DOCTORAL THESIS

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INTERNATIONAL DOCTORAL SCHOOL

Doctoral Programme in Health Sciences

RANDOMIZED CLINICAL STUDY IN SPLIT MOUTH: USE OF OZORAL PRO AND GEL® COMPARED WITH 1% CHLOREXIDINE GEL IN SITES AFFECTED BY PERI-IMPLANT MUCOSITIS

Author:

PhD Student Andrea Butera

Supervisors:

Prof. Dr. Carlos Pérez-Albacete Martinez

Prof. Andrea Scribante

Murcia, month de October 2024

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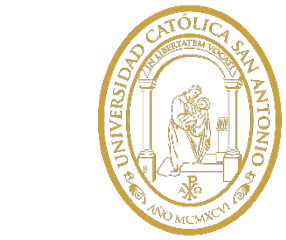

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Escuela Internacional de Doctorado

THESIS SUPERVISORS' AUTHORISATION FOR THESIS SUBMISSION

Prof. Andrea Scribante and Prof Carlos Pérez-Albacete Martinez as Supervisors[1] of the Doctoral Thesis RANDOMIZED CLINICAL STUDY IN SPLIT MOUTH: USE OF OZORAL PRO AND GEL® COMPARED WITH 1% CHLOREXIDINE GEL IN SITES AFFECTED BY PERI-IMPLANT MUCOSITIS by Mr Andrea Butera in the Doctorate Programme Health Science **authorise(s) its submission**, given that it meets the required conditions for its defence.

Which I hereby sign in compliance with Spanish Royal Decree 99/2011, of 28 January, in Murcia, on 11 October 2024

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[1] If the Thesis is supervised by more than one Supervisor, both must be mentioned and both must sign.

ABSTRACT

Peri-implant mucositis consists of a reversible inflammation of peri-implant tissues characterized by bleeding on gentle probing in the absence of bone loss. Ozone therapy is being extensively studied for its efficacy in treating different dental conditions. To date, few studies have evaluated ozone as an adjunct to the oral hygiene measures of peri-implant mucositis patients. The aim of the present study is to assess the efficacy of an ozonized gel (Trial group) compared to chlorhexidine (Control group) after a domiciliary protocol of oral hygiene in a 24-month study. According to a split-mouth study design, patients were divided into Group 1 for the application of chlorhexidine gel in peri-implant mucositis sites of quadrants Q1 and Q4, whereas in quadrants Q2 and Q3, the ozonized gel was administered in-office. For Group 2, the quadrants were reversed. At baseline (T0), and after 1 (T1), 2 (T2), and 3 (T3) months, At baseline (T0) and after one (T1), three (T2), six (T3), nine (T4), twelve (T5), fifteen (T6), eighteen (T7), twenty-one (T8), twenty-four (T9) months, the following indices of peri-implant mucositis were recorded: probing pocket depth (PPD), index plaque (PI), gingival bleeding index (GBI), bleeding score (BS), marginal mucosal condition (MMC).

Both for the ozonized gel and for chlorhexidine there was a significant decrease in all the peri-implant mucositis indices studied. In contrast, no significant variations were found in intergroup comparisons. The greater improvements for BS, GBI and MMC inflammatory indices of the ozonized gel gel compared to chlorhexidine suggest the importance of further studies to investigate the relevance of the product itself. The ozonized gel deserves particular attention, considering the better outcome than chlorhexidine on specific clinical periodontal parameters, as well as its lesser shortcomings.

RESUMEN

La mucositis periimplantaria consiste en una inflamación reversible de los tejidos periimplantarios caracterizada por sangrado al sondaje suave en ausencia de pérdida ósea. La ozonoterapia está siendo ampliamente estudiada por su eficacia en el tratamiento de diferentes afecciones dentales. Hasta la fecha, pocos estudios han evaluado el ozono como complemento de las medidas de higiene bucal de pacientes con mucositis periimplantaria. El objetivo del presente estudio es evaluar la eficacia de un gel ozonizado (grupo de prueba) en comparación con clorhexidina

(grupo de control) después de un protocolo domiciliario de higiene bucal en un estudio de 24 meses. Según un diseño de estudio de boca dividida, los pacientes se dividieron en el Grupo 1 para la aplicación del gel de clorhexidina en los sitios de mucositis periimplantaria de los cuadrantes Q1 y Q4, mientras que en los cuadrantes Q2 y Q3, el gel ozonizado se administró en el consultorio. Para el Grupo 2, los cuadrantes se invirtieron. Al inicio (T0) y después de 1 (T1), 2 (T2) y 3 (T3) meses. Al inicio (T0) y después de uno (T1), tres (T2), seis (T3), nueve (T4), doce (T5), quince (T6), dieciocho (T7), veintiún (T8), veinticuatro (T9) meses, se registraron los siguientes índices de mucositis periimplantaria: profundidad de la bolsa al sondaje (PPD), índice de placa (PI), índice de sangrado gingival (GBI), puntuación de sangrado (BS), condición de la mucosa marginal (MMC).

Tanto para el ozono como para la clorhexidina hubo una disminución significativa en todos los índices de mucositis periimplantaria estudiados. Por el contrario, no se encontraron variaciones significativas en las comparaciones intergrupales. Las mayores mejoras en los índices inflamatorios BS, GBI y MMC del gel ozono en comparación con la clorhexidina sugieren la importancia de realizar más estudios para investigar la relevancia del producto en sí. El gel ozonizado merece una atención especial, considerando que tiene mejores resultados que la clorhexidina en parámetros clínicos periodontales específicos, así como sus menores deficiencias.

KEY WORDS

Ozone; Ozonated Oils; Peri-implant mucositis; Chlorhexidine; Scaling and Root Planing; Implants; Periodontal Parameteres; Randomized Clinical Traial; Experimental Surgery;Gases

PALABRAS CLAVE

Ozono; Aceites Ozonizados; Mucositis periimplantaria; Clorhexidina; Raspado y Alisado Radicular; Parámetros Periodontales; Ensayo clínico aleatorizado, Cirurgia Experimental; Gases

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QUOTE

“Patience, perseverance and hard work create an unbeatable combination for success.”". Napoleon Hill

GENERAL INDEX

ACRONYMS AND ABBREVIATIONS

BS: Bleeding score.

CHX: Chlorhexidine.

GBI: Gingival Bleeding index.

H03: Trioxide.

IL: Interlukin.

MMC: Marginal Mucosal Condition.

OH: Hydroxyl Radical.

O_2: Oxygen.

O3: Ozone.

PEEK: Polyetheretherketone.

PI: Plaque Index.

PPD: Probing Pocket Dept.

SRP: Scaling Root Pleaning.

WHO: World Health Organization

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I – INTRODUCTION

1. INTRODUCTION

1. HISTOLOGY OF PERI-IMPLANT TISSUES

A dental implant is defined as a device made of one or more materials that is intentionally placed within the body, fully or partially submerged under an epithelial surface [Von Wilmowsky et al.,2013]. Dental endosseous implants are inserted during an osteotomy, surgically created in the alveolar bone (Figure1).

After implant surgery, epithelial cells at the edges of the wound gain the ability to adhere to the implant surface and produce a basal lamina and hemidesmosomes, creating an epithelial seal that resembles the junctional epithelium around teeth [Lindhe J et al.,1998]. The gingival junctional epithelium is part of the attachment apparatus between the tooth and the gum. The innermost cells of the junctional epithelium then form the epithelial attachment apparatus, which ensures a tight seal against the tooth surface. This adherence to the tooth surface means that the junctional epithelium plays a critical role in tissue homeostasis and in defense against microorganisms and their constituents; similarly, it is universally accepted that the integrity of the epithelial attachment between the implant and the peri-implant mucosa is fundamental for maintaining osseointegration [Bosshardt DD et al.,1998].

The term osseointegration was coined by Brånemark et al., and was described as a direct contact between bone and implant, without interposition of soft tissue [Atsuta I et al.,2015]. Subsequently, Albrektsson and Sennerby defined osseointegration as a direct functional and structural connection between living bone and the surface of a load-bearing implant [Albrektsson T et al.,1986].The peri-implant mucosa is made up of a well-keratinized oral epithelium on the external surface, continuous with a sulcular epithelium lining the lateral aspect of the gingival sulcus. This epithelial attachment of the inner lining of the peri-implant mucosa resembles the junctional epithelium of the teeth in its histological characteristics. The implant unit, however, lacks the periodontal ligament, periodontal vascular plexus, neurosensory system and cement, which instead surround the natural elements, and the absence of cement on the surface of the implant prevents its union with the collagen fibres. The connective tissue of the peri-implant mucosa has clinical and histological characteristics somewhat similar to those of the teeth; despite this, the main difference is observed in the cellular composition and fiber orientation. The connective tissue is richer in collagen fibres, but has a smaller cell population, represented by fibroblasts, and less vascularisation than that of natural teeth, thus taking on the characteristics of scar tissue. The connective tissue surrounding the dental implant is in direct contact with the surface of the titanium dioxide and contains a dense network of collagen fibers which, in larger bundles, originate from the periosteum of the alveolar bone crest extending to the margin of the mucosa. These fibers are oriented in a direction

parallel to the surface of the implant/ abutment. This is in contrast to the attachment of connective tissue to the teeth, whereby collagen fibers insert into the root cementum in a perpendicular direction [Berglundh T et al., 1991].

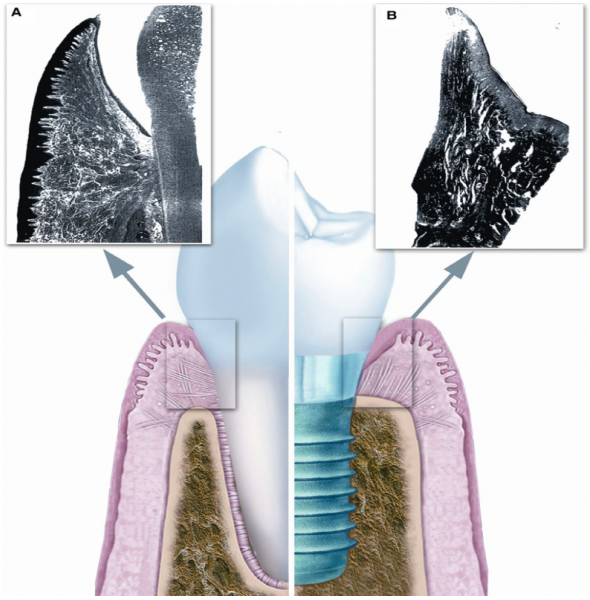


*Figure 1: Cross section of the buccal dento-alveolar region (A) and the buccal-coronal part of the peri-implant bone and mucosa (B). Similar anatomical components (i.e., sulcular epithelium, junctional epithelium, and connective tissue) are present in both the periodontal and peri-implant mucosa [*Pokrowiecki R et al., 2017].

A healthy peri-implant alveolus is colonized mainly by oral streptococci, which constitute 45%-86% of the supra- and subgingival peri-implant sulcus microbiota. Also *Actinomyces naeslundii*, *Actinomyces oris* and *Actinomyces meyeri*, as well as *Neisseria* and Rothia species, are frequently isolated. It has been observed that even the healthy peri-implant sulcus can rarely be colonized by *Fusobacterium nucleatum* , *Prevotella intermedia*, *Porphyromonas gin*givalis and *Aggregatibacter actinomycetemcomitans* . These pathogens have been identified in healthy peri-implant pockets without any symptoms of inflammation. For this reason, it seems that peri-implant infections are not a direct consequence of the presence of periodontopathogenic species per se. Instead, they are highly correlated with the host's response and changes in the composition of the individual's specific oral

microbiota. Consequently, potential periodontopathogenic species may not be invasive, as long as their proportion is below the critical level.

The transition from a healthy peri-implant sulcus to a diseased peri-implant pocket is associated with the increased presence of cocci, bacilli and spirochetes. A change in biofilm distribution is commonly more evident in subgingival rather than supragingival dental plaque. Therefore, peri-implant mucositis may result in an increase in the proportion of periodontopathogenic bacteria, mainly of the orange complex: *F. nucleatum, P specie intermedia and Eubacterium*. A decrease in *Streptococci spp.* and *Actinomyces spp*. it is also common. Peri-implantitis, on the other hand, appears to be associated with an increase in the level of pathogenic bacteria of the orange and red complexes*: P. gingivalis and Tannerella forsythia* are the most common and abundant species of the red complex, while *Prevotella nigrescens, Prevotella oris and F. nucleatum* are periodontal pathogens frequently isolated from the orange complex. The bacterial species associated with peri-implantitis may, however, differ significantly from those involved in periodontal disease [Herrera D et al., 2023].

2. PERI-IMPLANT HEALTH, PERI-IMPLANT MUCOSITIS AND PERI-IMPLANTITIS.

Dental implants have high long-term survival rates (≥10 years). However, functional implants and their restorations may be subject to mechanical and biological complications. The biological complications associated with dental implants are mainly due to the inflammatory conditions of the soft tissues and bone surrounding the implants, in which the accumulation of bacterial biofilm plays a fundamental role. These conditions, which have been called peri-implant mucositis and peri-implantitis, must be clearly defined and differentiated from a peri-implant health state, so that the clinician can assign a correct diagnosis and select an appropriate treatment modality in cases where the disease is present.

Araujo and Lindhe concluded that peri-implant health requires the absence of clinical signs of inflammation (e.g. erythema and swelling), including no bleeding on probing, although the latter may sometimes present as a consequence of a traumatic mechanical episode rather than as a sign of biofilm-induced inflammation; absence of suppuration following light probing; no increase in PD compared with previous tests; absence of radiographically visible bone loss in comparison with previous examinations [Araujo MG et al .,2018]. In clinical health, the peri-implant mucosa forms a tight seal around the transmucosal component of the implant itself, the abutment or restoration. The height of the soft tissue around the implant after placement influences the initial probing depth. In general, however, the probing depth associated with peri-implant health should be ≤5.0 mm. Intraoral radiographic evaluation of changes in bone levels around implants

is necessary to discriminate between health and disease states [Academy Report:.,2013].The American Academy of Periodontology has defined peri-implant mucositis as a disease involving inflammation of the soft tissues surrounding a dental implant, as well as the presence of bleeding and/or suppuration on light probing with or without increased PPD in comparison with previous examinations but without further bone loss after the initial remodeling that occurs during healing following surgical implant placement [Lindhe J et al ., 2008].

The etiology of peri-implant mucositis concerns the accumulation of bacterial biofilm around the implant. Peri-implantitis has instead been defined as an inflammatory lesion of the mucosa surrounding an endosseous implant with progressive loss of the supporting peri-implant bone [Lindquist LW et al., 1996]. It is generally verified that after implant installation and initial loading, some people lose crestal bone height (between 0.5 and 2 mm) in the healing process [Cochran DL, et al., 2009. Zitzmann NU et al., 2008] . Any additional radiographic evidence of bone loss suggests peri-implant disease. The conversion from an inflammatory process identified as peri-implant mucositis (without evidence of bone loss) to peri-implantitis (with bone loss) remains an enigma. However, it is generally accepted that both peri-implant mucositis and peri-implantitis have as their infectious etiology the development of biofilm composed of bacteria with known pathogenicity.

Clinical/radiographic findings and phases of the clinical examination necessary to detect the presence of peri-implant mucositis: (Figure 2A)

1. Visually, local swelling, redness and shine of the soft tissue surface are classic signs of clinical inflammation. A common symptom reported by patients is pain.

2. A local bleeding point resulting from the probing may be the result of a traumatic lesion which should not be considered, in the absence of other inflammatory alterations, a definitive criterion to characterize a lesion of the peri-implant soft tissues.

3. Presence of bleeding on probing, combined with visual inflammatory changes of the tissues at the probing site.

4. Clear evidence of bleeding, such as a line or drip hemorrhage, should be used as an indication of a peri-implant inflammatory soft tissue lesion.

5. Suppuration on clinical examination.

6. Intraoral radiographic evaluation of bone levels around implants should always be included in the presence of clinical signs of inflammation.

Furthermore, a prerequisite for evaluation is that a baseline radiograph is taken and used for future evaluation of mesial and distal bone levels in relation to defined

references. Taking into account the remodeling process of the alveolar bone during the first year after installation, the change in bone level from placement of the prosthetic superstructure should not be > 2.0 mm.crestal bone level changes resulting from the initial remodeling process after implant installation suggests progresive peri-implant-infection. (Figure 2B) [Gobbato, L et al.,2013].

