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# Ozone Therapy in Periodontitis

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**ABSTRACT:** Periodontitis is a chronic inflammatory condition affecting the supporting structures of teeth triggered by specific microorganisms, leading to progressive destruction of the periodontal ligament and alveolar bone, resulting in periodontal pocket formation, gingival recession or both. The effects of ozone therapy in any form (gaseous, aqueous or oil), in the treatment of dental diseases like caries, periodontitis, and hypersensitivity, have been explored and studied with promising outstanding outcomes. The usage of ozone as an adjunctive therapy characterizes a unique method in the management of chronic periodontal disease. It is well established in the management of gingival and periodontal diseases because of the antimicrobial and immunostimulating effects of ozone therapy. This review article outlines the clinical applications of ozone therapy in periodontal diseases.

## I. INTRODUCTION

Periodontitis is an inflammatory condition that affects the supporting tissues of teeth which is caused by particular microorganisms leading to progressive destruction of periodontal ligament and alveolar bone resulting in pocket formation, recession, or both. [1] Bacteria are the primary etiological agents causing periodontal disease. The oral biofilm formation and development, are correlated with the foremost common oral pathologies, like dental caries, periodontitis and peri-implantitis.[2] The conventional non-surgical periodontal therapy is considered as the gold standard therapy for periodontitis. Use of disinfectants or various antibiotics as an adjunct to SRP have been considered as the traditional methods for periodontal therapy.[3][4] Ozone therapy is one among the modern non-medication methods of treatment. Ozone is an unstable gas which rapidly releases nascent oxygen to produce oxygen gas. Ozone has been employed in human medicine to kill microorganisms, inactivate viruses and control hemorrhages over a long time[5]. Ozone is made by actinic ray (ultraviolet rays) and electrical discharges within the Earth's atmosphere from dioxygen.It is a compound made up of three oxygen atoms (O3-triatomic oxygen), with a higher source of energy than normal atmospheric oxygen (O2). The molecular weight of ozone is 41.98 g/mol and is a strong oxidizer. Medical grade ozone is a combination of pure oxygen and pure ozone in the ratio of 95-99.5% of $O_2$ and 0.05-5% of $O_3$. It must be prepared immediately before use due to the instability of ozone and thereby it cannot be stored for longer duration. [6] Within less than an hour after the preparation half of the mixture gets transformed to oxygen and only the remaining half is still ozone. Ozone effectively kills bacteria, fungi, viruses and parasites at a lower concentration and does not induce microbial resistance characteristics.[7]

Extensive research has been administered for the utilization of ozone for infection control and wound management and ozone treatment is gaining popularity in dentistry. The use of ozone has been proposed in dentistry due to its unique properties like its disinfectant, antimicrobial and healing properties. [8]

Ozone application for periodontal therapy is usually done in gaseous, and oil forms. When gaseous ozone was compared to ozonized water, the latter was safe.

Ozonated water inhibited the accumulation of experimental dental plaque in vitro. Ozonated water had strong bactericidal activity against bacteria in plaque biofilm. It was found that ozonated water (0.5–4 mg/L) was highly effective in killing of both gram-positive and gram-negative microorganisms. Gram-negative bacteria, such as Porphyromonasgingivalis, Porphyromonasendodontalis were more sensitive to ozonated water than gram-positive oral streptococci and Candida albicans in pure culture.[9]

The use of ozone around implants is supported by published research showing that ozone not only effectively sterilizes the surfaces of both the implant and bone, but also initiates the

reparative mechanisms allowing tissue regeneration around implant surface.[10,11]

## HISTORY

The word ozone originates from the Greek word "ozein", meaning odor and was first used by German chemist Christian Friedrich Schonbein, father of ozone therapy in 1840. In 1881, ozone was used in the treatment of diphtheria as a disinfectant. Food and Drug Act, revised the use of ozone and its effect in the medical field in the early 20th century. In 1950, Dr. Edward.A.Fisch, a German dentist was the first to use ozone to treat a gangrenous pulpite in the form of ozonated water. In 1958, Joachim Hansler, a German physicist and physician along with Hans Wolff, a German physician, developed the first ozone generator for medical purpose.The invention of a reliable ozoniser for medical use by the physicist Joachim Hansler (1908-1981) was a big step forward in the field of medicine and it is their design that is still used as the basis for modern equipment.

