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Ozone gel in chronic periodontal disease: a randomized clinical trial on anti-inflammatory and pro-regenerative effects of ozone application

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Abstract: Considering that antiseptics represent an aid to the gold standard nonsurgical treatment Scaling-and-Root-Planing (SRP) for periodontal disease, this study aims to assess the efficacy of the ozonized gel GeliO3 (Bioemmei Srl, Vicenza, Italy) plus SRP (experimental treatment), with respect to SRP + chlorhexidine gel. Ten participants were treated with SRP + chlorhexidine gel (control sites) and with SRP + ozone gel (trial sites). After 1 (T1) and 3 months (T2) from baseline (T0), patients were revisited. At each time-point, the following indexes were assessed: probing pocket depth (PPD), clinical attachment loss (CAL), gingival index (GI), plaque index (PI) and bleeding on probing (BoP). It has been assessed that the use of the ozonized gel GeliO3 in addition to SRP did not show significant differences if compared to conventional SRP + chlorhexidine. Chlorhexidine was found to be more effective than ozone only on the reduction of CAL and GI at T2. Despite this, the ozonized gel tested could be regarded as a valid support to SRP and as a substitute to chlorhexidine, especially because of the absence of the major shortcomings associated with the latter, besides the major applicability of ozonized products in dentistry. In this connection, we also glance at the latest research on ozone therapy.

Keywords: dentistry; periodontitis; scaling and root planing; ozone; chlorhexidine; periodontology; clinical trial; anti-infective; implant infections; antibiotic-resistance.

1. Introduction

In the last years, the use of ozone in medicine has significantly raised due to its recognized properties. Several in vitro studies have shown a wide antibacterial activity for ozonized vegetable oils against microorganisms, like bacteria, virus, protozoa and fungi [1,2]. In addition to that, ozone shows immunomodulatory, anti-hypoxic, biosynthetic, and anti-inflammatory properties which justifies its several applications both in medicine and dentistry [3]. As regards this latter, ozone therapy has been used to manage wounds healing, dental caries, oral lichen planus, gingivitis and periodontitis, halitosis, osteonecrosis of the jaw, post-surgical pain, plaque and biofilms, root canal treatment, dentin hypersensitivity, temporomandibular joint disorders, and teeth whitening [3,4].

Considering the abovementioned applications, the use of ozone for the treatment of gingivitis and periodontitis appears quite interesting for clinicians. In particular, this latter

condition is not only more and more frequent among patients but, in some cases, might be refractory to the treatment. Gingivitis arises from the accumulation on the teeth of dental plaque, corresponding to a complex biofilm of bacteria dipped into a polymeric matrix. If this biofilm is not properly removed by means of oral hygiene, gingivitis might develop into periodontitis, with a destruction of tooth-supporting tissues, in presence of other predisposing factors among which smoke, diabetes, immune disorders, etc. [5].

Scaling and root planing (SRP) is the gold standard non-surgical therapy which is aimed both to remove dental plaque and calculus as well as to smooth the root surfaces infected by bacteria [6]. In this context, however, the use of antimicrobic agents, like ozone, could be very useful considering the pathogenetic action exerted by bacteria in the development and maintaining of periodontal inflammation. In vitro exposition of bacteria to ozone causes the oxidation of phospholipids and lipoproteins constituting the bacterial cell envelope; this event leads to the disruption of the cytosolic membrane integrity, thus allowing ozone to infiltrate the microorganisms and oxidize glycoproteins and glycolipids, with a final block of the bacterial enzymatic function [3].

The aim of the present study is to evaluate the efficacy of the subgingival application of an experimental ozone gel in addition to standard SRP, as well as to compare this protocol with SRP plus a conventional chlorhexidine gel. The null hypothesis of the study is that there are no significant intergroup and intragroup differences between the two oral gel used for periodontal nonsurgical treatment.

2. Materials and Methods

2.1. Material

The products used for the experimentation and their characteristics are shown in Table 1.

Table 1. Products tested in the study.

Product	Description	Ingredients	Manufacturer
GeliO3	Ozonized gel	Bio-ozonized olive oil (20 mEq O_2/Kg), Hydrated Silica, Arnica	Bioemmei Srl, 36100 Vicenza, Italy
Curasept Parodontal gel 1% Ads	Chlorhexidine gel	Sorbitol, Aqua, Hydrated Silica, Glycerin, Xylitol, PEG-40 Hydrogenated Castor Oil, Cocamidopropyl Betaine, Aroma, Cellulose Gum, Chlorhexidine, Digluconate, Ascorbic Acid, Sodium Metabisulfite, Sodium Saccharin, Sodium Methylparaben, Sodium Citrate, CI 42090	Curasept SPA, 21047 Saronno, Varese, Italy

2.2. Trial Design

This study has been designed as a prospective single-group and single-center trial. No changes to the methods occurred after the commencement of the study. According to previous research, this study was designed as a split mouth study with the subdivision of the mouth into quadrants [7,8]. CONSORT guidelines have been followed to design this trial and to write the present report.