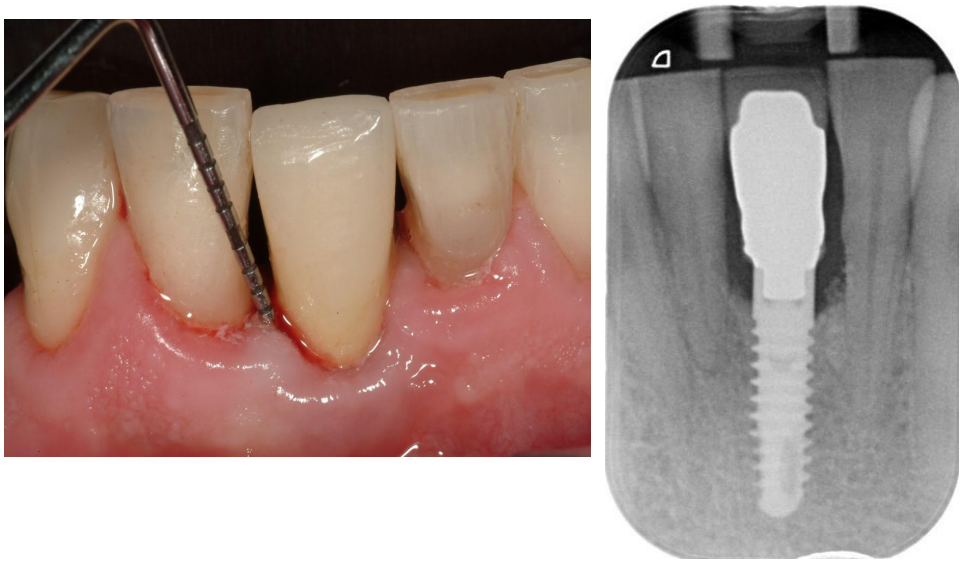


Figure 2A and 2 B: Photographic and radiographic investigations highlighting the presence of peri-implant mucosits [Gobbato, L et al.,2013].

The prevalence of peri-implant mucositis is approximately 80% in subjects and approximately 50% around implant sites. Without adequate professional and home maintenance, mucositis can progress to the much more serious condition of peri-implantitis, which is observed in 28% -56% of subjects and in 12%-43% of implant sites, a condition that can ultimately lead to implant failure [Laine, M.L et al .,2006]The risk factors related to peri-implant disease are: history of periodontitis [Korsch, M et al., 2014];smoke [Renvert , S. et al., 2014]; diabetes [Lang, N.P et al.,2011] ; poor plaque control / absence of regular maintenance therapy [Kozlovsky, A. et al., 2007] ; keratinized mucosa < 2 mm [Fretwurst , Tet al.,2006] ; genetic factors[Bassetti, M. et al.,2014] ; excess cement [Mombelli , A et al.,2018]; systemic conditions [Feres, M et al., 2008] ; iatrogenic factors [Butera, A et al.,2021] ; occlusal overload [Meimandi , M. et al., 2017] ; titanium particles [Invernici, MM et al.,2018]

Non-surgical treatment of peri-implant mucositis usually includes mechanical debridement of dental plaque and calculus, using professional or home oral hygiene techniques, with or without the additional use of antimicrobials [Francino , M. et al.,2016] . Scaling and root planing (SRP) of peri-implant mucositis sites,

using curettes and ultrasound devices with titanium- or polyetheretherketone (PEEK)-coated tips, with or without antimicrobials, has been shown to significantly reduce root inflammation. peri-implant tissues and bleeding on probing [Ince, G et al.,2015]. In the case of oral hygiene products for home use, mechanical plaque control, together with the use of an antiseptic, can provide benefits in the treatment of peri-implant mucositis, reducing bleeding on probing and sometimes even index of plate [Z˙ ółkiewicz et al., 2020] .SRP has some deficiencies, the most represented of which is bacterial recolonization [Laugisch , O et al., 2022]. Accordingly, antibiotics [O'Leary, T.J et al., 1972] , ozone application [Denton GW et al.,1991] , probiotics and postbiotics [Kuyyakamond T et al., 1992] have been proposed as additional therapeutic approaches.

3. ADJUVANTS TO CAUSAL THERAPY

3.1. CHLOREXIDINE

Chlorhexidine is an antimicrobial agent considered to date the gold standard in antimicrobial therapy of the oral cavity. It is a cationic bisbiguanide with broad antibacterial activity, low toxicity to mammals, and a strong affinity for binding to skin and mucous membranes. Chlorhexidine has a broad spectrum of activity that includes gram-positive and gram-negative bacteria, yeasts, dermatophytes and some lipophilic viruses. [Lindhe J et al., 1993].

Its antimicrobial activity is aimed at damaging the internal cytoplasmic membrane. Interestingly, chlorhexidine shows different effects at different concentrations; at low concentrations the agent is bacteriostatic, while at higher concentrations the agent is rapidly bactericidal. The actual levels at which bacteriostatic and bactericidal effects occur vary between bacterial species.

The antibacterial mode of action of chlorhexidine is believed to be as follows. The bacterial cell is typically negatively charged. The cationic chlorhexidine molecule is rapidly attracted to the negatively charged bacterial cell surface, which alters the integrity of the bacterial cell membrane. Chlorhexidine binds to phospholipids in the inner membrane, leading to increased membrane permeability and the loss of low molecular weight components, such as potassium ions. In this bacteriostatic stage the effects of chlorhexidine are reversible [Rolla G et al., 1975].

The increase in the concentration of chlorhexidine causes progressively greater damage to the membrane, there is coagulation and precipitation of the cytoplasm through the formation of phosphate complexes and the precipitation of proteins and nucleic acids: this bactericidal stage is irreversible [Lindhe J et al., 1993].

The chemical properties of chlorhexidine also allow it to bind to the different surfaces of the mouth: teeth, mucous membranes, salivary proteins and acquired film; after a single rinse with chlorhexidine, the saliva itself shows antibacterial activity for up to 5 hours, while persistence on oral surfaces has been shown to

suppress salivary bacterial load for over 12 hours. This adhesion by chlorhexidine to the surfaces of the enamel does not completely inhibit bacterial adhesion to the surface of the enamel, but bacterial growth is slowed down precisely due to the bacteriostatic properties of the molecule [Rolla G et al., 1975].

Therefore the property that allows chlorhexidine to be defined as the gold standard in anti-plaque chemical therapy can be explained in terms of its superior degree of persistence on the tooth surface.

When we talk about plaque, however, we must consider that the chlorhexidine molecule can bind not only to the proteins of the bacterial cell membrane, but also to other proteins resulting from bacterial metabolism [Burns DT et al .,1997].

To optimize its dose-dependent effects, therefore, it is necessary to remove the biofilm mechanically and prevent chlorhexidine from losing its effectiveness by binding to other proteins.

3.2. OZONE

The pathogenicity of biofilm in the oral cavity is amplified by two characteristics: increased antibiotic resistance and the inability of host inflammatory cells to phagocytose this aggregated community [Li Z, et al., 2013]. As periodontal researchers search for alternatives to antibiotic treatments, the emergence of ozone therapy appears to have a promising future.

Ozone (O_3) is a triatomic molecule, made up of three oxygen atoms. Its molecular weight is 47.98 g/mol and it is a thermodynamically highly unstable compound that, depending on system conditions (such as temperature and pressure), decomposes into pure oxygen with a short half-life [Gandhi KK et al.,2019]. It is produced naturally by the photodissociation of molecular oxygen (O_2) into activated oxygen atoms, which then react with other oxygen molecules; this transient radical anion rapidly becomes protonated, generating hydrogen trioxide (HO3) which, in turn, decomposes into an even more powerful oxidant, the hydroxyl radical (OH).

It is therefore a powerful oxidizing agent with a high antimicrobial power against oral pathogens. The antibacterial action of ozone is related to its ability to react with lipid double bonds, thus leading to the lysis of the bacterial wall and extravasation of the bacterial cellular contents. Upon entering the cell, ozone promotes the oxidation of nucleic acids and amino acids; furthermore, it inactivates viruses by diffusing through the protein coat into the nucleic acid core, resulting in damage to the viral nucleic acid. The antimicrobial action of ozone is non-specific and selective for microbial cells; does not damage the cells of the human body due to their greater antioxidant capacity.

Ozone also appears to have immunostimulatory, anti -hypoxic and biosynthetic effects on the human body. It influences the cellular and humoral immune system,

stimulates the proliferation of immunocompetent cells and the synthesis of immunoglobulins, activates the function of macrophages and increases the sensitivity of microorganisms and phagocytosis. Macrophage-derived mediators, such as tumor necrosis factor α (TNF-α), interleukin (IL)-1 β, IL-6, and IL-8, contribute to the biological response to ozone leading to the response inflammatory [(41)]. Improves the metabolism of inflamed tissues by increasing oxygenation and reducing local inflammatory processes. It also activates protein synthesis mechanisms and increases the number of ribosomes and mitochondria in cells. These changes at the cellular level explain the high functional activity and regeneration potential of tissues and organs [Nogales CG, et al., 2008].

All these characteristics of ozone justify the current interest in its application in medicine and dentistry; in fact, it has been indicated for the treatment of 260 different pathologies [Yu Y et al., 2022].

There are three basic forms of ozone application to oral tissue: ozonated water, ozonated oil and oxygen/ozone gas. Ozonated water and oil have the ability to trap and then release oxygen/ozone, an ideal delivery system [Li Z. et al., 2013].

Clinical implications of ozone in dentistry:

- Elimination of pathogenic bacteria and control of infections
- Disinfection of periodontal pockets and bone disinfection
- Prevention of dental caries
- Endodontic treatments
- Cons of dentin sensitivity
- Treatment of the temporomandibular joint
- Gingival recessions (exposed root surfaces)
- Tissue regeneration
- Control of bad breath
- Remineralization of the tooth surface
- Teeth whitening

Contraindications of ozone therapy:

- Pregnancy
- Autoimmune disorders
- Hyperthyroidism
- Anemia
- Myasthenia
- Alcohol intoxication
- Cardiovascular diseases and myocardial infarction

- Allergy to ozone

3.3. PROBIOTICS, PARAPROBIOTICS AND POSTBIOTICS

In recent years, probiotics have been increasingly used for the treatment of periodontal disease and, recently, peri-implant pathologies, thanks to the absence of side effects associated with conventional antibiotic therapy.

According to the World Health Organization (WHO), probiotics are "live microorganisms that, when administered in adequate quantities, confer a health benefit on the host." Probiotics are supposed to compete with pathogens for nutritional sources and adhesion sites, improving mucosal barrier function and producing antimicrobial and immunomodulatory substances.

New products have been proposed based on non-viable probiotics, such as paraprobiotics (tyndallized probiotics) and postbiotics. [Butera, A. et al.2022]. In particular, paraprobiotics are inactivated microbial cells which therefore confer a benefit to the consumer without presenting any health risk; they are able to regulate both the adaptive and innate immune system, to exert an antagonistic action against pathogens, as well as to carry out an anti-inflammatory, anti-proliferative and antioxidant action. Probiotics and paraprobiotics should not be confused with postbiotics which include any substance released or produced through the metabolic activity of microorganisms without containing the viable microorganisms themselves.

The concept of "biotics" must also be discussed taking into account the more general term of "metabiotics", used to describe the structural components of probiotic microorganisms and/or their metabolites and/or signaling molecules with a specific chemical structure capable of enhance host-specific physiological functions, regulatory, metabolic, and/or behavioral reactions related to the activity of the host's indigenous microbiota [Butera, A. et al.2022].

II – JUSTIFICATION

II-JUSTIFICATION

In the era of modern dentistry, implant rehabilitation has and has had a wide application that allows the rehabilitation of individual elements, hemi arches or entire arches. Therefore, the anatomical and structural characteristics vary according to the condition related to the alveolar bone, the type of implant, its position and the type of prosthetic artifact.

The risk of an imbalance of the oral microbiota is very frequent if there is not proper management of the bacterial biofilm at home, trying to exponentially reduce the risk of dysbiosis. It is also necessary to study well the type of transmucosal pathway that reduces the possibility of a lesion arising in the connective tissue around the implant. Therefore, to avoid peri-implantitis, it is essential to proactively study a protocol that can guarantee a state of eubiosis over time and can benefit both the patient and the operator. To date, the gold standard remains chlorexdine, but from the analysis of the reading, natural substances are becoming more and more popular, which have no contraindication of use and above all counteract pathogenic bacteria that at the peri-implant level do not change in quality compared to periodontal but in quantity, with a higher destructive percentage of the red complex. In the various classifications of periodontal and peri-implant disease both in 2018 and in the most recent one in June 2023, they included mucositis as a transient inflammation without loss of clinical attachment and as a predominant pathognomonic sign bleeding through the BOP (bleeding on probing) and BS (bleeding score) therefore both qualitative and quantitative level, in 2023 they also added suppuration, in our protocol drawn up and validated in 2021 suppuration had already been included, currently only the radiographic examination is missing which was included within the limits of the study.

The hypothesis of this study was to evaluate alternative substances to chlorhexidine, in this case ozone, capable of influencing long-term per-implantation mucositis, reducing the incidence of dysbiosis with natural substances after two years. Patients enrolled with overt mucositis have both the

upper and lower hemiarches, so that it is possible to evaluate how the same substance reacts on the same patients and evaluate its long-term recurrence through periodics recalls In the objective evaluation using reference indices, professional treatment, using minimally invasive instruments and the use of low-granulometry powders combined with ozonized substances at home, have shown statistically significant results in reducing the progression of mucositis itself and allow us to. Therefore, alternative substances such as ozone are able to reduce peri-implant mucositis in the long term.

III – OBJECTIVES

III-OBJECTIVES

The main objective of this study is to evaluate whether ozonated substances are without contraindications if used long-term, in patients with peri-implant mucositis in order to avoid the evolution into peri-implantitis by reducing the chemical-pharmacological action the chlorhexidine.
Specific objectives.

Primary objective:

- -Evaluate the effects of ozone by comparing it with that of chlorhexidine, evaluating the substantial difference, that is, the effectiveness of both substances on the same patient but above all having greater support for alternative substances for implant maintenance.

Secondary objective:

- -Evaluate how ozone is able to maintain eubiosis within the oral cavity over time without side effects.
- Evaluate the patient's perception based on the characteristics of the tested material.
- There will be no contraindications as the timing of use has been maintained at 14 days post-treatment like chlorhexidine, in order to avoid pigmentation, taste alterations, and dysgeusia.

IV – MATERIAL AND METHODS

IV-MATERIALS AND METHODS

This was a single-center, split-mouth randomized controlled trial with a 1:1 allocation ratio.The study covered by this paper was approved by both Ethics Committees with an opinion expressed in the session of 01/12/2021 proposal number 2021-1201 at the University of Pavia and in the session of 03/25/2022 proposal number CE032212 at Universidad Catolica San Antonio. The study was conducted at the Unit of Dental Hygiene, Section of Dentistry, Department of Clinical, Surgical, Diagnostic and Pediatric Sciences of the University of Pavia (Pavia, Italy), starting in January 2022 and ending in February 2024. Patients were asked to sign an informed consent document before participating. Both the interventions and the outcomes assessments were conducted at the same unit.After signing the informed consent document (baseline, T0), patients were visited and the following indexes were collected by an instructed operator by means of a probe (UNC probe 15; Hu-Friedy, Chicago, IL, USA): PD, Probing Depth (distance between soft margin of the gum and base of the pocket) [Butera, A. et al.2022].; PI, Plaque Index (percentage of sites with plaque with respect to total dental sites) [Silness, J. et al. 1964]; SI, Suppuration Index (presence or absence of suppuration in the peri-implant site); BS, Bleeding Score (presence of bleeding on probing on a scale of 0–3) [Ainamo, J.et al 1975]; and MMC, Marginal Mucosa Condition (presence of qualitative changes in the mucosa on a scale of 0–3) [Butera, A. et al.2022].. Then, a professional supragingival and subgingival oral hygiene appointment was conducted with a piezoelectric instrument (Multipiezo, Mectron S.p.a., Carasco, Italy), Gracey curettes (Hu-Friedy, Chicago, IL, USA), PEEK ultrasonic tip (Implant Cleaning Set S, Mectron S.p.a., Carasco, Italy), and a titanium curette for implant sites (Implant Curette TIS2CN, Arnold Deppeler SA, Rolle, Switzerland); this was followed by decontamination with glycine powder (Mectron S.p.a., Carasco, Italy).According to the split-mouth study, patients were divided into two groups: Group 1 received the application of Curasept Periodontal Gel in peri-implant mucositis sites of quadrants Q1 and Q4, whereas for quadrants Q2 and Q3, Ozoral Pro was administered in-office. For Group 2, the quadrants were inverted shows the compositions of the two products used. Following the

professional oral hygiene appointment, each peri-implant mucositis site was rinsed, air-dried, and isolated by means of cotton rolls so that the assigned gel could be applied and left for at least 2 min. Patients were visited after 1 (T1), 3 (T2), and 6 (T3) months, up to (T9) 24 months. All of the clinical procedures were repeated for each time frame, except for the professional oral hygiene appointment, which was repeated at T9 at the end of the visit. For the duration of the study, patients applied Curasept Periodontal Gel and Ozoral Gel to the same quadrants as the in-office administration once a day for the next 14 days (based on the recommended chlorhexidine protocol). Patients were given two different syringes with a blunt plastic needle of 5–6 mm in diameter for the domiciliary administration.The sample size calculation (alpha = 0.05; power = 95%) for two independent study groups and a continuous primary endpoint was calculated.The following mathematical formula was used for the sample size calculation:

$$\text{Sample size}=Z2(1-\alpha\,2)p(1-p)/d2$$

Where Z is the standard normal variate corresponding to 1.96 at 5% type 1 error, *p* is the expected proportion of the population expressed as a decimal and based on previous studies, and finally d is the confidence level determined by the researcher and expressed as a decimal, too. The variable Probing Depth was chosen as the primary outcome. A mean of 3.35 was expected, and a difference between the means of 0.59, with a standard deviation of 1.10 [Isler, S.C et al. 2018]. Therefore, 90 peri-implant mucositis sites per group were required for the split-mouth study. With a block randomization table, the data analyst generated a randomization sequence considering a permuted block of 90 peri-implant sites due to the split-mouth design. After the random assignment of the Trial treatment for one quadrant, the contralateral one was allocated to the Control treatment. Opaque envelopes were previously prepared, sealed, and numbered sequentially (SNOSE); afterward, an operator performed the procedures and the index collection after assigning the quadrants to the respective treatments. For the home oral hygiene procedures, the two gels were concealed. Patients and the data analyst were blinded for the allocation. For the domiciliary protocol the two gels had different colors to help the participants, and written instructions were left on the packaging to avoid mistakes due to the split-mouth design.

