patient that must extend 2-3 mm beyond the affected gingival area, leaving a free space for gas circulation. Two outlets must be connected for the gas inlet and outlet, at the distal and mesial level of the treatment area, respectively. As a safety precaution to completely seal the edges, a photopolymerisable or silicone dam can be applied. This procedure will treat both hard and soft tissues of the area concerned.

Experimental study

The use of oxygen-ozone therapy in the treatment of periodontal disease has now been clinically and scientifically consolidated in a number of studies; however, its use inside narrow and deep periodontal pockets is not always so easy, because the frequent presence of blood leads to continuous obliteration of the needles used for insufflation.

This study proposes a new protocol, which foresees both the use of oxygen-ozone in the oral cavity, constructing special customised masks for the widespread treatment of both dental arches, and the use of a Superpulse Light Diode Laser of 980 nm with 400-micron fibre, undoubtedly easier for the treatment of periodontal pockets.

The purpose of this study is to evaluate its effectiveness, comparing it with conventional causal therapy and photodynamic therapy (PDT).

Materials and Methods

Two impressions were taken with alginate (Hydrogum Fast Setting, Zhermack SpA, Badia Polesine (RO), Italy) of the patient’s dental arches. The impressions had to have a wide extension in order to be able to read and extend the masks beyond the dental arches, so as to ensure they have a good margin of closure on the gum tissue, to achieve a sealing effect.

In patients in whom it was not possible to take alginate impressions due to a marked emetic reflex, an intra-oral 3D laser scanner was used (Align Technology, Inc 2820 Orchard Parkway, San Jose, CA, USA). The scans were processed with EXOCAD software (ver. MATERA, prototype-2018-11-08, Darmstadt, Germany; Figure 1A) and later models with resin Pro3dure Gr-13 (MICROTECNOR, Buccinasco, Italy) were produced using a 3D printer (ASIGA MAX, Sydney, Australia; Figure 1B).

On the models obtained, discharge wax was applied to the dental arches, with the aim of creating a circulation chamber for the ozone gas below the masks (Figure 2).

The models thus prepared were duplicated in plaster, because the wax could not withstand the high temperatures for the thermomoulding of the masks (Figure 3).

The masks were produced with an Erkoform-3d motion thermo-printing machine using two transparent Erkoloc-pro disks with a diameter of 120 mm and a thickness of 3 mm Ref. 595130 (Erkodent Erich Kopp GmbH, Pfalzgrafenweiler, Germany), and using an occluder (Occluform-3, Erkodent Erich Kopp GmbH, Germany).

Figure 1. A) Intraoral scans with iTero Align Technology scanner and processed with Exocad software; B) Models produced with resin Pro3dure Gr-13 via 3D printer.
Pfalzgrafenweiler, Germany), to ensure that they could stabilise in the oral cavity. Next, the masks were finished in the laboratory as regards their extension, so as to have an optimal seal and ensure there were no positioning interference with respect to muscular insertions.

Then, two Ø 4 mm x ID 3 mm soft copper tubes were applied for hydraulic refrigeration devices in the most distal areas, to ensure that the ozone gas was forced to circulate around the entire arch. In addition, a cylindrical brass tube was applied to the upper mask, which acts as a safety suction duct, to intercept accidental ozone gas leakage from the devices (Figure 4).

The masks were then tested in the mouth, to verify that they had a good marginal seal and did not leak. They were then brought locked in place by asking patients to close their teeth forcefully. The copper and brass tubes were attached with Fr 20 (copper tubes) and Fr 10 (brass tube) Nelaton catheters. Traditionally, it has been established that the ozone gas circulation circuit had: i) an inlet through

Figure 2. Exhaust wax applied to the models of the dental arches, with the aim of creating a circulation chamber for the gaseous ozone below the masks.

Figure 3. Duplication of plaster models.