## MECHANISM OF ACTION

Ozone therapy has a broad spectrum of applications in the treatment of various diseases. The unique properties of ozone include antimicrobial, immunostimulant, analgesic, antihypnotic, detoxicating, bioenergetic and biosynthetic actions.

### A. Anti-microbial action:-

The antimicrobial effect of ozone acts by damaging the cytoplasmic membrane of cells. This occurs by ozonolysis of dual bonds and modification of intracellular contents because of secondary oxidant effect leading to oxidation of protein and loss of organelle function. Ozone is very efficient against antibiotic resistant strains. The acidic pH environment enhances the antimicrobial property. The application of ozone for a few seconds results in the stoppage of all vital functions of bacteria. Gram-positive bacteria are more sensitive than Gram-negative bacteria to the action of ozone.

### B. Immunostimulating Effect:-

Ozone has the ability to influence cellular and humoral immune system thereby it stimulates the synthesis of immunoglobulins and proliferation of immunocompetent cells. It also increases sensitivity of micro-organisms to phagocytosis and activates the function of macrophages.[12] Ozone is responsible for the synthesis of biologically active substances like interleukins, leukotrienes and prostaglandins which are favourable in reducing inflammation and wound healing and finally leads to improve wound healing. Ozone in its low concentration causes immunostimulating effect and in its high concentration causes immunosuppressive effect. [13]

### C. Anti-hypoxic action:-

Ozone helps in improving the oxygen transport in blood, leading to a change in cellular metabolism, activation of aerobic processes like Krebs cycle, glycolysis, β-oxidation of fatty acids, etc and causes increased pO2 in tissues and the use of energetic resources.

Continuous low dosage of ozone stimulate enzymes like super-oxide dismutases, catalases, dehydrogenase, and glutatione peroxidases. It prevents the formation of erythrocyte aggregation and increases the contact surface for the transport of oxygen. The flexibility of erythrocytes is restored as well as the surface structures of the erythrocytes are changed. This enables blood flow in capillary vessels and leads to reduction of blood cell rolling. Moreover ozone facilitates the metabolism of inflamed tissues by increasing their oxygenation and reducing local inflammatory processes.

### D. Bioenergetic and biosynthetic effect:-

Ozone activates protein synthesis, increases the number of ribosomes and mitochondria in cells. (12)As a result of these changes on the cellular level, the functional activity of the cells and the regeneration potential of tissues and organs are elevated

### E. Detoxicating action:-

Ozone results in the secretion of vasodilators such as NO, which are responsible for the dilatation of arterioles and venules, and releasing growth factors like Vascular Endothelial Growth Factor (VEGF) that causes the activation of angiogenesis.

### F. Remineralization action:-

Ozone intensifies the remineralization potential when acting on the organic substance of mineralized tooth structures. It enables the diffusion of calcium and phosphorus ions to the deeper layers of carious cavities by the opening of the dentinal tubules.