1.1. CLINICAL PROTOCOL

1.1. INDEXES USED FOR PERI-IMPLANT MUCOSITIS DETECTION

- PPD (Probing Pocket Depth)
  Quantitative evaluation in mm
- PI (Plaque Index)
  Quantitative evaluation in O'leary index, each dental element is divided into 4 surfaces, application of the formula:(no. of sites with plaque / total no. of tooth surfaces) X 100
- BOP (Bleeding on Probing)
  Quantitative evaluation in %Each element is divided into 6 surfaces, application of the formula:(no. of sites with bleeding / total no. of tooth surfaces) X 100
- BS (Bleeding Score)
  Quantitative evaluation in ptu Mombelli et al. records bleeding on probing with a scale from 0 to 3 according to the following criteria:
  - 0 no bleeding when the probe passes along the peri-implant margin
  - 1 isolated bleeding in single points
  - 2 bleeding that forms a line along the entire peri-implant margin
  - 3 Profuse or profuse bleeding
- Suppuration
  Quantitative evaluation
  - 0 no suppuration
  - 1 suppuration present
- MMC (Marginal Mucosal Conditions)
  Qualitative evaluation in ptu . Apse et al. Index, records the qualitative changes in the mucosa with a scale from 0 to 3. The criteria are:
  - 0 normal mucosa
  - 1 minimal inflammation, with mild color change
  - 2 moderate inflammation with redness and edema

  -3 severe inflammation with redness, edema, ulceration and spontaneous bleeding

1.2. EQUIPMENT AND PROTOCOLS USED

PROFESSIONAL SUPRAGENGIVAL HYGIENE	Professional hygiene session with tools for scaling and deplaquing using: - sonic scaler (power from 2 to 6 Khz) / ultrasonic (up to 30 KHz [Kayahan E et al.2022,] ; Mectron Multipiezo Pro [Butera, A et al., 2022. Kayahan E et al.2022] or EMS AIRFLOW Profilaxis Master [Reinhart D et al., 2022]. - manual scaler
PROFESSIONAL SUBGINGIVAL HYGIENE	Professional oral hygiene session with scaling and deplaquing instruments using: - ultrasonic scaler in the subgingival portion of the tooth (up to 30 KHz; Mectron Multipiezo Pro or EMS AIRFLOW Profilaxis Master) - instruments for manual finishing (Gracey Curettes)in the subgingival portion - use of ultrasonic peek supports and manual peek or titanium instruments for the implant sites
Airflow powders	Deplaquing and decontamination of the site by applying glycine powder [Liu CC et al., 2024] using an Airflow handpiece
Chlorhexidine Digluconate gel 1%	Ingredients: purified water, propylene glycol, hydroxy - ethyl - cellulose, PVP/VA copolymer, castor oil polyethylene glycol esters, chlorhexidine digluconate, sodium acetate, aroma, acetic acid, sodium metabisulfite, ascorbic acid.(Figure3)
OZORAL PRO ®	Ingredients: water, ozonated sunflower oil, flavoring, glycerin, carbomer, polycarbophils, sodium hydroxide, sodium saccharin, star anise fruit/seed oil, glyceryl caprylate, tocopherol, ascorbyl palmitate, disodium EDTA, limonene, linalool (Figure 3)

Figure 3: Ozoral PRO ®, Ozoral GEL ® and *1% Chlorhexidine Digluconate Gel.*

Patients weretreated in 10 sessions.

1) ***Baseline (T0)***

- Collection of medical history data, dental charting, index detection;
- Education and motivation for individual home oral hygiene;
- Signature of the informed consent to the treatment;
- Professional supragingival and subgingival oral hygiene carried out using sonic / ultrasonic instrumentation, PEEK inserts and manual peek or titanium instrumentation;

- Decontamination of periodontal and peri-implant pockets with glycine-based Airflow powders;

- Application of 1% Chlorhexidine digluconate gel (in the sites of the Q1 and/or Q4 quadrants, to the right of the median sagittal plane) and of Ozoral PRO ® (in the sites of the Q2 and/or Q3 quadrants, to the left of the median sagittal plane) via syringe with blunt needle;

- Instructions for home treatment and delivery of the necessary aids: Chlorhexidine Gel digluconate 1% and Ozoral GEL ®.

2) ***Recall 1 month (T_1):***

- Review of medical history data, dental charting and re-evaluation of indices;

- Reinforcement of education and motivation for home oral hygiene;

- Decontamination of periodontal and peri-implant pockets with glycine-based Airflow powders;

- Application of 1% Chlorhexidine digluconate gel (in the sites of the Q1 and/or Q4 quadrants, to the right of the median sagittal plane) and of Ozoral PRO ® (in the sites of the Q2 and/or Q3 quadrants, to the left of the median sagittal plane) via syringe with blunt needle;

- Instructions for home treatment and delivery of the necessary aids: Chlorhexidine Gel digluconate 1% and Ozoral GEL ® .

3) ***3 month recall (T2):***

- Review of medical history data, dental charting and re-evaluation of indices;
- Reinforcement of education and motivation for home oral hygiene;
- Possible professional supragingival oral hygiene and decontamination of periodontal and peri-implant pockets with glycine-based Airflow powders;

- Application of 1% Chlorhexidine digluconate gel (in the sites of the Q1 and/or Q4 quadrants, to the right of the median sagittal plane) and of Ozoral PRO ® (in the sites of the Q2 and/or Q3 quadrants, to the left of the median sagittal plane) via syringe with blunt needle;

- Instructions for home treatment and delivery of the necessary aids: Chlorhexidine Gel digluconate 1% and Ozoral GEL ®.

4) ***6 month recall (T3):***

- Review of medical history data, dental charting and re-evaluation of indices;
- Reinforcement of education and motivation for home oral hygiene;

- Professional supragingival oral hygiene carried out using sonic / ultrasonic instrumentation, PEEK inserts and manual peek or titanium instrumentation;
- Decontamination of periodontal and peri-implant pockets with glycine-based Airflow powders;

- Application of 1% Chlorhexidine digluconate gel (in the sites of the Q1 and/or Q4 quadrants, to the right of the median sagittal plane) and of Ozoral PRO ®

(in the sites of the Q2 and/or Q3 quadrants, to the left of the median sagittal plane) via syringe with blunt needle;

- Instructions for home treatment and delivery of the necessary aids: Chlorhexidine Gel digluconate 1% and Ozoral GEL ® ;

- Final considerations;

- Programming of maintenance calls.

5) ***9 month recall (T4):***

- Review of medical history data, dental charting and re-evaluation of indices;
- Reinforcement of education and motivation for home oral hygiene;

- Professional supragingival oral hygiene carried out using sonic / ultrasonic instrumentation, PEEK inserts and manual peek or titanium instrumentation;
- Decontamination of periodontal and peri-implant pockets with glycine-based Airflow powders;

- Application of 1% Chlorhexidine digluconate gel (in the sites of the Q1 and/or Q4 quadrants, to the right of the median sagittal plane) and of Ozoral PRO ® (in the sites of the Q2 and/or Q3 quadrants, to the left of the median sagittal plane) via syringe with blunt needle;

- Instructions for home treatment and delivery of the necessary aids: Chlorhexidine Gel digluconate 1% and Ozoral GEL ® ;

- Final considerations;

- Programming of maintenance calls

6) ***12 month recall (T5):***

- Review of medical history data, dental charting and re-evaluation of indices;
- Reinforcement of education and motivation for home oral hygiene;

- Professional supragingival oral hygiene carried out using sonic / ultrasonic instrumentation, PEEK inserts and manual peek or titanium instrumentation;
- Decontamination of periodontal and peri-implant pockets with glycine-based Airflow powders;

- Application of 1% Chlorhexidine digluconate gel (in the sites of the Q1 and/or Q4 quadrants, to the right of the median sagittal plane) and of Ozoral PRO ® (in the sites of the Q2 and/or Q3 quadrants, to the left of the median sagittal plane) via syringe with blunt needle;

- Instructions for home treatment and delivery of the necessary aids: Chlorhexidine Gel digluconate 1% and Ozoral GEL ® ;

- Final considerations;

- Programming of maintenance calls

7) ***15 month recall (T6):***

- Review of medical history data, dental charting and re-evaluation of indices;
- Reinforcement of education and motivation for home oral hygiene;
- Professional supragingival oral hygiene carried out using sonic / ultrasonic instrumentation, PEEK inserts and manual peek or titanium instrumentation;
- Decontamination of periodontal and peri-implant pockets with glycine-based Airflow powders;
- Application of 1% Chlorhexidine digluconate gel (in the sites of the Q1 and/or Q4 quadrants, to the right of the median sagittal plane) and of Ozoral PRO ® (in the sites of the Q2 and/or Q3 quadrants, to the left of the median sagittal plane) via syringe with blunt needle;
- Instructions for home treatment and delivery of the necessary aids: Chlorhexidine Gel digluconate 1% and Ozoral GEL ® ;
- Final considerations;
- Programming of maintenance calls

8) ***18 month recall (T7):***

- Review of medical history data, dental charting and re-evaluation of indices;
- Reinforcement of education and motivation for home oral hygiene;
- Professional supragingival oral hygiene carried out using sonic / ultrasonic instrumentation, PEEK inserts and manual peek or titanium instrumentation;
- Decontamination of periodontal and peri-implant pockets with glycine-based Airflow powders;
- Application of 1% Chlorhexidine digluconate gel (in the sites of the Q1 and/or Q4 quadrants, to the right of the median sagittal plane) and of Ozoral PRO ® (in the sites of the Q2 and/or Q3 quadrants, to the left of the median sagittal plane) via syringe with blunt needle;
- Instructions for home treatment and delivery of the necessary aids: Chlorhexidine Gel digluconate 1% and Ozoral GEL ® ;
- Final considerations;
- Programming of maintenance calls

9) ***21 month recall (T8):***

- Review of medical history data, dental charting and re-evaluation of indices;
- Reinforcement of education and motivation for home oral hygiene;
- Professional supragingival oral hygiene carried out using sonic / ultrasonic instrumentation, PEEK inserts and manual peek or titanium instrumentation;
- Decontamination of periodontal and peri-implant pockets with glycine-based Airflow powders;

- Application of 1% Chlorhexidine digluconate gel (in the sites of the Q1 and/or Q4 quadrants, to the right of the median sagittal plane) and of Ozoral PRO ® (in the sites of the Q2 and/or Q3 quadrants, to the left of the median sagittal plane) via syringe with blunt needle;
- Instructions for home treatment and delivery of the necessary aids: Chlorhexidine Gel digluconate 1% and Ozoral GEL ® ;
- Final considerations;
- Programming of maintenance calls

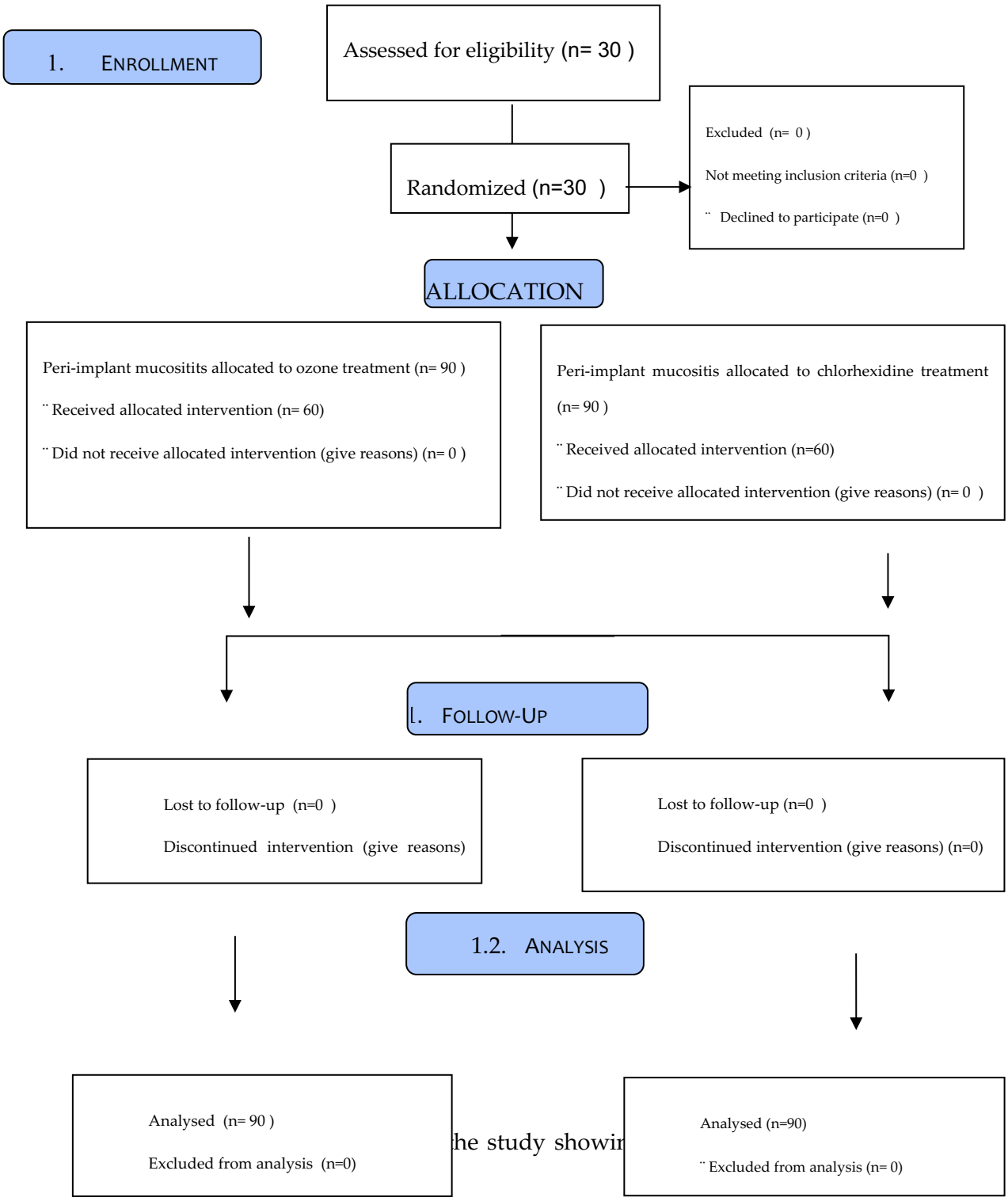
10) 24 month recall (T9):

- Review of medical history data, dental charting and re-evaluation of indices;
- Reinforcement of education and motivation for home oral hygiene;
- Professional supragingival oral hygiene carried out using sonic / ultrasonic instrumentation, PEEK inserts and manual peek or titanium instrumentation;
- Decontamination of periodontal and peri-implant pockets with glycine-based Airflow powders;
- Application of 1% Chlorhexidine digluconate gel (in the sites of the Q1 and/or Q4 quadrants, to the right of the median sagittal plane) and of Ozoral PRO ® (in the sites of the Q2 and/or Q3 quadrants, to the left of the median sagittal plane) via syringe with blunt needle;
- Instructions for home treatment and delivery of the necessary aids: Chlorhexidine Gel digluconate 1% and Ozoral GEL ® ;
- Final considerations;

11) Home treatment:

- After having carried out correct oral hygiene at home, apply once a day, in the evening, for a period of 14 days Chlorhexidine Gel digluconate 1% on the sites of the Q1 and/or Q4 quadrants, and Ozoral GEL ® on the sites of the Q2 and /or Q3 via syringe or brush;
- Do not introduce liquid or solid substances into your mouth for at least 30 minutes;
- Starting from the 15th day, interruption of the application of the gels

CONSORT 2010 Flow Diagram



he study showir

The study in question is illustrated in the consort flow chart cited above. 30 patients with peri-implant mucositis were selected and randomized, divided into two groups with split-mouth: Q1 and Q4, whereas in quadrants Q2 and Q3, the ozonized gel was administered in-office. The case group involves the treatment of sites with peri-implantitis with ozone; the control group involves the treatment of sites with peri-implantitis with chlorhexidine.(Figure 4)

2.STATISTIC ANALYSIS

The data analysis was conducted at the Experimental Tests Laboratory of the UDA of Orthodontics and Children's Dentistry of the Department of Clinical-Surgical, Diagnostic and Pediatric Sciences of the University of Pavia. The analysis was carried out with the R software (R version 3.1.3, R Development Core Team, R Foundation for Statistical Computing, Wien, Austria) calculating the descriptive statistics for each variable which included: mean, standard deviation, median, values minimum and maximum, measured for each group. The normality of the distributions was assessed using Kolmogorov and Smirnov tests. Subsequently, a Friedman test for repeated measures was applied followed by Dunn's post hoc test. For all tests significance was set at $P < 0.05$.