2.3. Participants, eligibility criteria, and settings

This study obtained the approval of the Internal Review Board (2020-0708) and participants signed an informed consent to take part to the experimentation and to allow the publication of the results obtained. Starting from September 2020 until November 2020, 10 patients with periodontal disease were recruited at the affiliation of the Authors where all the experimental phases have taken place until the end of the study (February 2021). Selected participants of both sexes with periodontitis (stage: grade III / severity: grade I-II) had to show a minimum of 10 teeth in the mouth, a probing depth > 5 mm at least in one site in each quadrant (minimum four pathological sites in four different teeth) and bleeding on probing [7]. People were excluded in case of the following situations: systemic diseases (e.g. uncontrolled diabetes, anaemia, cardiovascular diseases, infectious diseases), systemic diseases-related periodontitis, pathologic conditions of the oral mucosa, presence of fixed prostheses and orthodontic appliances, untreated decays, use of chewing tobacco, smokers, alcoholics, treatment with chlorhexidine in the last 6 weeks, pregnancy or feeding, use of systemic drugs in the last 3 months (antibiotics, FANS, steroids, inhibitors of the salivary flow, and anticoagulant / immunostimulant / immunosuppressive / antimycotic drugs) and contemporary use of topical drugs for the oral cavity. Additionally, people were excluded in case of concomitant participation to other clinical trials or lack of telephone contact.

2.4. Interventions and outcomes

During the first visit, participants underwent a professional oral hygiene and chairside instructions were also given at this appointment.

Two weeks later participants underwent another appointment (considered as baseline) in which the following clinical indexes were assessed: probing pocket depth (PPD) (distance from the gingival margin to the pocket base), clinical attachment loss (CAL) (differences between the position of the soft tissue in relation to the cement-enamel junction), gingival index (GI, Löe and Silness) (index 1-3, proportional to gingival inflammation), plaque index (PI - O'Leary) (percentage of sites with plaque) and bleeding on probing (BoP - Ainamo and Bay) (percentage of sites showing bleeding on probing) [5,9]. The two former indexes were respectively assessed on 4 sites for each tooth (mesial, buccal, distal and lingual) by means of a dental probe (UNC probe 15, Hu-Friedy, Chicago, IL), whereas the other three indexes were measured on 6 sites (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual).

At this same appointment, patients were randomly allocated to the respective treatment using a randomization table. Each quadrant of the mouth of the participants was randomly assigned to a treatment with SRP + chlorhexidine gel (control sites) and with SRP + ozone gel (trial sites), according to split mouth design. Only two or all the four quadrants of each patient's mouth were treated, depending on the number of sites with periodontal disease. SRP was conducted using a piezoelectric (Mini Piezon, EMS; Nyon, Switzerland) and Gracey curettes (Hu-Friedy, Chicago, IL), whereas the treatment with the oral gels consisted of a subgingival application by means of a syringe.

After 1 (T1) and 3 months (T2) from baseline (T0), patients were revisited; in case of necessity, a further professional supragingival oral hygiene was conducted at these appointments. In addition to that, periodontal clinical indexes were assessed again as previously described.

Chairside instructions for a correct domiciliary oral hygiene were repeated to participants at each appointment.