### Effect on bacteria, virus, fungus, protozoa

Ozone oxidises the lipid and lipoprotein elements of bacterial cell membranes. [14],[15] Ozone causes spores to be defective in germination, likely due to damage to the spore's inner membrane. [16] While all viruses are susceptible to ozone, their susceptibility varies

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greatly. [17] Ozone is especially harmful to lipid-enveloped viruses.[18],[19] Viral component analysis showed damage to polypeptide chains and envelope proteins that impair viral attachment capabilities, and viral RNA breakdown. At certain stages the ozone inhibits cell growth. [6]

**Effect on blood cells**

Ozone reduces or eliminates red blood cell clumping, restoring red blood cell durability and oxygen-carrying capacity. (20) Ozone promotes the production of glutathione peroxidase, catalase, and superoxide dismutase, which function as free radical scavengers. [21]

**Effect on leukocytes**

TNF-α, interleukin-2, interleukin-6, interleukin-8, and transforming growth factor [TGF-]) are all induced by ozone, which is a weak cytokine. [22],[23],[24],[25] When ozone interacts with unsaturated fatty acids in the lipid layer of cellular membranes, hydrogen peroxide is formed, which is one of the most strong cytokine inducers. [26]

**Effect on Platelets**

Blood ozonation activates phospholipase C, phospholipase A2, cyclo-oxygenases and lipo-oxygenases, and thromboxane synthetase, allowing prostaglandin E2 and prostaglandin F2a to be published. With irreversible platelet aggregation, this raises intracellular Ca2 and thromboxane A2. [27],[28],[29]

**OZONE GENERATORS**

In 1857, Werner Von Siemens developed the first ozone generator in Germany. German physicists named Joachim Hansler and Hans Wolff invented the first ozone generator for medical use. Due to a short half-life, ozone cannot be stored, and must be generated on site and on demand. Hence the most critical part of any effective ozone system is the ozone generator. Therapeutic grade ozone can be produced in three different systems: [30]

• **Ultraviolet System:** This system produces low ozone concentrations used in esthetics, saunas, and air purification.(Fig.1)

Fig.1:**Ultraviolet System**

• **Cold Plasma System**: A unique patented technology for the easy and safe generation of ozone inside sealed containers. External ozone supply is not needed;the cold plasma technology harnesses the air within the container to directly generate ozone**.** Ozone produced from this system is used in air and water purification.( Fig.2)


Fig.2:Cold plasma ozone generator

• **Corona Discharge System:** It is the most common system used in the medical/ dental field that produces high concentrations of ozone. It is easy to handle and has a controlled ozone production rate. Ozone is generated from electrical discharge, commonly known as a spark. A single spark from an anode to a cathode will find a few oxygen molecules in between and will produce certain amount of ozone. But, if this spark is dispersed over a larger area, there would be more interaction with oxygen molecules. That is why the dielectric barrier is used to create a true corona in an ozone generator.(Fig.3)

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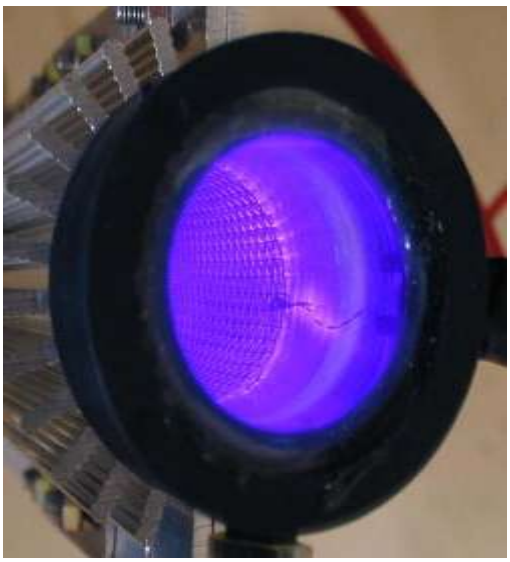

Fig.3:Corona discharge ozone generator

The electrical discharge will take place in a corona ozone generator, in an air gap within the corona cell specifically designed to split the oxygen molecule and create ozone. A dielectric is used in this air gap to disperse the electron flow uniformly through this gap to spread the electron flow to as great a volume as possible of oxygen.(Fig.4)