V – RESULTS

V-RESULTS

5.RESULTS

5.1 INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria:

- Patients aged between 18-70 years.
- Presence of at least one site with peri-implant mucositis (PPD>5) per side with respect to the median sagittal plane.
- Collaborative patients with good compliance.

Exclusion criteria:

- Patients suffering from neurological and/or psychological disorders.
- Patients with systemic, metabolic or autoimmune diseases.
- Patients who have taken bisphosphonates in the last 12 months.
- Pregnant and/or breastfeeding patients.
- Patients undergoing tumor therapy.

5.2 DESCRIPTION OF THE SAMPLE

At the Dental Hygiene Unit, Section of Dentistry, Department of Clinical, Surgical, Diagnostic and Pediatric Sciences of the University of Pavia, a total of 30 patients were recruited for the following study, of which 16 males and 14 females.

The average age of the males was 59.14yrs, while that of the females was 58.94yrs.

Table 1: Summary table of average patient age

Age	Average	Min. Age	Max age	SD
Males	59.14	37	70	7.25
Females	58.94	44	69	9.38

Descriptive and inferential statistics are reported below. The graphs show the mean and confidence interval. The Friedman test demonstrated the presence of significant differences (P<0.05) for some tested variables. Post-hoc testing demonstrated significant differences in some tested groups.

1.PPD

Regarding the probing depth, after 24 months, notable intragroup improvements were detected between all measurement times, both with the use of chlorhexidine and with the use of ozone gel. Comparing the T9 of both products revealed statistically significant differences between the two treatments (p<0.05) (Figure 5.) However, considering that at T0 the PPD value was higher in the trial group, the improvement at T9 in the trial group was greater than in the control group.

Table 2: Summary table of PPD statistical values in the control group.

	Control T0	Control T1	Control T2	Control T3	Control T4	Control T5	Control T6	Control T7	Control T8	Control T9
Mean	6,55	5,22	4,49	4,23	3,98	3,95	3,89	3,81	3,75	3,72
SD	1,18	1,17	1,16	1,13	0,94	0,92	0,92	0,89	0,87	0,86
Min	5	3	2	2	2	2	2	2	2	2
Mdn	6	5	4	4	4	4	4	4	4	4
Max	10	8	7	7	6	6	6	6	6	6

Table 3: Summary table of PPD statistical values in the case group.

	Trial T0	Trial T1	Trial T2	Trial T3	Trial T4	Trial T5	Trial T6	Trial T7	Trial T8	TrialT9
Mean	6,55	5,38	4,41	3,92	3,43	3,37	3,29	3,16	3,04	2,97
SD	1,21	1,36	1,4	1,42	1,05	1,02	1,04	1	0,93	0,92
Min	5	3	2	2	2	2	2	2	2	1
Mdn	7	5	4	4	3	3	3	3	3	3
Max	9	9	9	8	7	7	7	6	6	6

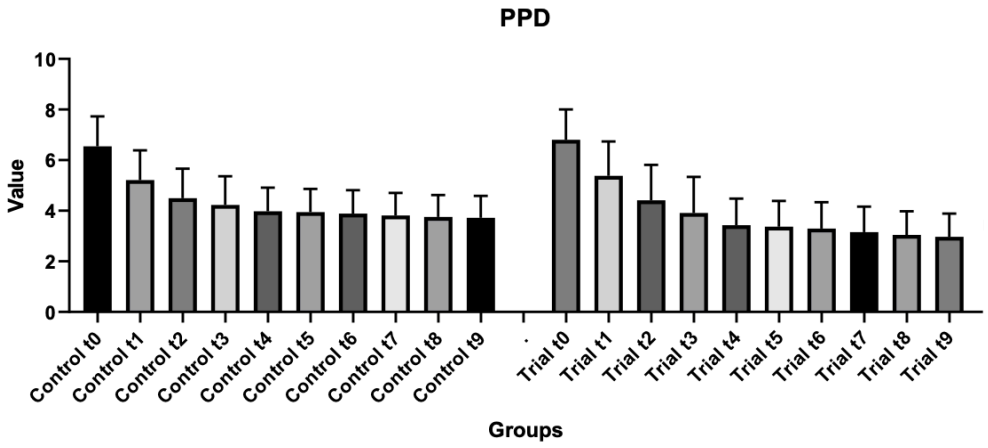


Figure 5: Descriptive histogram of PPD values between groups.

Table 4: Tukey's multiple comparisons test (PPD).

Dunn'smultiple comparisons test	Mean rank diff,	Significant?	Summary	Adjusted P Value
Control t0 vs. Trial t1	298,4	Yes	*	0,025
Control t0 vs. Trial t2	633,3	Yes	****	<0,0001
Control t0 vs. Trial t3	830,9	Yes	****	<0,0001
Control t0 vs. Trial t4	1024	Yes	****	<0,0001
Control t0 vs. Trial t5	1046	Yes	****	<0,0001
Control t0 vs. Trial t6	1077	Yes	****	<0,0001
Control t0 vs. Trial t7	1134	Yes	****	<0,0001
Control t0 vs. Trial t8	1186	Yes	****	<0,0001
Control t0 vs. Trial t9	1218	Yes	****	<0,0001
Control t1 vs. Trial t0	-351	Yes	**	0,001
Control t1 vs. Trial t2	313,8	Yes	*	0,011
Control t1 vs. Trial t3	511,4	Yes	****	<0,0001

Control t1 vs. Trial t4	704,9	Yes	****	<0,0001
Control t1 vs. Trial t5	726,3	Yes	****	<0,0001
Control t1 vs. Trial t6	757,1	Yes	****	<0,0001
Control t1 vs. Trial t7	814,3	Yes	****	<0,0001
Control t1 vs. Trial t8	866,9	Yes	****	<0,0001
Control t1 vs. Trial t9	898,1	Yes	****	<0,0001
Control t2 vs. Trial t0	-604	Yes	****	<0,0001
Control t2 vs. Trial t4	451,6	Yes	****	<0,0001
Control t2 vs. Trial t5	473,1	Yes	****	<0,0001
Control t2 vs. Trial t6	503,9	Yes	****	<0,0001
Control t2 vs. Trial t7	561	Yes	****	<0,0001
Control t2 vs. Trial t8	613,6	Yes	****	<0,0001
Control t2 vs. Trial t9	644,8	Yes	****	<0,0001
Control t3 vs. Trial t0	-704	Yes	****	<0,0001
Control t3 vs. Trial t1	-374	Yes	***	3E-04
Control t3 vs. Trial t4	351,7	Yes	**	0,001
Control t3 vs. Trial t	373,2	Yes	***	3E-04
Control t3 vs. Trial t6	404	Yes	****	<0,0001
Control t3 vs. Trial t7	461,1	Yes	****	<0,0001
Control t3 vs. Trial t8	513,8	Yes	****	<0,0001
Control t3 vs. Trial t9	545	Yes	****	<0,0001
Control t4 vs. Trial t0	-793	Yes	****	<0,0001
Control t4 vs. Trial t1	-463	Yes	****	<0,0001
Control t4 vs. Trial t5	284,8	Yes	*	0,05
Control t4 vs. Trial t6	315,6	Yes	**	0,01
Control t4 vs. Trial t7	372,7	Yes	***	3E-04
Control t4 vs. Trial t8	425,3	Yes	****	<0,0001
Control t4 vs. Trial t9	456,5	Yes	****	<0,0001

Control t5 vs. Trial t0	-806	Yes	****	<0,0001
Control t5 vs. Trial t1	-476	Yes	****	<0,0001
Control t5 vs. Trial t6	302,3	Yes	*	0,02
Control t5 vs. Trial t7	359,4	Yes	***	8E-04
Control t5 vs. Trial t8	412,1	Yes	****	<0,0001
Control t5 vs. Trial t9	443,3	Yes	****	<0,0001
Control t6 vs. Trial t0	-836	Yes	****	<0,0001
Control t6 vs. Trial t1	-506	Yes	****	<0,0001
Control t6 vs. Trial t7	329,6	Yes	**	0,005
Control t6 vs. Trial t8	382,2	Yes	***	2E-04
Control t6 vs. Trial t9	413,4	Yes	****	<0,0001
Control t7 vs. Trial t0	-868	Yes	****	<0,0001
Control t7 vs. Trial t1	-538	Yes	****	<0,0001
Control t7 vs. Trial t7	297,4	Yes	*	0,026
Control t7 vs. Trial t8	350	Yes	**	0,001
Control t7 vs. Trial t9	381,2	Yes	***	2E-04
Control t8 vs. Trial t0	-896	Yes	****	<0,0001
Control t8 vs. Trial t1	-566	Yes	****	<0,0001
Control t8 vs. Trial t8	322,2	Yes	**	0,007
Control t8 vs. Trial t9	353,4	Yes	**	0,001
Control t9 vs. Trial t0	-911	Yes	****	<0,0001
Control t9 vs. Trial t1	-581	Yes	****	<0,0001
Control t9 vs. Trial t8	306,9	Yes	*	0,016
Control t9 vs. Trial t9	338,1	Yes	**	0,003
Trial t0 vs. Trial t1	329,8	Yes	**	0,005
Trial t0 vs. Trial t2	664,7	Yes	****	<0,0001
Trial t0 vs. Trial t3	862,4	Yes	****	<0,0001
Trial t0 vs. Trial t4	1056	Yes	****	<0,0001

Trial t0 vs. Trial t5	1077	Yes	****	<0,0001
Trial t0 vs. Trial t6	1108	Yes	****	<0,0001
Trial t0 vs. Trial t7	1165	Yes	****	<0,0001
Trial t0 vs. Trial t8	1218	Yes	****	<0,0001
Trial t0 vs. Trial t9	1249	Yes	****	<0,0001
Trial t1 vs. Trial t2	334,9	Yes	**	0,004
Trial t1 vs. Trial t3	532,5	Yes	****	<0,0001
Trial t1 vs. Trial t4	726	Yes	****	<0,0001
Trial t1 vs. Trial t5	747,4	Yes	****	<0,0001
Trial t1 vs. Trial t6	778,2	Yes	****	<0,0001
Trial t1 vs. Trial t7	835,4	Yes	****	<0,0001
Trial t1 vs. Trial t8	888	Yes	****	<0,0001
Trial t1 vs. Trial t9	919,2	Yes	****	<0,0001
Trial t2 vs. Trial t4	391,1	Yes	***	1E-04
Trial t2 vs. Trial t5	412,6	Yes	****	<0,0001
Trial t2 vs. Trial t6	443,4	Yes	****	<0,0001
Trial t2 vs. Trial t7	500,5	Yes	****	<0,0001
Trial t2 vs. Trial t8	553,1	Yes	****	<0,0001
Trial t2 vs. Trial t9	584,3	Yes	****	<0,0001
Trial t3 vs. Trial t7	302,9	Yes	*	0,021
Trial t3 vs. Trial t8	355,5	Yes	**	0,001
Trial t3 vs. Trial t9	386,7	Yes	***	2E-04

Table 4 shows all the comparisons of intragroup and intergroup timing. All statistical significances ($p<0.05$) present within the group can be deduced. In summary, a statistical significance can be seen for the control group at T0 examined with all the other times of the control group; statistical significance is evident for the control group at T0 under examination with all the times of the case group, with the exception of the control group T0 vs case group T0; statistical significance is evident for the control group at T1 in question with all control group times with the

exception of the T1 control group vs T2 control group; statistical significance is evident for the T1 control group in question with all case group times with the exception of the T1 control group vs T1 case group; statistical significance is evident for the T2 control group under examination with all the times of the case group with the exception of the T2 control group vs T1, T2 and T3 case group; statistical significance is evident for the control group at T3 examined with all the times of the case group with the exception of the control group T3 vs case group T2 and T3; statistical significance is evident for the control group at T4 examined with all times of the case group with the exception of the control group T4 vs case group T2, T3 and T4; statistical significance is evident in the case group at T0 under examination with all the times of the case group; statistical significance is evident in the case group at T1 under examination with all the times of the case group; statistical significance of the case group is evident at T2 examined with all the times of the case group with the exception of T3; statistical significance of the case group is evident at T3 examined with all the times of the case group with the exception of T4, T5 and T6.

1. PI

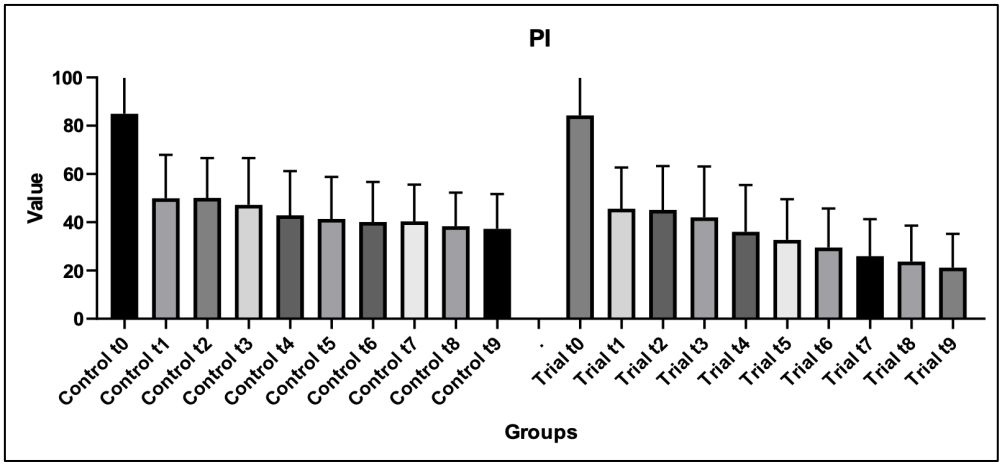
In the analysis of the plaque index, statistically significant differences were highlighted between the T9 control group and the T9 trial group ($p<0.05$) (Figure 6) .Although both products led to a reduction in index values from time T0 to time T9, the trial group showed a notable reduction in the plaque index over 24 months.

Table 5: Summary table of PI values of the control group.

	Control T0	Control T1	Control T2	Control T3	Control T4	Control T5	Control T6	Control T7	Control T9
Mean	84,9	49,9	50,1	47,2	42,9	41,4	40,1	40,3	37,3
SD	16,7	1,8	16,5	19,4	18,3	17,4	16,6	15,3	14,4
Min	45	20	15	15	10	10	10	10	10
Mdn	89,5	46,5	46,5	42	36,5	38	37,5	40	33,5
Max	100	100	88	81	77	75	70	67	62

Table 6: Summary table of PI values of the case group.

	Trial T0	Trial T1	Trial T2	Trial T3	Trial T4	Trial T5	Trial T6	Trial T7	Trial T8	Trial T9
Mean	84,3	45,6	45,1	42	36,1	32,7	29,5	25,9	23,7	21,2
SD	16,2	17,1	18,2	21,1	19,4	16,9	16,2	15,3	14,9	14
Min	45	10	10	0	5	5	5	1	0	0
Mdn	87,5	43	43	41	36	33,5	29,5	28	23,5	20
Max	100	88	79	83	76	70	73	72	68	65



Figures 6: Descriptive histogram of PI values between groups.

Table 7: Tukey's multiple comparisons test (PI).