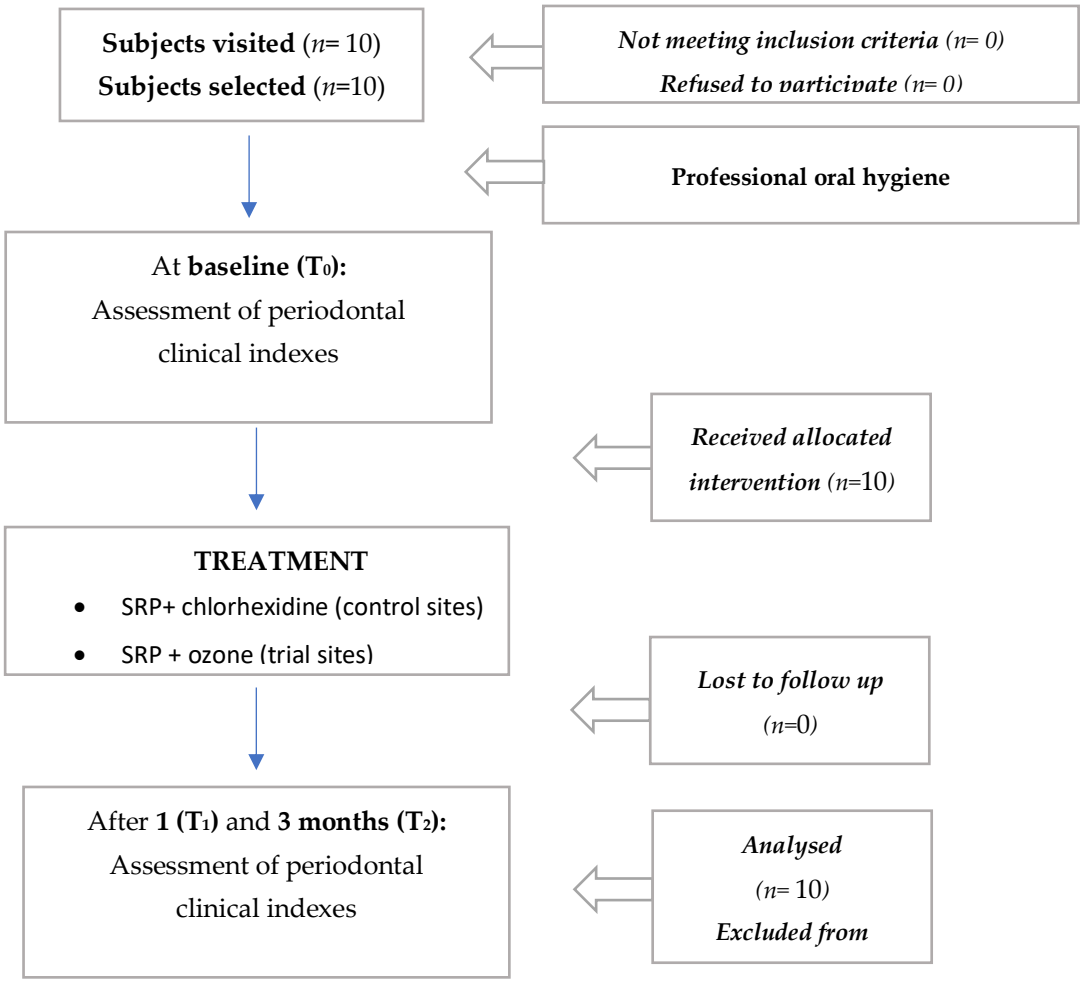
2.5. Sample size calculation

Sample size calculation (Alpha = 0.05; Power = 90%) for an independent study group and a continuous primary endpoint was performed. Concerning the variable gingival index (primary outcome) an expected mean of 1.80 was hypothesized, with a standard deviation of 0.60 [10]. The expected difference between the means was supposed to be 1.2 therefore 10 patients were requested. Loss to follow-up and incomplete compliance with therapy were excluded.

A total of 10 patients (4 males and 6 females, mean age 50 years old) was visited before the trial commencement and then selected for the study according to the sample size calculation. No one refused to participate or did not meet the inclusion criteria.

The flow chart of the study is shown in Figure 1.

Figure 1. Flow-chart of the study



2.6. Blinding

Professional oral procedures and outcomes assessment were respectively executed by two operators. Blinding the operator administering the treatment assigned was not technically possible but this one was not involved in any other phase of the study and was not in contact with the other researchers. Conversely, data assessor and data analyst were

always blinded during the study since none of them knew the treatment administered to each participant. Patients were asked not to reveal their respective treatment to the data assessor.

2.7. Statistical methods

Data were submitted to statistical analysis with R Software (R version 3.1.3, R Development Core 150 Team, R Foundation for Statistical Computing, Wien, Austria). For each variable, descriptive statistics (mean, standard deviation, median, minimum and maximum value) were calculated. PPD and CAL were measured in millimetres (mm), whereas PI and BoP as percentage, and GI with the relative score (0-3).

Data normality was calculated using the Kolmogorov–Smirnov test and subsequently a T test was applied. Significance for all statistical tests was predetermined at $P < 0.05$.

3. Results

The descriptive statistics of the clinical indexes assessed is shown in Table 2.

Table 2. Descriptive statistics of the clinical indexes assessed in the study.

Clinical index	Treatment	Time	Mean	SD	Min	Median	Max	Significance*
PPD	SRP + Ozone	T_0	6,21	0,92	5,25	6,27	8,40	A
		T_1	4,66	0,74	3,83	4,48	5,50	B
		T_2	4,20	0,48	3,50	4,15	5,20	B
	SRP + Chlorhexidine	T_0	5,94	0,89	5,00	5,89	7,50	A
		T_1	4,42	0,76	3,37	4,35	5,78	B
		T_2	3,95	0,52	3,25	3,90	4,95	B
CAL	SRP + Ozone	T_0	6,00	0,83	5,10	5,89	7,50	A
		T_1	4,42	0,76	3,37	4,35	5,78	B
		T_2	4,32	0,47	3,45	4,32	4,94	B
	SRP + Chlorhexidine	T_0	6,13	0,81	5,25	6,06	8,00	A
		T_1	4,85	0,90	3,83	4,54	6,50	B
		T_2	3,99	0,56	3,15	4,05	4,91	C
GI	SRP + Ozone	T_0	1,67	0,56	0,80	1,54	2,56	A
		T_1	1,01	0,38	0,50	0,95	1,60	B
		T_2	0,91	0,35	0,37	0,93	1,45	B
	SRP + Chlorhexidine	T_0	1,67	0,39	0,87	1,80	2,14	A
		T_1	1,06	0,38	0,45	1,15	1,50	B
		T_2	0,71	0,36	0,05	0,75	1,30	C
PI	SRP + Ozone	T_0	0,85	0,18	0,55	0,90	1,00	A
		T_1	0,54	0,09	0,40	0,50	0,70	B
		T_2	0,39	0,07	0,27	0,40	0,50	C
	SRP + Chlorhexidine	T_0	0,86	0,16	0,60	0,90	1,00	A
		T_1	0,52	0,07	0,40	0,50	0,65	B
		T_2	0,36	0,08	0,25	0,34	0,50	C
BOP	SRP + Ozone	T_0	0,43	0,27	0,07	0,40	0,87	A
		T_1	0,15	0,06	0,05	0,17	0,24	B
		T_2	0,09	0,04	0,02	0,09	0,15	C
	SRP + Chlorhexidine	T_0	0,33	0,13	0,18	0,31	0,50	A
		T_1	0,11	0,07	0,02	0,10	0,24	B
		T_2	0,09	0,06	0,02	0,07	0,17	C

*Different letters between the groups show statistically significant differences ($P < 0.05$).