Figure 4. Masks produced with Erkoform-3d motion thermo-moulding machine using two transparent Erkoloc-pro disks with diameter 120 mm and thickness 3 mm.
the copper pipes distal to quadrant 1; ii) an outlet distal to quadrant 2; iii) a connection between the outlet of quadrant 2 and an inlet distally to quadrant 3; iv) a final outlet distal to quadrant 4.

The last outlet was then connected to a high-speed aspirator, together with the Nelaton pipe coming from the brass safety circuit (Figure 5).

A vacuum test was then performed with ozonated water and oxygen gas to check the tightness of the entire system. For the treatment of periodontal pockets only, a galenic topical cream composed of hydrogen peroxide at 3% 10 vol. stabilised with PISL lipids (Galenico Laboratory Dr. Ternelli, Bibbiano, Reggio Emilia, Italy) was applied. After this application, the pockets were irradiated using a 980 nm diode laser (Wiser Doctor Smile, Lambda SpA, Italy) with 400-micron fibre, set to 2.5 Watts (average energy 0.625 W) and TON5 milliseconds and TOFF15 milliseconds, with 50 Hertz frequency and 50 seconds application time.

**Experimental protocol**

Five patients affected by different forms of periodontal disease were selected, two as a control group and 3 as a study group (Table 1).

Traditional periodontal therapy with only mechanical causal treatment was performed in the control group. In the study group, an experimental treatment lasting six weeks was performed with a weekly session. Table 2 shows all the procedures used step by step: during the first five weeks the operative protocol was applied, while at the sixth week the end-of-treatment parametric diagnostic data were compared to those before treatment.

The goal of the treatment was to reset the habitat of the microorganisms of the oral cavity, to put patients in the ideal anatomical-morphological conditions to be able to perform home hygiene manoeuvres with the best possible performance, and to motivate them to maintain the skills acquired over time, both from a quantitative and qualitative point of view.

![Figure 5. Connection of tubes with catheters in Nelaton to create a unidirectional circuit of oxygen-ozone; this allows a single application of therapy to both arches.](image)

In this regard, as a means of communication, viewing through an optical microscope (Leica DM750, Leica ICC50W camera, 1000x) images obtained from pre- and post-treatment observation of microbiological observations on slides (Gram-Hücker staining, DDKItalia, Vigevano, Pavia, Italy), and the subsequent discussion with patients proved very useful. This gave them direct awareness of the results obtained, closely related to the reduction of bacterial load (PI) and the consequent reduction of the bleeding index (BI). On average, after the third and fourth session, all patients stated that they perceived a marked improvement in the health condition of their mouth (Figure 6).

**Results**

In the control group, subjected only to causal therapy, the mean PPD and CAL, pre and post treatment, was calculated. A total of 54 teeth were assessed, of which 34 single-rooted and 20 multi-rooted, and the clinical percentage gain was calculated one year after the end of treatment (Figure 7A).

The data of the study group were then compared with the data

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<th>Post</th>
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<td>6th session</td>
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Figure 6. Images taken from the observation of slides (Gram-Hücker staining, DDKItalia, Vigevano, Pavia, Italy) under an optical microscope (Leica DM750, Leica ICC50W camera, 1000x) before and after treatment. This gave them direct awareness of the results obtained, closely related to the reduction of bacterial load (PI) and the consequent reduction of the bleeding index (BI).
Table 1. Selection of 5 patients with different forms of periodontal disease, two as a control group and 3 as a study group.

<table>
<thead>
<tr>
<th>S/C</th>
<th>Sex</th>
<th>Age</th>
<th>Risk factor</th>
<th>Diagnosis</th>
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<td>F</td>
<td>56</td>
<td>Smoker</td>
<td>Chronic moderate periodontitis</td>
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<tr>
<td>Control</td>
<td>F</td>
<td>59</td>
<td>None</td>
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</tr>
<tr>
<td>Study</td>
<td>F</td>
<td>39</td>
<td>Type 1 diabetes</td>
<td>Severe chronic periodontitis</td>
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<tr>
<td>Study</td>
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<td>Severe chronic periodontitis</td>
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<tr>
<td>Study</td>
<td>M</td>
<td>59</td>
<td>None</td>
<td>Severe chronic periodontitis</td>
</tr>
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Table 2. Procedures used phase by phase during the six weeks of treatment.