Dunn's multiple comparisons test	Rank sum diff,	Significant?	Summary	Adjusted P Value
Control t0 vs. Control t9	336	Yes	****	<0,0001
Control t0 vs. Trial t1	231	Yes	****	<0,0001
Control t0 vs. Trial t2	191	Yes	**	0,006
Control t0 vs. Trial t3	200,5	Yes	**	0,002
Control t0 vs. Trial t4	323	Yes	****	<0,0001
Control t0 vs. Trial t5	371,5	Yes	****	<0,0001
Control t0 vs. Trial t6	430,5	Yes	****	<0,0001
Control t0 vs. Trial t7	478,5	Yes	****	<0,0001
Control t0 vs. Trial t8	501,5	Yes	****	<0,0001
Control t0 vs. Trial t9	532	Yes	****	<0,0001
Control t1 vs. Trial t0	-188	Yes	**	0,008

Control t1 vs. Trial t5	184,5	Yes	*	0,011
Control t1 vs. Trial t6	243,5	Yes	****	<0,0001
Control t1 vs. Trial t7	291,5	Yes	****	<0,0001
Control t1 vs. Trial t8	314,5	Yes	****	<0,0001
Control t1 vs. Trial t9	345	Yes	****	<0,0001
Control t2 vs. Control t8	184	Yes	*	0,011
Control t2 vs. Control t9	213,5	Yes	***	6E-04
Control t2 vs. Trial t4	200,5	Yes	**	0,002
Control t2 vs. Trial t5	249	Yes	****	<0,0001
Control t2 vs. Trial t6	308	Yes	****	<0,0001
Control t2 vs. Trial t7	356	Yes	****	<0,0001
Control t2 vs. Trial t8	379	Yes	****	<0,0001
Control t2 vs. Trial t9	409,5	Yes	****	<0,0001
Control t3 vs. Control t8	177,5	Yes	*	0,02
Control t3 vs. Control t9	207	Yes	**	0,001
Control t3 vs. Trial t4	194	Yes	**	0,004
Control t3 vs. Trial t5	242,5	Yes	****	<0,0001
Control t3 vs. Trial t6	301,5	Yes	****	<0,0001
Control t3 vs. Trial t7	349,5	Yes	****	<0,0001
Control t3 vs. Trial t8	372,5	Yes	****	<0,0001
Control t3 vs. Trial t9	403	Yes	****	<0,0001
Control t4 vs. Trial t6	201	Yes	**	0,002
Control t4 vs. Trial t7	249	Yes	****	<0,0001
Control t4 vs. Trial t8	272	Yes	****	<0,0001
Control t4 vs. Trial t9	302,5	Yes	****	<0,0001
Control t5 vs. Trial t0	-257	Yes	****	<0,0001
Control t5 vs. Trial t6	175	Yes	*	0,026

Control t5 vs. Trial t7	223	Yes	***	2E-04
Control t5 vs. Trial t8	246	Yes	****	<0,0001
Control t5 vs. Trial t9	276,5	Yes	****	<0,0001
Control t6 vs. Trial t0	-286	Yes	****	<0,0001
Control t6 vs. Trial t7	193,5	Yes	**	0,005
Control t6 vs. Trial t8	216,5	Yes	***	4E-04
Control t6 vs. Trial t9	247	Yes	****	<0,0001
Control t7 vs. Trial t0	-272	Yes	****	<0,0001
Control t7 vs. Trial t7	208	Yes	**	0,001
Control t7 vs. Trial t8	231	Yes	****	<0,0001
Control t7 vs. Trial t9	261,5	Yes	****	<0,0001
Control t8 vs. Trial t0	-308	Yes	****	<0,0001
Control t8 vs. Trial t7	172	Yes	*	0,033
Control t8 vs. Trial t8	195	Yes	**	0,004
Control t8 vs. Trial t9	225,5	Yes	***	2E-04
Control t9 vs. Trial t0	-337	Yes	****	<0,0001
Control t9 vs. Trial t9	196	Yes	**	0,004
Trial t0 vs. Trial t1	232	Yes	****	<0,0001
Trial t0 vs. Trial t2	192	Yes	**	0,005
Trial t0 vs. Trial t3	201,5	Yes	**	0,002
Trial t0 vs. Trial t4	324	Yes	****	<0,0001
Trial t0 vs. Trial t5	372,5	Yes	****	<0,0001
Trial t0 vs. Trial t6	431,5	Yes	****	<0,0001
Trial t0 vs. Trial t7	479,5	Yes	****	<0,0001
Trial t0 vs. Trial t8	502,5	Yes	****	<0,0001
Trial t0 vs. Trial t9	533	Yes	****	<0,0001
Trial t1 vs. Trial t6	199,5	Yes	**	0,003
Trial t1 vs. Trial t7	247,5	Yes	****	<0,0001

Trial t1 vs. Trial t8	270,5	Yes	****	<0,0001
Trial t1 vs. Trial t9	301	Yes	****	<0,0001

Table 7 compares all the intragroup and intergroup times.

In summary, statistical significance is evident for the control group at T0 examined with all the other times of the control group with the exception of the control group at T2 and T3; statistical significance is evident for the control group at T0 examined with all the times of the case group with the exception of the case group T0; statistical significance is evident for the control group at T1 examined with all the times of the case group with the exception of the case group T1, T2, T3 and T4; statistical significance is evident for the control group at T2 examined with all the times of the case group with the exception of the case group T0, T1, T2 and T3; statistical significance is evident for the control group at T3 examined with all the times of the case group with the exception of the case group T0, T1, T2 and T3; statistical significance is evident for the case group at T0 examined with all the times of the case group with the exception of the case group; statistical significance is evident for the case group at T1 examined with all the times of the case group with the exception of the case group T2, T3, T4 and T5; statistical significance is evident for the case group at T2 examined with all the times of the case group with the exception of the case group T3 and T4; statistical significance is evident for the case group at T3 examined with all the times of the case group with the exception of the case group T4; statistical significance is evident for the case group at T4 examined with all the times of the case group with the exception of the case group T5, T6 and T7.

3. BOP

Regarding the bleeding index, a statistically significant intergroup difference was highlighted between the T9 control group and the T9 trial group ($p<0.05$) (Figure 7). Furthermore, it emerges that there are clear improvements from T0 to T1, from T0 to T2 and from T0 to T3 with both ozone and chlorhexidine, but also that from T3 to T9 the difference is no longer significant in the control group, something that instead persists in the trial group. Therefore, there is a clear long-term improvement with the use of ozone gel.

Table 8: Summary table of the BOP statistical values of the control group.

	Control T0	Control T1	Control T2	Control T3	Control T4	Control T5	Control T6	Control T7	Control T8	Control T9
Mean	44,6	27,1	18,4	16,1	14,1	13,9	13,8	13,4	13,2	13,3
SD	26,9	17,2	12,3	10,2	9	8,73	9,05	8,76	8,38	8,46
Min	8,33	0	1,19	1,1	1	1	1	0	0	0
Mdn	44	23,7	19,3	16,5	15	15	11,5	11	12,5	12,5
Max	100	55	40,3	36	33	32	35	35	30	31

Table 9: Summary table of the BOP statistical values of the case group.

	Trial T0	Trial T1	Trial T2	Trial T3	Trial T4	Trial T5	Trial T6	Trial T7	Trial T8	TrialT9
Mean	46,1	21,7	14,1	10,8	7,6	5,8	4,95	4,4	3,57	3,33
SD	27,1	13,7	9,15	7,72	6,61	5,49	5,49	4,87	4,25	4,01
Min	5,95	0	0	0	0	0	0	0	0	0
Mdn	42,5	21	13,5	10,1	5	5	3,5	3,5	2,5	2
Max	100	48	37,1	29,4	25	20	22,5	20	18	15

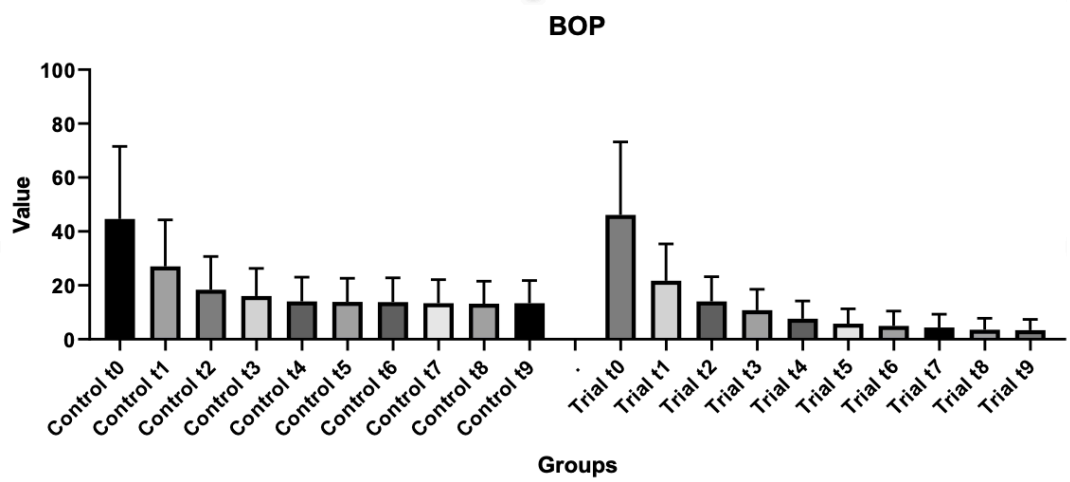


Figure 7: Descriptive histogram of BOP values across groups.

Table 10: Tukey's multiple comparisons test (BOP).

Dunn's multiple comparisons test	Rank sum diff,	Significant?	Summary	Adjusted P Value
Control t0 vs. Trial t2	242	Yes	****	<0,0001
Control t0 vs. Trial t3	286,5	Yes	****	<0,0001
Control t0 vs. Trial t4	375,5	Yes	****	<0,0001
Control t0 vs. Trial t5	426,5	Yes	****	<0,0001
Control t0 vs. Trial t6	451	Yes	****	<0,0001
Control t0 vs. Trial t7	459,5	Yes	****	<0,0001
Control t0 vs. Trial t8	475,5	Yes	****	<0,0001
Control t0 vs. Trial t9	493,5	Yes	****	<0,0001
Control t1 vs. Control t8	173	Yes	*	0,03
Control t1 vs. Trial t3	189	Yes	**	0,007
Control t1 vs. Trial t4	278	Yes	****	<0,0001
Control t1 vs. Trial t5	329	Yes	****	<0,0001

Control t1 vs. Trial t6	353,5	Yes	****	<0,0001
Control t1 vs. Trial t7	362	Yes	****	<0,0001
Control t1 vs. Trial t8	378	Yes	****	<0,0001
Control t1 vs. Trial t9	396	Yes	****	<0,0001
Control t2 vs. Trial t4	236	Yes	****	<0,0001
Control t2 vs. Trial t5	287	Yes	****	<0,0001
Control t2 vs. Trial t6	311,5	Yes	****	<0,0001
Control t2 vs. Trial t7	320	Yes	****	<0,0001
Control t2 vs. Trial t8	336	Yes	****	<0,0001
Control t2 vs. Trial t9	354	Yes	****	<0,0001
Control t3 vs. Trial t0	181	Yes	*	0,015
Control t3 vs. Trial t4	204,5	Yes	**	0,002
Control t3 vs. Trial t5	255,5	Yes	****	<0,0001
Control t3 vs. Trial t6	280	Yes	****	<0,0001
Control t3 vs. Trial t7	288,5	Yes	****	<0,0001
Control t3 vs. Trial t8	304,5	Yes	****	<0,0001
Control t3 vs. Trial t9	322,5	Yes	****	<0,0001
Control t4 vs. Trial t0	240	Yes	****	<0,0001
Control t4 vs. Trial t5	197	Yes	**	0,003
Control t4 vs. Trial t6	221,5	Yes	***	3E-04
Control t4 vs. Trial t7	230	Yes	****	<0,0001
Control t4 vs. Trial t8	246	Yes	****	<0,0001
Control t4 vs. Trial t9	264	Yes	****	<0,0001
Control t5 vs. Trial t5	180,5	Yes	*	0,016
Control t5 vs. Trial t6	205	Yes	**	0,002
Control t5 vs. Trial t7	213,5	Yes	***	6E-04
Control t5 vs. Trial t8	229,5	Yes	***	1E-04
Control t5 vs. Trial t9	247,5	Yes	****	<0,0001

Control t6 vs. Trial t0	-248	Yes	****	<0,0001
Control t6 vs. Trial t5	189	Yes	**	0,007
Control t6 vs. Trial t6	213,5	Yes	***	6E-04
Control t6 vs. Trial t7	222	Yes	***	2E-04
Control t6 vs. Trial t8	238	Yes	****	<0,0001
Control t6 vs. Trial t9	256	Yes	****	<0,0001
Control t7 vs. Trial t0	-273	Yes	****	<0,0001
Control t7 vs. Trial t6	188	Yes	**	0,008
Control t7 vs. Trial t7	196,5	Yes	**	0,003
Control t7 vs. Trial t8	212,5	Yes	***	7E-04
Control t7 vs. Trial t9	230,5	Yes	****	<0,0001
Control t8 vs. Trial t0	-281	Yes	****	<0,0001
Control t8 vs. Trial t6	180,5	Yes	*	0,016
Control t8 vs. Trial t7	189	Yes	**	0,007
Control t8 vs. Trial t8	205	Yes	**	0,002
Control t8 vs. Trial t9	223	Yes	***	2E-04
Control t9 vs. Trial t0	272	Yes	****	<0,0001
Control t9 vs. Trial t6	189,5	Yes	**	0,007
Control t9 vs. Trial t7	198	Yes	**	0,003
Control t9 vs. Trial t8	214	Yes	***	6E-04
Control t9 vs. Trial t9	232	Yes	****	<0,0001
Trial t0 vs. Trial t2	252	Yes	****	<0,0001
Trial t0 vs. Trial t3	296,5	Yes	****	<0,0001
Trial t0 vs. Trial t4	385,5	Yes	****	<0,0001
Trial t0 vs. Trial t5	436,5	Yes	****	<0,0001
Trial t0 vs. Trial t6	461	Yes	****	<0,0001
Trial t0 vs. Trial t7	469,5	Yes	****	<0,0001
Trial t0 vs. Trial t8	485,5	Yes	****	<0,0001

Trial t0 vs. Trial t9	503,5	Yes	****	<0,0001
Trial t1 vs. Trial t4	241,5	Yes	****	<0,0001
Trial t1 vs. Trial t5	292,5	Yes	****	<0,0001
Trial t1 vs. Trial t6	317	Yes	****	<0,0001
Trial t1 vs. Trial t7	325,5	Yes	****	<0,0001
Trial t1 vs. Trial t8	341,5	Yes	****	<0,0001
Trial t1 vs. Trial t9	359,5	Yes	****	<0,0001
Trial t2 vs. Trial t5	184,5	Yes	*	0,011
Trial t2 vs. Trial t6	209	Yes	***	0,001
Trial t2 vs. Trial t7	217,5	Yes	***	4E-04
Trial t2 vs. Trial t8	233,5	Yes	****	<0,0001
Trial t2 vs. Trial t9	251,5	Yes	****	<0,0001
Trial t3 vs. Trial t7	173	Yes	*	0,03
Trial t3 vs. Trial t8	189	Yes	**	0,007
Trial t3 vs. Trial t9	207	Yes	**	0,001

Table 10 compares all intra-group and inter-group timescales.

In summary, statistical significance is evident for the control group at T0 examined with all the other times of the control group with the exception of the control group at T2 and T3; statistical significance is evident for the control group at T0 examined with all the times of the case group with the exception of the case group T0; statistical significance is evident for the control group at T1 examined with all the times of the case group with the exception of the case group T1, T2, T3 and T4; statistical significance is evident for the control group at T2 examined with all the times of the case group with the exception of the case group T0, T1, T2 and T3; statistical significance is evident for the control group at T3 examined with all the times of the case group with the exception of the case group T0, T1, T2 and T3; statistical significance is evident for the case group at T0 examined with all the times of the case group with the exception of the case group; statistical significance is evident for the case group at T1 examined with all the times of the case group with the exception of the case group T2, T3, T4 and T5; statistical significance is evident for the case group at T2 examined with all the times of the case group with the exception of the case group T3 and T4; statistical significance is evident for the

case group at T3 examined with all the times of the case group with the exception of the case group T4; statistical significance is evident for the case group at T4 examined with all the times of the case group with the exception of the case group T5, T6 and T7.

4. BS

Bleeding score analysis revealed a statistically significant difference between T3 control group and T3 trial group and at both T9s detected ($p<0.05$).(Figure 8) With Ozoral Pro ® it was also possible to observe a progressive improvement in all data collection times, providing feedback on the effectiveness of the ozone gel. With chlorhexidine there were improvements between the timescales but significantly lower than in the trial group

Table 11: Summary table of the BS statistical values of the control group.