3.1. Probing pocket depth (PPD)

Significant intragroup differences were found between each timepoint both for the sites treated with SRP plus ozone and for the sites treated with SRP plus chlorhexidine (P<0.05); no significant intergroup differences were found between the sites (P>0.05) (Table 2 and Figure 2).

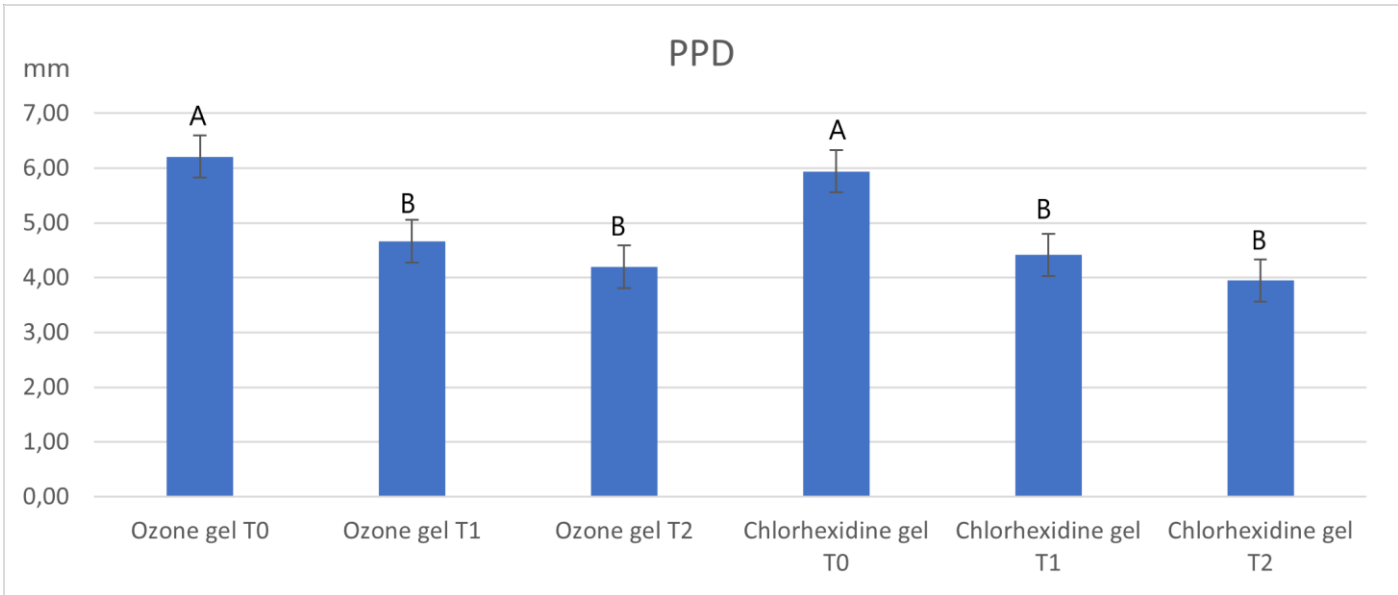


Figure 2. Probing Pocket Depth (PPD): this graph is showing PPD values in group treated with ozone vs chlorhexidine. Significant differences were found at $P < 0.05$.

3.2. Clinical attachment loss (CAL)

Significant intragroup differences were found between each timepoint both for the sites treated with SRP and ozone (except between T1 and T2) and for the sites treated with SRP plus chlorhexidine (P<0.05); no significant intergroup differences were found between the sites (P>0.05), except at T2 (Table 2 and Figure 3).