<table>
<thead>
<tr>
<th>1st session</th>
<th>Periodontal examination with recording of diagnostic parameters</th>
<th>Collection using BPA Premium Specific site microbiological analysis (Biomolecular Diagnostic, Florence, Italy) pre-treatment Pre-treatment sample inside the pocket, with smear on slide for Gram staining PPD (Probing Pocket Depth) REC (Recession) CAL: (Clinical Attachment Level) BI (Bleeding Index) PI (Plaque Index)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-3-4-5 sessions</td>
<td>Periodontal examination with recording of diagnostic parameters</td>
<td>Pre-treatment sample inside the pocket, with smear on slide for Gram staining BI (Bleeding Index) PI (Plaque Index)</td>
</tr>
<tr>
<td>6th session</td>
<td>Periodontal examination with recording of diagnostic parameters</td>
<td>Sample using BPA Premium Specific site microbiological analysis (Biomolecular Diagnostic, Florence, Italy) post treatment PPD (Probing Pocket Depth) REC (Recession) CAL: (Clinical Attachment Level) BI (Bleeding Index) PI (Plaque Index)</td>
</tr>
</tbody>
</table>

Scaling: With ultrasound and ozonated water

Treatment of periodontal pockets: Curettage, Topical application of the PISL compound, Pocket irradiation with HLLT super-pulsed diode laser, Pocket washing with ozonated water

Treatment of dental arches with masks: Application of 250 cc of ozonated osmosis water with OM3 High program (Multiossigen, Bergamo, Italy), Application of oxygen ozone gas at 35 y in a continuous flow for 4 minutes with Medical 95 Computerized Photometric System with high precision UV Photometric control (Multiossigen, Bergamo, Italy), Application of 250 cc of ozonated osmosis water with OM3 High program (Multiossigen, Bergamo, Italy), Post-treatment sample inside the pocket, with smear on slide for Gram staining

Home hygiene instructions for the patient: Super-soft toothbrushes, Interdental picks, Dental floss, Plaque disclosing tablets, Ozodent ozonated toothpaste (Laboratorio Terapeutico M. R., Florence, Italy), Topical application of ozonated oil in the pockets

Instructions to the patient: Motivational reinforcement for home hygiene, Correction of home hygiene procedures
on PDT LLLT (low level laser therapy) found in the literature. The study in question investigated 433 teeth in total, of which 309 single-rooted and 124 multi-rooted and the reduction in PPD was calculated at one year from the end of therapy (Figure 7B).

All the data were then compared with those obtained in our study group, supported by PDT HLLT (high level laser therapy) in association with topical oxygen-ozone applications with the aid of special customised masks (Figure 7C).

Furthermore, the trend of BI and PI was monitored throughout the six-week experimental treatment route (Figure 8).

### Conclusions

Although the results are statistically insignificant due to the low number of cases treated, from the clinical point of view the association of photodynamic therapy with HLLT diode laser with (PIGF) and oxygen-ozone therapy seems to lead to more promising and faster therapeutic results, suggesting that ozone plays a catalytic role in the healing processes of the periodontal ligament.

Given the modulating role of ozone in the humoral mediators involved in periodontal disease and participating in the host response, additional systemic administration routes, such as the Great Auto Emo Infusion, the Small Auto Emo Infusion (GAEI, PAEI) and rectal insufflation, could implement and improve healing processes, so they should be investigated by further targeted studies.

Despite the application difficulties, the procedure proved to be very well appreciated by the patients, who were able to note the progressive and immediate improvement in the state of their health.

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44. Martinez-Abreu J, Weisser MT, Menendez-Cerero S. Therapeutic effects of Ozone therapy in adult periodontitis treatment, subtypes I and II; 5 December, 2015.