	Control T0	Control T1	Control T2	Control T3	Control T5	Control T6	Control T7	Control T8	Control T9
Mean	2,13	1,81	1,5	1,31	0,91	0,8	0,68	0,59	0,53
SD	0,66	0,75	0,76	0,68	0,47	0,49	0,52	0,55	0,5
Min	1	0	0	0	0	0	0	0	0
Mdn	2	2	2	1	1	1	1	1	1
Max	3	3	3	2	2	2	2	2	1

Table 12: Summary table of the BS statistical values of the case group.

	Trial T0	Trial T1	Trial T 2	Trial T3	Trial T4	Trial T5	Trial T6	Trial T7	Trial T8	Trial T9
Mean	2,05	1,57	1,17	0,84	0,6	0,52	0,37	0,25	0,14	0,06
SD	0,71	0,72	0,7	0,68	0,49	0,5	0,49	0,43	0,34	0,24
Min	1	0	0	0	0	0	0	0	0	0
Mdn	2	2	1	1	1	1	0	0	0	0
Max	3	3	3	2	1	1	1	1	1	1

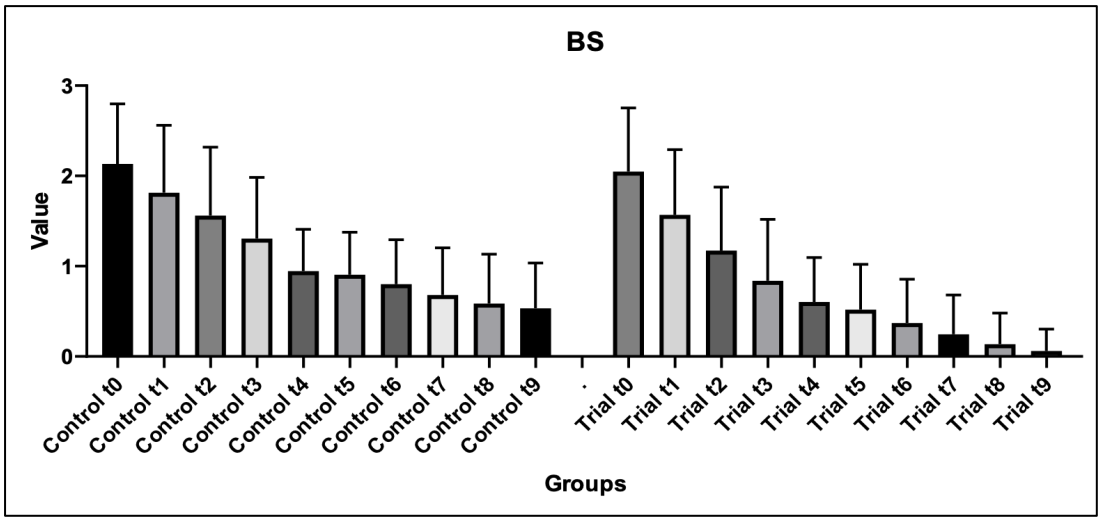


Figure 8: Descriptive histogram of BS values between groups

Table 13: Dunn's multiple comparisons test (BS)

Dunn's multiple comparisons test	Mean rank diff,	Significant?	Summary	Adjusted P Value
Control t0 vs. Trial t2	397,4	Yes	****	<0,0001
Control t0 vs. Trial t3	573,6	Yes	****	<0,0001
Control t0 vs. Trial t4	695,7	Yes	****	<0,0001
Control t0 vs. Trial t5	747,6	Yes	****	<0,0001
Control t0 vs. Trial t6	836,6	Yes	****	<0,0001
Control t0 vs. Trial t7	910,8	Yes	****	<0,0001
Control t0 vs. Trial t8	977,6	Yes	****	<0,0001
Control t0 vs. Trial t9	1022	Yes	****	<0,0001
Control t1 vs. Control t4	383,5	Yes	****	<0,0001
Control t1 vs. Control t5	405,9	Yes	****	<0,0001
Control t1 vs. Trial t3	457,4	Yes	****	<0,0001
Control t1 vs. Trial t4	579,4	Yes	****	<0,0001
Control t1 vs. Trial t5	631,3	Yes	****	<0,0001
Control t1 vs. Trial t6	720,4	Yes	****	<0,0001
Control t1 vs. Trial t7	794,6	Yes	****	<0,0001

Control t1 vs. Trial t8	861,3	Yes	****	<0,0001
Control t1 vs. Trial t9	905,9	Yes	****	<0,0001
Control t2 vs. Trial t9	803,1	Yes	****	<0,0001
Control t3 vs. Control t6	258,9	Yes	*	0,032
Control t3 vs. Trial t0	-290	Yes	**	0,003
Control t3 vs. Trial t3	249,4	Yes	*	0,042
Control t3 vs. Trial t4	371,4	Yes	****	<0,0001
Control t3 vs. Trial t5	423,3	Yes	****	<0,0001
Control t3 vs. Trial t6	512,4	Yes	****	<0,0001
Control t3 vs. Trial t7	586,6	Yes	****	<0,0001
Control t3 vs. Trial t8	653,3	Yes	****	<0,0001
Control t3 vs. Trial t9	697,9	Yes	****	<0,0001
Control t4 vs. Trial t	-466	Yes	****	<0,0001
Control t4 vs. Trial t1	-286	Yes	**	0,004
Control t4 vs. Trial t5	247,9	Yes	*	0,046
Control t4 vs. Trial t6	336,9	Yes	***	1E-04
Control t4 vs. Trial t7	411,1	Yes	****	<0,0001
Control t4 vs. Trial t8	477,9	Yes	****	<0,0001
Control t4 vs. Trial t9	522,4	Yes	****	<0,0001
Control t5 vs. Trial t0	-488	Yes	****	<0,0001
Control t5 vs. Trial t1	-309	Yes	***	9E-04
Control t5 vs. Trial t6	314,4	Yes	***	6E-04
Control t5 vs. Trial t7	388,6	Yes	****	<0,0001
Control t5 vs. Trial t8	455,4	Yes	****	<0,0001
Control t5 vs. Trial t9	499,9	Yes	****	<0,0001
Control t6 vs. Trial t0	-549	Yes	****	<0,0001
Control t6 vs. Trial t1	-369	Yes	****	<0,0001
Control t6 vs. Trial t6	253,5	Yes	*	0,033

Control t6 vs. Trial t7	327,7	Yes	***	2E-04
Control t6 vs. Trial t8	394,5	Yes	****	<0,0001
Control t6 vs. Trial t9	439	Yes	****	<0,0001
Control t7 vs. Trial t0	-620	Yes	****	<0,0001
Control t7 vs. Trial t1	-440	Yes	****	<0,0001
Control t7 vs. Trial t2	-256	Yes	*	0,028
Control t7 vs. Trial t7	257,1	Yes	*	0,026
Control t7 vs. Trial t8	323,9	Yes	***	3E-04
Control t7 vs. Trial t9	368,4	Yes	****	<0,0001
Control t8 vs. Trial t0	-676	Yes	****	<0,0001
Control t8 vs. Trial t1	-496	Yes	****	<0,0001
Control t8 vs. Trial t2	-312	Yes	***	7E-04
Control t8 vs. Trial t8	267,8	Yes	*	0,014
Control t8 vs. Trial t9	312,3	Yes	***	7E-04
Control t9 vs. Trial t0	-705	Yes	****	<0,0001
Control t9 vs. Trial t1	-525	Yes	****	<0,0001
Control t9 vs. Trial t2	-341	Yes	****	<0,0001
Trial t0 vs. Trial t2	363,5	Yes	****	<0,0001
Trial t1 vs. Trial t3	359,9	Yes	****	<0,0001
Trial t1 vs. Trial t4	481,9	Yes	****	<0,0001
Trial t1 vs. Trial t5	533,9	Yes	****	<0,0001
Trial t1 vs. Trial t6	622,9	Yes	****	<0,0001
Trial t1 vs. Trial t7	697,1	Yes	****	<0,0001
Trial t1 vs. Trial t8	763,9	Yes	****	<0,0001
Trial t1 vs. Trial t9	808,4	Yes	****	<0,0001
Trial t2 vs. Trial t3	176,2	No	ns	>0,9999
Trial t2 vs. Trial t4	298,2	Yes	**	0,001
Trial t2 vs. Trial t5	350,2	Yes	****	<0,0001

Trial t2 vs. Trial t6	439,2	Yes	****	<0,0001
Trial t2 vs. Trial t7	513,4	Yes	****	<0,0001
Trial t2 vs. Trial t8	580,2	Yes	****	<0,0001
Trial t2 vs. Trial t9	624,7	Yes	****	<0,0001
Trial t4 vs. Trial t8	282	Yes	**	0,004
Trial t4 vs. Trial t9	326,5	Yes	***	2E-04

Table 13 compares all intra-group and inter-group timescales. In summary, statistical significance is evident for the control group at T0 examined with all the other times of the control group with the exception of the control group at T1 and T2; statistical significance is evident for the control group at T0 examined with all the times of the case group with the exception of the case group T0 and T1; statistical significance is evident for the control group at T1 examined with all the times of the control group with the exception of the control group T2 and T3; statistical significance is evident for the control group at T1 examined with all the times of the case group with the exception of the case group T0 and T1; statistical significance is evident for the control group at T2 examined with all the times of the control group with the exception of the control group T3; statistical significance is evident for the control group at T2 examined with all the times of the case group with the exception of the case group T0, T1 and T2; statistical significance is evident for the control group at T3 examined with all the times of the case group with the exception of the case group T1 and T2; statistical significance is evident for the control group at T4 examined with all the times of the case group with the exception of the case group T2, T3 and T4; statistical significance is evident for the control group at T5 examined with all the times of the case group with the exception of the case group T2, T3, T4 and T5; statistical significance is evident for the control group at T6 examined with all the times of the case group with the exception of the case group T2, T3, T4 and T5; statistical significance is evident for the control group at T7 examined with all the times of the case group with the exception of the case group T3, T4, T5 and T6; statistical significance is evident for the control group at T8 examined with all the times of the case group with the exception of the case group T3, T4, T5, T6 and T7; statistical significance is evident for the case group at T0 examined with all the times of the case group with the exception of the case group T1; statistical significance is evident for the case group at T1 examined with all the times of the case group with the exception of T2; statistical significance is evident for the case group at T2 examined with all the times of the case group with the exception of the case group T3; statistical significance is evident for the case group at T3 examined with all the times of the case group with the exception of the case group T4 and T5; statistical significance is evident for the case group at T4

examined with all the times of the case group with the exception of the case group T5, T6 and T7; statistical significance is evident for the case group at T5 examined with all the times of the case group with the exception of the case group T6, T7 and T9.

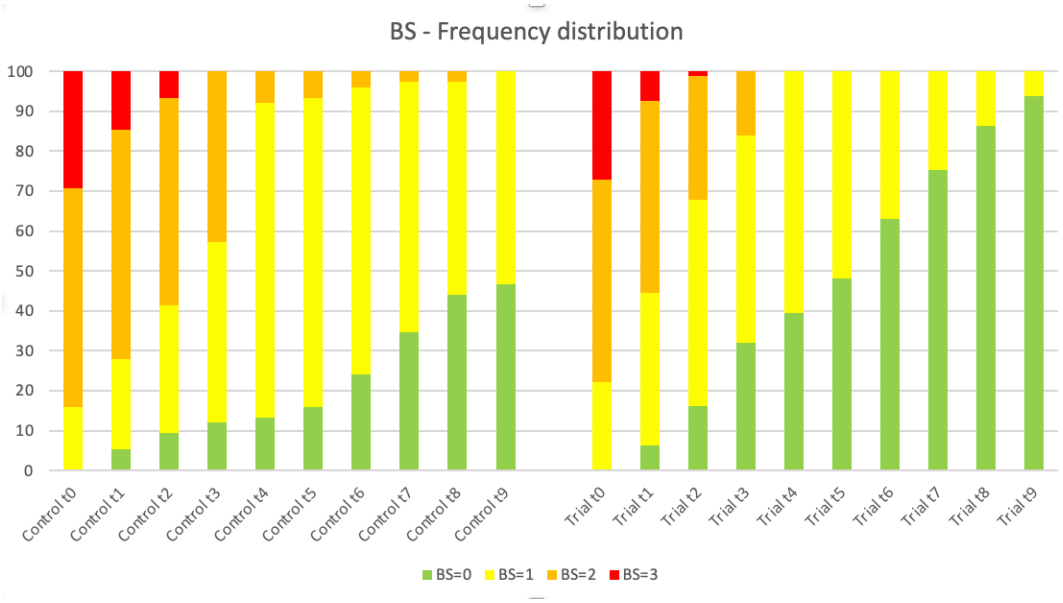
Frequency distribution

Table 14: Summary table of the BS statistical values of the control group.

	Control T0	Control T1	Control T2	Control T3	Control T4	Control T5	Control T6	Control T7	Control T8	Control T9
0	0	4	7	9	10	12	18	26	33	35
1	12	17	24	34	59	58	54	47	40	40
2	41	43	39	32	6	5	3	2	2	0
3	22	11	5	0	0	0	0	0	0	0
Tot	75	75	75	75	75	75	75	75	75	75

Table 15: Summary table of the BS statistical values of the trial group.

	Trial T0	Trial T1	Trial T2	Trial T3	Trial T4	Trial T5	Trial T6	Trial T7	Trial T8	TrialT9
0	0	5	13	26	32	39	51	61	70	76
1	18	31	42	42	49	42	30	20	11	5
2	41	39	25	13	0	0	0	0	0	0
3	22	6	1	0	0	0	0	0	0	0
Tot	81	81	81	81	81	81	81	81	81	81



Graph 1: Frequency distribution of the BS

5. SUPPURATION

An analysis of the suppuration revealed no significant difference between chlorhexidine and ozone gel. However, as regards the control group, improvements were only observed from T0 to T3, while as regards the trial group the improvements were already detected starting from T2.(Figure 9)

Table 16: Summary table of the statistical values of Suppuration of the control group.

	Control T0	Control T1	Control T2	Control T3	Control T4	Control T5	Control T6	Control T7	Control T8	Control T9
Mean	0,16	0,13	0,05	0,04	0	0	0	0	0	0
SD	0,37	0,34	0,23	0,2	0	0	0	0	0	0
Min	0	0	0	0	0	0	0	0	0	0
Mdn	0	0	0	0	0	0	0	0	0	0
Max	1	1	1	1	0	0	0	0	0	0

Table 17: Summary table of the statistical values of Suppuration of the trial group.

	Trial T0	Trial T1	Trial T2	Trial T3	Trial T4	Trial T5	Trial T6	Trial T7	Trial T8	TrialT9
Mean	0,17	0,07	0,05	0,02	0,01	0	0	0	0	0
SD	0,37	0,26	0,21	0,15	0,11	0	0	0	0	0
Min	0	0	0	0	0	0	0	0	0	0
Mdn	0	0	0	0	0	0	0	0	0	0
Max	1	1	1	1	1	0	0	0	0	0

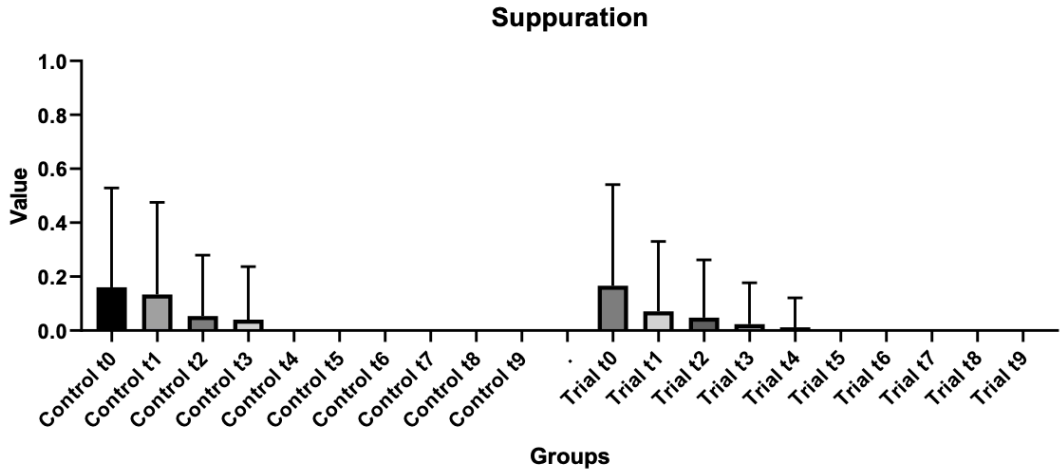


Figure 9: Descriptive histogram of Suppuration values between groups.