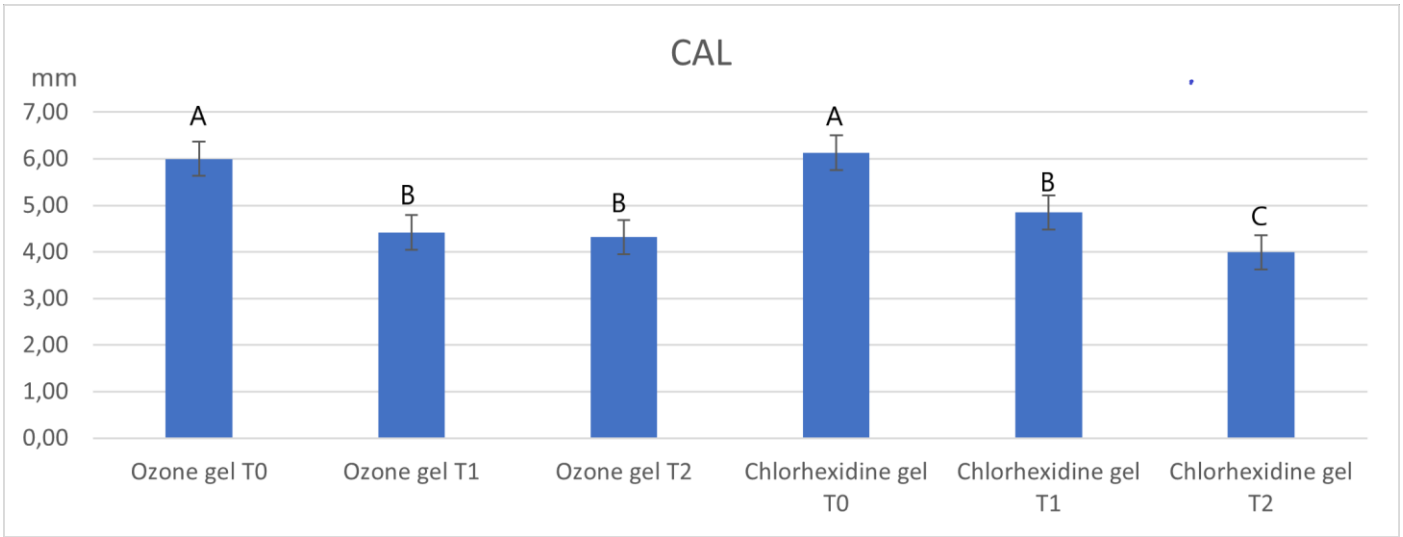


Figure 3. Clinical Attachment Loss (CAL): this graph is showing CAL values in group treated with ozone vs chlorhexidine. Significant differences were found at $P < 0.05$.

3.3. Gingival Index (GI)

Significant intragroup differences were found between each timepoint both for the sites treated with SRP and ozone (except between T1 and T2) and for the sites treated with SRP plus chlorhexidine (P<0.05); no significant intergroup differences were found between the sites (P>0.05), except at T2 (Table 2 and Figure 4).

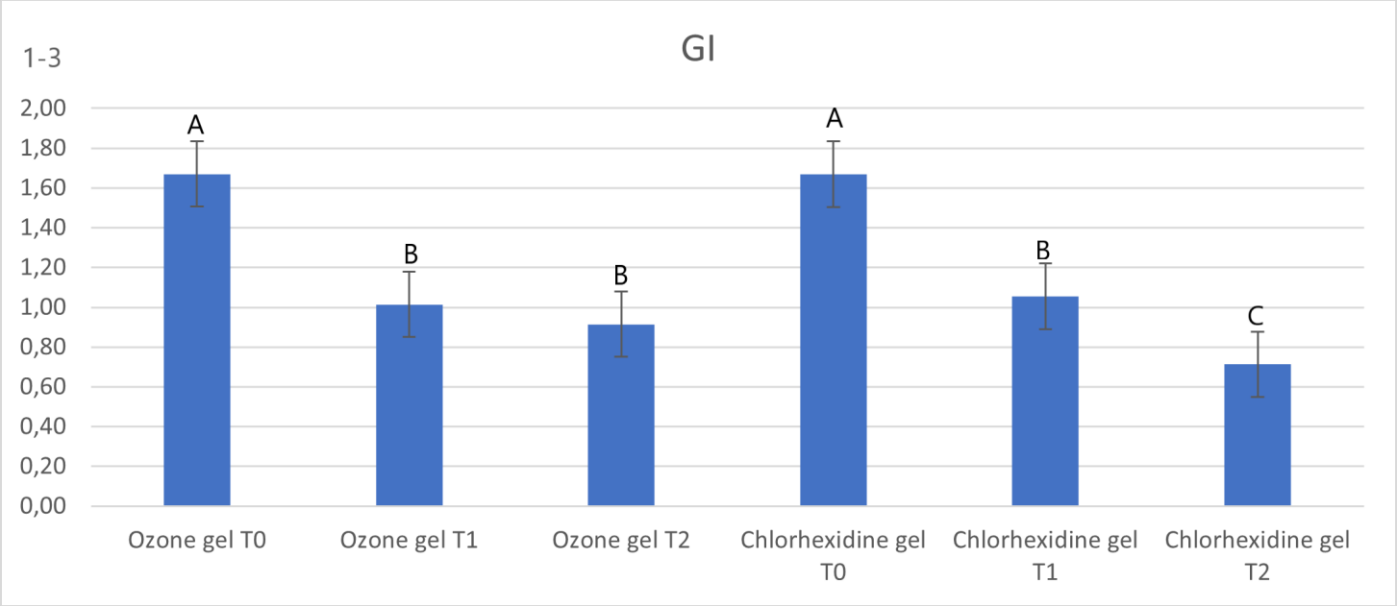


Figure 4. Gingival Index (GI): this graph is showing GI values in group treated with ozone vs chlorhexidine. Significant differences were found at $P < 0.05$.

3.4. Plaque index (PI)

Significant intragroup differences were found between each timepoint both for the sites treated with SRP plus ozone and for the sites treated with SRP plus chlorhexidine (P<0.05); no significant intergroup differences were found between the sites (P>0.05) (Table 2 and Figure 5).

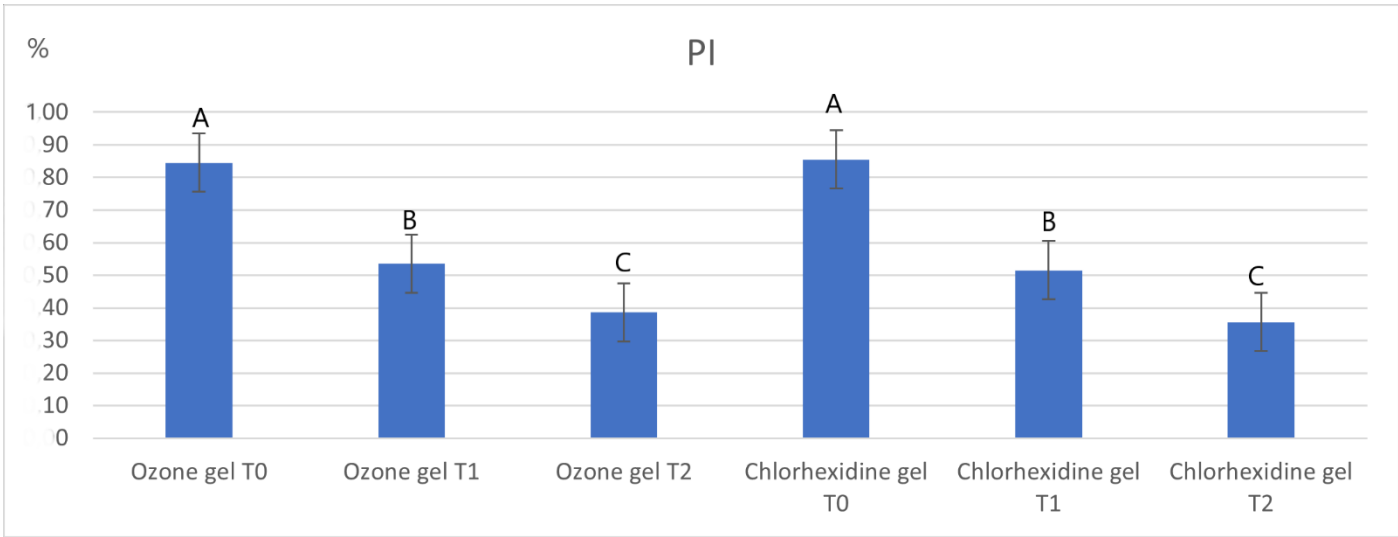


Figure 5. Plaque Index (PI) : this graph is showing PI values in group treated with ozone vs chlorhexidine. Significant differences were found at $P < 0.05$.

3.5. Bleeding on Probing (BoP)

Significant intragroup differences were found between each timepoint both for the sites treated with SRP plus ozone and for the sites treated with SRP plus chlorhexidine (P<0.05); no significant intergroup differences were found between the sites (P>0.05) (Table 2 and Figure 6).