Table 18: Tukey's multiple comparisons test (Suppuration)

Dunn's multiple comparisons test	Mean rank diff,	Significant?	Summary	Adjusted P Value
Control t0 vs. Control t3	95,4	Yes	*	0,013
Control t0 vs. Control t4	127,2	Yes	****	<0,0001
Control t0 vs. Control t5	127,2	Yes	****	<0,0001
Control t0 vs. Control t6	127,2	Yes	****	<0,0001
Control t0 vs. Control t7	127,2	Yes	****	<0,0001
Control t0 vs. Control t8	127,2	Yes	****	<0,0001
Control t0 vs. Control t9	127,2	Yes	****	<0,0001
Control t0 vs. Trial t2	89,34	Yes	*	0,024
Control t0 vs. Trial t3	108,3	Yes	***	6E-04
Control t0 vs. Trial t4	117,7	Yes	****	<0,0001
Control t0 vs. Trial t5	127,2	Yes	****	<0,0001
Control t0 vs. Trial t6	127,2	Yes	****	<0,0001
Control t0 vs. Trial t7	127,2	Yes	****	<0,0001
Control t0 vs. Trial t8	127,2	Yes	****	<0,0001
Control t0 vs. Trial t9	127,2	Yes	****	<0,0001
Control t1 vs. Control t4	106	Yes	**	0,002
Control t1 vs. Control t5	106	Yes	**	0,002
Control t1 vs. Control t6	106	Yes	**	0,002
Control t1 vs. Control t7	106	Yes	**	0,002
Control t1 vs. Control t8	106	Yes	**	0,002
Control t1 vs. Control t9	106	Yes	**	0,002
Control t1 vs. Trial t3	87,07	Yes	*	0,035
Control t1 vs. Trial t4	96,54	Yes	**	0,007
Control t1 vs. Trial t5	106	Yes	**	0,001

Control t1 vs. Trial t6	106	Yes	**	0,001
Control t1 vs. Trial t7	106	Yes	**	0,001
Control t1 vs. Trial t8	106	Yes	**	0,001
Control t1 vs. Trial t9	106	Yes	**	0,001
Control t2 vs. Trial t0	-90,1	Yes	*	0,021
Control t3 vs. Trial t0	-101	Yes	**	0,003
Control t4 vs. Trial t0	-133	Yes	****	<0,0001
Control t6 vs. Trial t0	-133	Yes	****	<0,0001
Control t7 vs. Trial t0	-133	Yes	****	<0,0001
Control t8 vs. Trial t0	-133	Yes	****	<0,0001
Control t9 vs. Trial t0	-133	Yes	****	<0,0001
Trial t0 vs. Trial t2	94,64	Yes	**	0,005
Trial t0 vs. Trial t3	113,6	Yes	****	<0,0001
Trial t0 vs. Trial t4	123	Yes	****	<0,0001
Trial t0 vs. Trial t5	132,5	Yes	****	<0,0001
Trial t0 vs. Trial t6	132,5	Yes	****	<0,0001
Trial t0 vs. Trial t7	132,5	Yes	****	<0,0001
Trial t0 vs. Trial t8	132,5	Yes	****	<0,0001
Trial t0 vs. Trial t9	132,5	Yes	****	<0,0001

Table 18 compares all intra-group and inter-group timescales. In summary, in the case of suppuration there are very few statistical significances: statistical significance is evident for the control group at T0 examined with all the other times of the control group with the exception of the control group at T1 and T2; statistical significance is evident for the control group at T0 examined with all the times of the case group with the exception of the case group T0 and T1; statistical significance is evident for the control group at T1 examined with all the times of the control group with the exception of the control group T2 and T3; statistical significance is evident for the control group at T1 examined with all the times of the case group with the exception of the case group T0, T1 and T2.

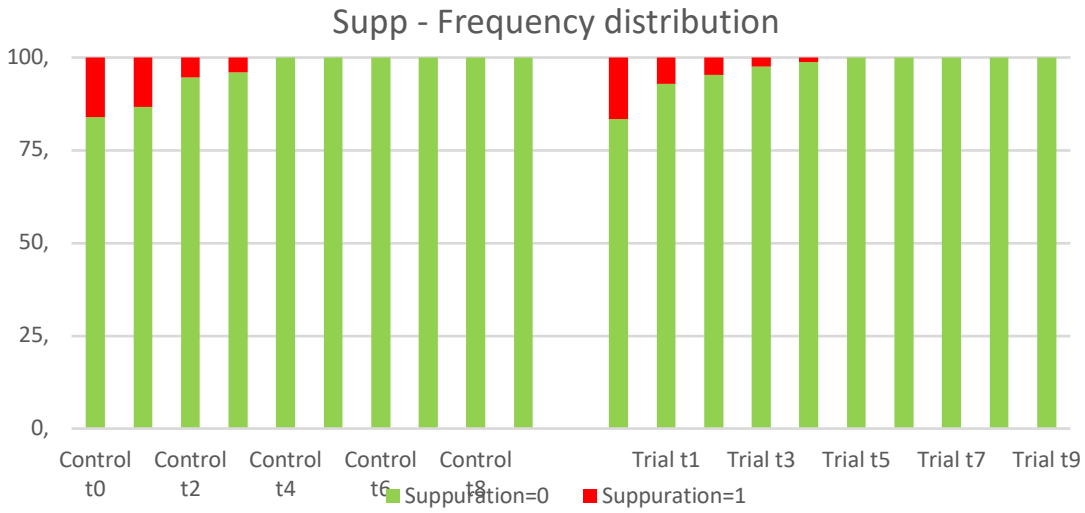
Frequency distribution

Table 19: Frequency distribution Suppuration statistical values of the trial group.

	Control T0	Control T1	Control T2	Control T3	Control T4	Control T5	Control T6	Control T7	Control T8	Control T9
0	63	65	71	72	75	75	75	75	75	75
1	12	10	4	3	0	0	0	0	0	0
Tot	75	75	75	75	75	75	75	75	75	75

Table 20 Summary table of the Suppuration statistical values of the trial group.

	Trial T0	Trial T1	Trial T2	Trial T3	Trial T4	Trial T5	Trial T6	Trial T7	Trial T8	TrialT9
0	70	78	80	82	83	84	84	84	84	84
1	14	6	4	2	1	0	0	0	0	0
Tot	84	84	84	84	84	84	84	84	84	84



Graph 2: Frequency distribution of Suppuration

6. MMC

Regarding the analysis of the condition of the marginal mucosa, no significant difference was detected between the two treatments. However, intragroup differences emerged from T0 to T1, from T0 to T2 and from T0 to T3 in both the use of chlorhexidine and ozone.(Figure 10)

Table 21: Summary table of the MMC statistical values of the control group.

	Control T0	Control T1	Control T2	Control T3	Control T4	Control T5	Control T6	Control T7	Control T8	Control T9
Mean	2,29	1,63	1,32	1,09	0,88	0,81	0,72	0,59	0,49	0,43
SD	0,49	0,75	0,6	0,68	0,59	0,56	0,48	0,5	0,5	0,5
Min	1	0	0	0	0	0	0	0	0	0
Mdn	2	2	1	1	1	1	1	1	0	0
Max	3	3	2	2	2	2	2	1	1	1

Table 22: Summary table of the MMC statistical values of the trial group.

	Trial T0	Trial T1	Trial T2	Trial T3	Trial T4	Trial T5	Trial T6	Trial T7	Trial T8	TrialT9
Mean	2,27	1,61	1,22	0,96	0,51	0,41	0,34	0,13	0,06	0,02
SD	0,47	0,71	0,63	0,72	0,53	0,49	0,48	0,34	0,24	0,15
Min	1	1	0	0	0	0	0	0	0	0
Mdn	2	1	1	1	0	0	0	0	0	0
Max	3	3	2	2	2	1	1	1	1	1

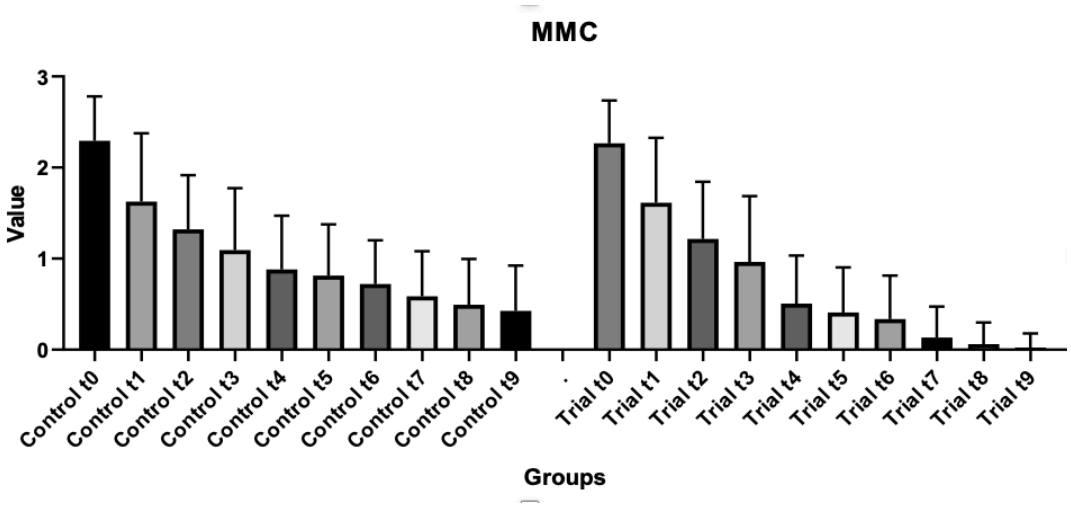


Figure 10: Descriptive histogram of MMC values between groups.

Table 23: Dunn's multiple comparisons test (MMC).

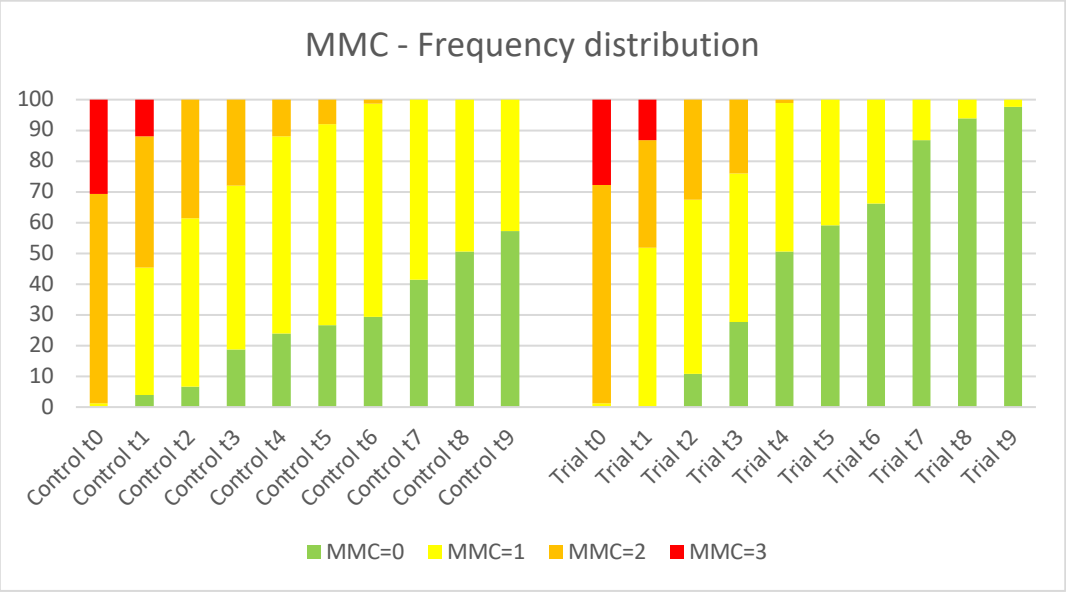
Dunn's multiple comparisons test	Mean rank diff,	Significant?	Summary	Adjusted P Value
Control t0 vs. Control t1	257,8	Yes	*	0,042
Control t0 vs. Control t2	368,1	Yes	****	<0,0001
Control t0 vs. Trial t2	421,7	Yes	****	<0,0001
Control t0 vs. Trial t3	563,8	Yes	****	<0,0001
Control t0 vs. Trial t4	808,7	Yes	****	<0,0001
Control t0 vs. Trial t5	866	Yes	****	<0,0001
Control t0 vs. Trial t6	910,4	Yes	****	<0,0001
Control t0 vs. Trial t7	1036	Yes	****	<0,0001
Control t0 vs. Trial t8	1081	Yes	****	<0,0001
Control t0 vs. Trial t9	1103	Yes	****	<0,0001
Control t1 vs. Trial t0	-253	Yes	*	0,038
Control t1 vs. Trial t3	306	Yes	**	0,001
Control t1 vs. Trial t4	551	Yes	****	<0,0001
Control t1 vs. Trial t5	608,3	Yes	****	<0,0001
Control t1 vs. Trial t6	652,7	Yes	****	<0,0001
Control t1 vs. Trial t7	778,5	Yes	****	<0,0001
Control t1 vs. Trial t8	823	Yes	****	<0,0001
Control t1 vs. Trial t9	845,2	Yes	****	<0,0001
Control t2 vs. Trial t0	-364	Yes	****	<0,0001
Control t2 vs. Trial t4	440,6	Yes	****	<0,0001
Control t2 vs. Trial t5	497,9	Yes	****	<0,0001
Control t2 vs. Trial t6	542,4	Yes	****	<0,0001
Control t2 vs. Trial t7	668,2	Yes	****	<0,0001
Control t3 vs. Trial t7	545,9	Yes	****	<0,0001

Control t3 vs. Trial t8	590,3	Yes	****	<0,0001
Control t3 vs. Trial t9	612,5	Yes	****	<0,0001
Control t4 vs. Trial t0	-592	Yes	****	<0,0001
Control t4 vs. Trial t1	-336	Yes	***	2E-04
Control t4 vs. Trial t5	270	Yes	*	0,014
Control t4 vs. Trial t6	314,4	Yes	***	7E-04
Control t4 vs. Trial t7	440,2	Yes	****	<0,0001
Control t4 vs. Trial t8	484,7	Yes	****	<0,0001
Control t4 vs. Trial t9	506,9	Yes	****	<0,0001
Control t5 vs. Trial t0	-626	Yes	****	<0,0001
Control t5 vs. Trial t1	-370	Yes	****	<0,0001
Control t5 vs. Trial t6	279,8	Yes	**	0,008
Control t6 vs. Trial t1	-417	Yes	****	<0,0001
Control t6 vs. Trial t2	-256	Yes	*	0,033
Control t6 vs. Trial t7	358,9	Yes	****	<0,0001
Control t6 vs. Trial t8	403,3	Yes	****	<0,0001
Control t6 vs. Trial t9	425,5	Yes	****	<0,0001
Control t7 vs. Trial t1	-497	Yes	****	<0,0001
Control t7 vs. Trial t2	-336	Yes	***	2E-04
Control t7 vs. Trial t7	279,1	Yes	**	0,008
Control t7 vs. Trial t8	323,5	Yes	***	4E-04
Control t7 vs. Trial t9	345,7	Yes	****	<0,0001
Control t8 vs. Trial t0	-810	Yes	****	<0,0001
Control t8 vs. Trial t1	-554	Yes	****	<0,0001
Control t8 vs. Trial t8	266,1	Yes	*	0,017
Control t8 vs. Trial t9	288,3	Yes	**	0,004
Control t9 vs. Trial t0	-851	Yes	****	<0,0001
Control t9 vs. Trial t1	-595	Yes	****	<0,0001

Control t9 vs. Trial t2	-434	Yes	****	<0,0001
Control t9 vs. Trial t3	-292	Yes	**	0,003
Trial t0 vs. Trial t1	255,9	Yes	*	0,022
Trial t0 vs. Trial t2	417,1	Yes	****	<0,0001
Trial t0 vs. Trial t7	1032	Yes	****	<0,0001
Trial t0 vs. Trial t8	1076	Yes	****	<0,0001
Trial t0 vs. Trial t9	1098	Yes	****	<0,0001
Trial t1 vs. Trial t3	303,3	Yes	***	9E-04
Trial t1 vs. Trial t4	548,2	Yes	****	<0,0001
Trial t1 vs. Trial t5	605,6	Yes	****	<0,0001
Trial t1 vs. Trial t6	650	Yes	****	<0,0001
Trial t1 vs. Trial t7	775,8	Yes	****	<0,0001
Trial t1 vs. Trial t8	820,3	Yes	****	<0,0001
Trial t1 vs. Trial t9	842,5	Yes	****	<0,0001
Trial t2 vs. Trial t4	387	Yes	****	<0,0001
Trial t2 vs. Trial t5	444,3	Yes	****	<0,0001
Trial t3 vs. Trial t8	517	Yes	****	<0,0001
Trial t3 vs. Trial t9	539,2	Yes	****	<0,0001
Trial t4 vs. Trial t8	272	Yes	**	0,008

Table 23 compares all intra-group and inter-group timescales. In summary, in the case of MMC there are very few statistical significances: statistical significance is evident for the control group at T0 examined with all the other times of the control group; statistical significance is evident for the control group at T0 examined with all the times of the case group with the exception of the case group T0; statistical significance is evident for the control group at T1 examined with all the times of the control group with the exception of the control group T2 and T3; statistical significance is evident for the control group at T1 examined with all the times of the case group with the exception of the case group T1 and T2; statistical significance is evident for the control group at T2 examined with all the times of the control group with the exception of the control group T2 and T4; statistical

significance is evident for the control group at T2 examined with all the times of the case group with the exception of the case group T1, T2 and T3; statistical significance is evident for the control group at T3 examined with all the times of the control group with the exception of the control group T4, T5 and T6; statistical significance is evident for the control group at T3 examined with all the times of the case group with the exception of the case group T1, T2 and T3; statistical significance is evident for the control group at T4 examined with all the times of the case group with the exception of the case group T2, T3 and T4; statistical significance is evident for the control group at T5 examined with all the times of the case group with the exception of the case group T2, T3, T4 and T5; statistical significance is evident for the control group at T6 examined with all the times of the case group with the exception of the case group T3, T4, T5 and T6; statistical significance is evident for the control group at T7 examined with all the times of the case group with the exception of the case group T3, T4, T5 and T6; statistical significance is evident for the control group at T8 examined with all the times of the case group with the exception of the case group T4, T5, T6 and T7; statistical significance is evident for the control group at T9 examined with all the times of the case group with the exception of the case group T4, T5, T6, T7, T8 and T9; statistical significance is evident for the case group at T0 examined with all the times of the case group; statistical significance is evident for the case group at T1 examined with all the times of the case group with the exception of T2; statistical significance is evident for the case group at T2 examined with all the times of the case group with the exception of T3; statistical significance is evident for the case group at T3 examined with all the times of the case group; statistical significance is evident for the case group at T4 examined with all the times of the case group with the exception of T5, T6 and T7.