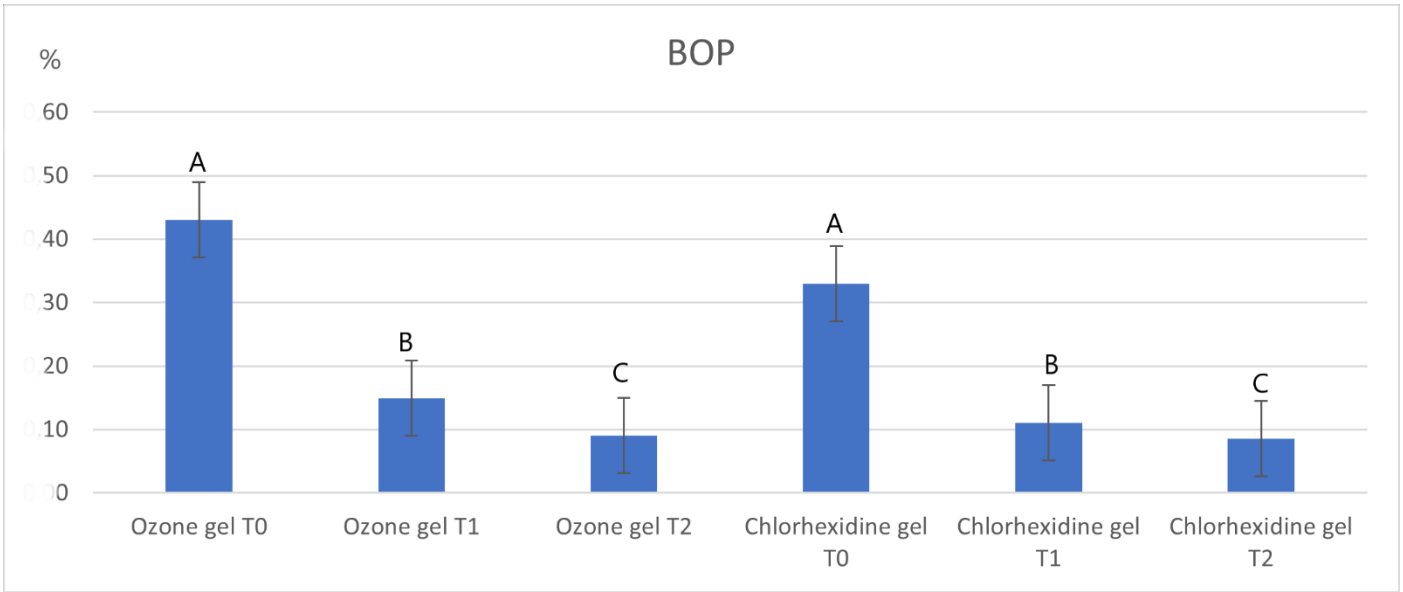


Figure 6. Bleeding on Probing (BoP): this graph is showing BoP values in group treated with ozone vs chlorhexidine. Significant differences were found at $P < 0.05$.

3. Discussion

Scaling and root planing (SRP) represents the gold standard therapy for the treatment of periodontal disease, along with the concomitant use of antibiotics and/or antiseptics [5,11]. In order to propose new chemical compounds, the major goal of the present study was to assess the efficacy of subgingival applications of ozone gel in addition to SRP, with respect to SRP plus a conventional chlorhexidine gel. Intergroup and intragroup differences at the various times have been conducted in order to assess which chemical compound could be more beneficial for the treatment of periodontitis in addition to SRP. The null hypotheses of the study were that no significant intergroup and intragroup differences occur between the experimental treatment and the control one, which were both partially refused.

Our results show that all clinical indexes tested (Probing pocket depth, PPD; Clinical Attachment Loss, CAL; Gingival Index, GI; Plaque Index, PI; and Bleeding on Probing, BoP) significantly improved after 1 and 3 months, with respect to baseline. This tendency was confirmed both for the experimental and the control condition in the split mouth study design considered. In addition to that, intragroup differences were generally significant, differently from intergroup differences. According to these results, the experimental protocol combining ozone to conventional SRP seems to be a reliable option for the nonsurgical management of the periodontal disease. The improvement of all the clinical indexes following the treatment with SRP plus subgingival applications of ozone might be due to the antimicrobial effects of this latter being an oxidant [12]. However, this chemical compound can also induce the release of growth factors, cause a vascular and hematological modulation, stimulate the immune system, and activate local antioxidant mechanisms if administered at low doses [13,14]. In particular, despite no intragroup differences with chlorhexidine occur at any time for BoP, the reduction of

this index in the quadrants treated with GeliO3 might be due not only to an antimicrobial effect (as also happens for chlorhexidine) but especially to the anti-inflammatory and antioxidant action.

Conversely, focusing on the significant improvement for PPD and CAL, this is due to the repair of connective tissue, ascribable to the stimulating action of ozone towards fibroblasts, but also to an increase angiogenesis with revascularization of the gingival tissue [15].