Graph 3: Frequency distribution of MMC

VI – DISCUSSION

VI-DISCUSSION

The aim of the following study was to compare the therapeutic efficacy of two gels, Ozoral PRO ® at a professional level and Ozoral GEL ® at home level, with 1% Chlorhexidine digluconate Gel, post mechanical debridement, evaluating their capabilities in improve the indices of peri-implant mucositis.

To date, there is still no precise therapy aimed at resolving peri-implant mucositis: the addition of topical antimicrobials is certainly able to increase the chances of success of the treatment and consequently lead to a reduction in the bacterial load, which is difficult to achieve with drugs alone. mechanical means.

Ozone is used in various branches of medicine, especially dentistry, for its antimicrobial properties as it has been observed to have an effect on bacteria, viruses, and fungi [Gupta G et al.,2012. Sechi LA et al., 2001].

Regarding its antibacterial properties, ozone has been used in various formulations from gas to irrigation with ozonated water to the application of ozonated oil and ozone-based gel for the treatment of periodontal diseases and peri-implantitis according to many authors [Moreo G et al., 2020]

Dengizek and Tasdemir found no significant differences in CAL values between the groups treated without ozone and the group treated with ozone gas [Rapone B. et al., 2022]. However, they considered short follow-up periods of 1 month and 3 months, respectively. In contrast, Rapone and Isler found statistically significant differences between groups, and Isler considered a 12-month follow-up [Seydanur Dengizek E et al., 2019].

McKenna instead compared treatment with O 3 + NaCl with treatment with O 3 + H 2 O 2 and found that they were equally the most effective treatments, while O 2 + NaCl was the least effective in controlling bleeding around the implants [Vasthavi C., et al.,2020].Yilmaz compared three different groups: an SRP-only group, an SRP + ozone group, and an SRP + laser group, and only the laser + SRP group showed statistically significant differences between the groups [Tasdemir Z. et al., 2019].

Tasdemir and Dengizek found no statistically significant differences in PI between groups. McKenna found that treatment with O 3 + NaCl and O 3 + H 2 O 2 were equally the most effective treatments for GI and PI, while O 2 + NaCl was the least effective [Ranjith A et al., 2022]. Ozone is used in various branches of medicine, particularly in dentistry, for its antimicrobial properties as it has been observed to have an effect on bacteria, viruses and fungi [Sechi LA, et al. 2001]

Ozoral PRO ® comes in a pre -filled syringe, and is reserved exclusively for the operator in the studio. It is a medical device in the form of a hydrogel containing 15% ozonated sunflower seed oil called Ozonia 3000, produced, titrated, stabilized and standardized by Innovares . It promotes the maintenance of a healthy oral environment and is indicated in people susceptible to the development of phlogistic alterations of the oral cavity. Similarly, Ozoral GEL ® is a 15% hydrogel of ozonated sunflower seed oil, packaged in a tube and aimed at home therapy [Tetè G et al., 2023- Butera A, et al., 2023].

The objectives set in this study were almost completely achieved. From the baseline application of the two gels up to 24 months later, the results obtained suggest how these products are valid tools in the regression of peri-implant mucositis.

The most satisfactory outcomes were achieved in the evaluation of the BOP, as well as in the BS.

From the intergroup comparison Control T9 VS Trial T9 a statistically significant difference emerged ($p<0.05$): more specifically $p<0.0001$ regarding the BOP index and $p=0.005$ for the BS index; this shows that ozone led to a decrease in bleeding which is relevant compared to chlorhexidine, both in terms of frequency and quantity. This represents a fundamental fact in a healing process, as the first sign of inflammation in the oral cavity is determined by the presence of bleeding. Furthermore, with ozone there was a progressive decrease in the index in all detection phases, which was instead observed in a smaller quantity from T2 to T9 with chlorhexidine. From these data it can be deduced how much ozone is able to bring about a decrease in the percentage of bleeding over time.

For all other indices, the trends in values were found to be significant within the group. In particular, in the PPD there was significance not only from T0 to T9 but also from one recall interval to another. Analyzing specifically the control group Control T0 vs. Control T9 with a $p<0.0001$; the trial group with Trial T0 vs. Trial T9 $p<0.0001$; control group and trial group with Control T9 vs. Trial T9 $p=0.003$. It would have been interesting to carry out a radiographic check to evaluate the different probing depths in relation to the patient's implant situation and how this influenced the evaluation of the index.In the detection of the PI, however, significance was highlighted only in the transition from T0 to T1 ($p<0.0001$), from T0 to T2 ($p=0.006$) and from T0 to T3 ($p=0.002$) and so on up from T0 at T9 ($p<0.0001$), but not between one booster session and another, as plaque control after months appears to be more complicated to maintain for all patients, both following the lack of motivation as the months pass, but also following the different conditions of the implant rehabilitations existing between one patient and another, capable of favoring a more or less substantial accumulation of plaque. A limitation of the study can certainly be found in the fact that the positioning of the implants, the type of connection, the shape of the prostheses and the width of the mucosal tunnel decided during the surgery were not taken into consideration.

As regards Suppuration, the data shows us improvements only within its reference group from baseline to 24 months. From the statistical evaluation table n. 16, the decrease in the suppuration data detected already from T0 to T1 is evident as regards the case group, which was slightly detected in the control group. Although suppuration occurred at T0 on 12 sites in the control group and on 14 sites in the trial group, this albeit minimal difference in level to the detriment of ozone led to obtaining significance already in the comparison from T0 to T2 ($p=0.005$), whereas with chlorhexidine it was achieved from T0 to T3 ($p=0.01$). Finally, it must be said that suppuration, associated in all respects with the definition of peri-implant mucositis, appears in the study sample taken into consideration only in 10 out of 30 patients; consequently, significance on such a small number is difficult to obtain. In the analysis of the condition of the marginal mucosa, comparing the data found in tables no. 19 and 20, no notable differences were highlighted as previously described.

The strong evidence that Ozoral PRO ® and Ozoral GEL ® are effective in improving inflammatory indices should be further investigated in order to investigate the actual relevance of the product alone [15].

Therefore, in the complete management of the implant patient it is essential to understand his state of health and restore a restitutio ad integrum in case of mucositis so that it does not lead to peri-implantitis, because many times mucositis is the antechamber of peri-implantitis, it is It is essential to manage the microbiota in the best possible way because unlike the periodontal patient it has fewer species of the orange and red complex but they are quantitatively greater, therefore a proactive long-term approach allows us to guarantee a predictable result over time and reduce discomfort for the patient, to date we have evaluated the ozonated substances after two years but in future objectives it will also be necessary to evaluate photodynamic therapy, the use of all natural substances which are becoming increasingly popular, in fact in the new classifications of peri-implant diseases they are included among those not recommended compared to gold standard, however, when evaluating all randomized clinical trials, they have statistical significance in the reduction of clinical indices such as those we obtained in this work [Herrera D et al., 2023].

In the current study, the home protocol was effective in achieving better clinical outcomes for peri-implant sites treated with ozonated gel compared to CHX after 24 months of treatment. The protocol was different from others in previous studies, as the products had to be used for the first 14 days after the study time. The rationale behind this choice is that prolonged use of chlorhexidine should be avoided due to its shortcomings [Poppolo Deus and Ouanounou, 2022]. It should be noted that patient compliance with hygiene maneuvers should be assessed and that the undeniable effect of non-surgical mechanical debridement should be taken into account [Renvert et al., 2019]. However, the two products were used for the

same period of time, so a direct comparison can be made. Virtually, taking into account that patients were instructed on the correct home maneuversand that hygienic control was maintained, ozone seems to have a beneficial effect over a long period of time, probably because the continuous stimuli exerted during brushing and the oral ecosystem can be attributed to prolonged inflammatory states and ozone promotes tissue healing; CHX, on the other hand, although exerting antimicrobial activity, may be less useful if hygienic control is maintained. However, to confirm these hypotheses, the evaluation of pro-inflammatory mediators in the gingival crevicular fluid should be added [Sánchez-Fernández et al., 2021]. This hypothesis is confirmed by PI and BoP that improved significantly from T0 to T9, while for the other time points no significant differences were found, although a continuous reduction was found in these parameters throughout the study. The healing properties of ozone can be also explained by the MMC index, which was found significantly lower in the intergroup comparison T4 compared to CHX. Considering the periodontal setting studies as the closest context to make comparisons, the systematic review with meta-analysis by Bertl and [Bertl et al., 2015] found that a beneficial effect could be attributed to ozone for specific variables, such as BoP and PD. In the present study, significant intergroup differences were found at T5 for PD and BS, therefore the results can be confirmed; however, the heterogeneity of the studies lowers the quality of the evidence. The same problem can be found for a recent systematic review with meta-analysis on the clinical evaluation of ozone in periodontal surgery for gingival recessions [Kalimeri et al., 2024]; the studies included were only three, therefore the evidence is still low. The present report has some limitations. The evaluation of home treatments is not free from bias, since adherence to the protocol cannot be properly verified. Ozone in the form of gel may have a lower adherence to mucosal tissues in the long term, therefore the administration may be incomplete or must be repeated over time. Further studies should include the evaluation of ozone in the form of other forms of administration, such as film-type material [Lee et al., 2024], with the aim of improving the adhesion of the molecule on soft tissues. Furthermore, comparison with other adjuvant products/devices such as hyaluronic acid [Pilloni et al., 2021], air polishing powders [Nicola et al., 2024] and antiseptics [Shreenidhi et al., 2024], or combined administration, should be proposed.

VII – CONCLUSIONS

VII-CONCLUSIONS

Based on the results obtained, the use of ozone-based gels Ozoral PRO ® and Ozoral GEL ® can be a valid aid for the long-term professional and home treatment of peri-implant mucositis, also having an advantageous role in maintaining the homeostasis of the oral microbiome.

- The use of gel based on chlorhexidine digluconate 1% showed slight improvements in the data but only in the first months of its application. However, it must be taken into consideration that chlorhexidine cannot be used for long periods to avoid side effects such as dysgeusia, oral burning, bacterial resistance and inflammation of the mucosa.
- The effectiveness of these new products in the form of ozonated gels in the resolution of peri-implant diseases is still little demonstrated in the literature to date, so their role should be further investigated. Evaluating the limits of the study, being a split-mouth with a very long follow up we found a statistically significant variation for the respective BS, GBI and MMC indices in the trial group compared to the control group.
- These indices are very relevant as they quantitatively measure the degree of inflammation present through bleeding and the margin of the mucosa around the implant itself, therefore looking at a proactive perspective, since for the moment the ozonized substances have been used with the same timing as chlorhexidine, having no contraindications, they can be used in causal therapy to reduce the concentration of inflammation in the long term compared to chlorhexidine, and can also be used on the intact implant not only in the form of gel, but also as a mouthwash and/or toothpaste to avoid and reduce the incidence of mucositis, the same procedure with chlorhexidine would not be applicable. Therefore, once the effectiveness of ozone on mucositis has been demonstrated, new protocols will be developed on the management of intact dental implants.

VIII – LIMITATIONS AND FUTURE WORK

VII-LIMITATIONS AND FUTURE WORK

The limitations of this study, being operator dependent, may vary based on the operator's ability to find data and reevaluate them over time. Long-term patient compliance, in this case two years, could fluctuate with an abandonment rate of 0. however, the clinical indices in the interdental spaces and lingual areas increased.

Being a split-mouth study, by not dosing the product well, some small portions of the gel itself could contaminate the opposite surface.

Therefore, as future objectives we have prepared ourselves to increase the sample and proceed with a further study, again on the management of peri-implant mucositis but in two distinct groups and not on pathological sites at the level of the same mouth in order to see if the data in the long term they are superimposable

In the meantime, our research group has carried out parallel studies on other natural substances and we have set ourselves the goal of continuing along this line so as to be a point of reference among our colleagues.

The future objective of the study is to propose the evaluation, based on the new 2023 classification and the guidelines of the European Federation of Periodontology, through radiographic examination

IX – BIBLIOGRAPHICAL REFERENCES

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X – APPENDIXES

APPENDIX 1. UCAM Ethics Committee Approval

UCAM
UNIVERSIDAD CATÓLICA SAN ANTONIO

UCAM ETHICS COMMITTEE

Suggestions for the researcher

In view of the application of the attached report by the Researcher and the above mentioned recommendations, the opinion of the Committee is to:

Issue a favorable report	X
Issue an unfavorable report	
Issue a favorable report with subject to correction	
MOTIVATION	
It will increase knowledge in this area	

Approved by the President,

Sig.: José Alberto Cánovas Sánchez

UNIVERSIDAD CATÓLICA SAN ANTONIO UCAM COMITÉ DE ÉTICA MURCIA

Approved by the Secretary,

Sig.: José Alarcón Teruel

APPENDIX 2. UNIPV Ethics Commitee Approval

Università degli Studi di Pavia
Dipartimento di Scienze Clinico Chirurgiche, Diagnostiche e Pediatriche
Sezione di Odontoiatria

UDA di Ortognatodonzia e Odontoiatria Infantile

COMITATO DI REVISIONE INTERNA (INTERNAL REVIEW BOARD)
Giudizio analitico preliminare per la valutazione di studi sperimentali non invasivi

Pavia, 1 dicembre 2021

^^^ Oggetto: Proposta numero: **2021-1201**

Titolo dello studio	RANDOMIZED CLINICAL STUDY IN SPLIT MOUTH: USE OF OZORAL PRO® AND GEL® COMPARED WITH 1% CHLOREXIDINE GEL IN SITES AFFECTED BY PERI-IMPLANT MUCOSITIS.
Referente interno	Andrea Scribante, DDS PhD MHA MSc
Investigatore principale	Andrea Butera, RDH
Durata stimata	24 mesi
Materiali utilizzati	Ozoral PRO®, at a professional level, Ozoral GEL®, at home, 1% Chlorhexidine Gel.
Intervento in vitro	Non Presente
Tipologia	-
Variabili	-
Intervento clinico	Presente
Tipologia	Studio osservazionale su due differenti trattamenti della mucosite perimplatare
Variabili	• BOP (bleeding on probing) ang GBI (gingival bleeding index) • Conditions of the marginal mucosa (swelling and erythema) • Suppuration • Migration of the marginal mucosa • PPD (probing pocket depth) • PI (plaque index) • BS (bleeding score)
Randomizzazione partecipanti	Si mediante split mouth
Dichiarazione di Helsinki	Rispettata
Consenso informato	Si
Presenza di procedure invasive	No
Presenza di interventi non codificati	No
Software Analisi Dati	R (R version 3.1.3, R Development Core Team, R Foundation for Statistical Computing, Wien, Austria)
Calcolo Numerosità campionaria	Si
Statistica descrittiva	Media, deviazione standard, minimo, mediana e massimo
Statistica inferenziale	Kolmogorov-Smirnov e analisi di varianza
Significatività	$P<0.05$

Parere Eseguibile