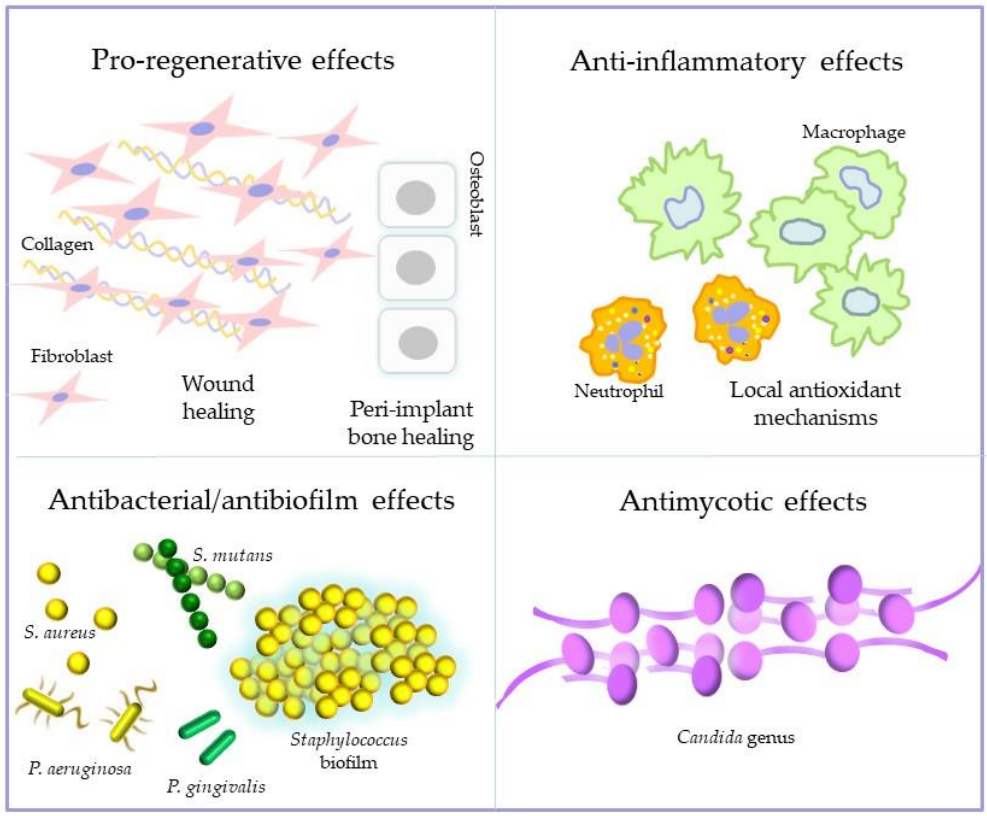


Figure 7. Principal biological effects reported for ozone treatments

In this study, no significant intergroup differences were assessed for most of the indexes assessed. According to this outcome, despite the valuable effect of ozone in addition to SRP, the results obtained after 1 and 3 months were generally the same assessed for SRP plus chlorhexidine. Previous studies in literature were carried out to compare the efficacy of ozonized and chlorhexidine-based products to deal with periodontitis. Most of these reports generally agree with our results by showing no statistical differences between the two antiseptics or a slightly better improvement for ozone with respect to chlorhexidine [16]. In other cases, greater outcomes have been described for ozone therapy [17]. According to the recent systematic review and metanalysis of Moraschini et al., [18], no significant differences occur when comparing the effect of chlorhexidine or ozone in addition to SRP; however, the same authors suggest that, due to the potential heterogeneity across the studies considered, the presence of confounding factors, and the short follow-up of some included RCTs, their results should not be considered definitive.

According to the results reported in this study, the use of the ozonized gel GeliO3 inside a protocol for the nonsurgical management of periodontal disease represents a valid approach, despite without a greater effect with respect to standard SRP plus chlorhexidine. However, it should be taken into account that chlorhexidine has several shortcomings like a higher cytotoxic effect which might be a valid reason to prefer the use of ozone in nonsurgical periodontal therapy instead of the former [3,19-21]. Despite the cheaper use of chlorhexidine, the recourse to ozone in the dental clinic by means of ozone generators could be justified, also considering the wider range of applicability of the latter substance.

The mouth houses a diverse symbiotic microbiota organized in biofilms that colonize the mucous membranes and dental surfaces. The oral microbiota exerts beneficial effects on the host, as it resists and counteracts colonization by pathogenic microorganisms, (b) attenuates the host's inflammatory responses and (c) participates in the physiological development of the immune defenses of the mouth. Under pathological conditions, such as a decreased pH level, this harmonious symbiotic relationship fails and a condition of dysbiosis occurs. In dysbiosis, the proportion of different bacterial species changes with the transition to a higher prevalence of anaerobic and proteolytic species endowed with high destructive potential. They can damage tissues and cause diseases of the teeth and mouth, such as tooth decay, periodontitis, pocket formation and loss of attachment [22].

Ozone is a broad spectrum antimicrobial agent (like chlorhexidine), which proved to be able to reduce the periodontitis bacterial burden. Moreover, as mentioned above, ozone appears to be worthy of particular consideration for its low toxicity compared to chlorhexidine. New agents more specifically active on periodontopathogens are now emerging and are being studied, such as Oxysafe [23].

The major limitation of this study is that only clinical parameters have been tested. It would be interesting to even perform microbiological tests to compare *in vitro* the antimicrobial action of the two products tested. In addition to that, further randomized clinical trials should be performed to evaluate a longer follow up to verify whether a long-term effect can be guaranteed as well.

Although these findings relate to the specific aim of this research, they provide some food for thought for more general reflection. In the last few years, a renewed interest in the therapeutic potential of ozone has emerged. In particular, the focus is on the ability to promote wound healing, to attenuate the adverse effects of inflammation by reducing the oxidative activities of inflammatory cells, to express antimicrobial activities against various bacterial species and mycetes pathogenic for humans, and also against biofilm-producing and antibiotic-resistant bacteria, thus offering chances of overcoming antibiotic-resistance issues [24-32] (see also Figure 7). Interestingly, some studies show that ozone treatments could be useful in combating implant-associated infections, exhibiting antibacterial activity and promoting osseointegration [26,27]. Furthermore, ozone-functionalized implant materials seem favorably influence the behavior of bone marrow cells and macrophages [28]. Ozone is also considered attractive for veterinary and food applications, which is important in the new era of the holistic "one health" view [34,35]. Finally, ozone is also considered with particular attention for its potential beneficial effects in environmental and hand hygiene to counter the spread of SARS-CoV-2, responsible for COVID-19, and as an adjuvant therapy in affected patients [36-40].

5. Conclusions

The additional use of the ozonized gel GeliO3 in addition to SRP does not show significant differences compared to SRP plus chlorhexidine, except considering GI and CAL. Despite chlorhexidine had a more evident effect only on these two last parameters, the ozonized gel tested could be a valid support for the nonsurgical treatment of periodontal disease as well, especially because of the absence of the major shortcomings associated with conventional chlorhexidine-based gels.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Internal Review Board.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding authors.

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