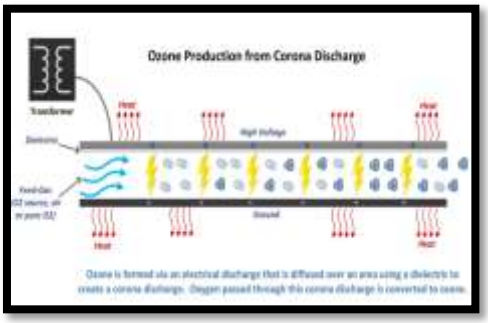


Fig.4: Ozone production from corona discharge system

**Appliances Producing Ozone For Dental Use**

Dental ozone generators which apply corona discharge system in the field of dentistry are as follows:[31]

**HEALOZONE**: It is an air-based system by which gas is applied in a closed circuit with the help of a tight cap. Perfect air-tightness of the cap is essential for the application of ozone. Therefore, the application is possible only on the surfaces where such air-tightness can be provided. The concentration of gas produced = 2100ppm adjacent to the tissue. The cost ranges from Rs. 60,000 and Rs. 65,000.

Disadvantage: Its application is limited as it can be applied to only those areas where such air-tightness is possible.

**OZONYTRON :**

This system consisting of a glass probe uses the power of high frequency and voltage. Inside the glass probe, which is formed by a double glass camera, is a mixture of noble gases that conducts and emits electromagnetic energy around the treated area splitting the environmental diatomic oxygen into single oxygen atom and ozone. Energy is emitted around the treated area as the tip of the probe gets in contact with the body that causes the splitting of environmental diatomic oxygen in singular atomic oxygen and ozone. The concentration of ozone gas in the operation field of =10-100µg/ml. Therefore, ozone may be applied to hard-to-reach and inaccessible areas such as gingival pockets or root canals, as there is no closed circuit here. The cost ranges from Rs. 13,000 and Rs. 15,000.

Advantage-There is no airtight cap in this device to allow gas to be applied to gingival / periodontal pockets or root canals

**PROZONE :**

A system producing preset tissue-compatible dosages of ozone gas in the indicated areas of periodontitis with the help of exchangeable plastic attachments (Perio tips). Because of its exchangeable plastic attachments, Prozone guarantees a hygienic procedure when gassing the pockets. As the plastic attachments (Perio tips or Endo tips) are exchangeable, a hygienic procedure is maintained during the gassing of the pockets. The cost ranges between Rs. 5000 and Rs. 10,000.
Advantage- It is user-friendly and safe in application
It is a portable and easy-to-use tabletop device with a free ozone flow system that uses corona discharge

**OZOTOP**

It is a portable and easy-to-use tabletop device with a free flow ozone delivery system that uses the application of corona discharge. Periodontal pockets and root canals could be easily penetrated. In this system, ambient air is utilized over a ceramic plate that is filtered and dried before it is passed. Later, high voltage is applied which then finally results in the formation of ozone. High volume suction is required as this is an open system. Depending on the requirement of treatment, ozone is applied at 6, 12, 18, 24 s (e.g.,

12 s – for surgical disinfection, 18 s – for periodontal disinfection, etc).

**USE OF OZONE IN DENTISTRY**

Ozone is a better alternative to standard antiseptics due to its undeniable disinfection power over other antiseptics. According to Krammer et al, aqueous ozone can be used:[5]

1. As a powerful disinfectant
2. Control bleeding
3. Increase the metabolic processes related to wound healing as ozonated water can increase the temperature in the wound area.
4. Improve healing by increasing the local supply of oxygen to the wound area.
5. Cleanse wounds in bones as well as in soft tissues

In cases of gingivitis, oral thrush, or stomatitis, ozonated water may be used as a mouth rinse, a spray to clean the affected area and disinfect the oral mucosa, or a water jet to treat painful gingivitis and stomatitis.

**GOALS OF OZONE THERAPY**

The goals of oxygen/ozone therapy are: [8]

1. Immune system activation.
2. Elimination and inactivation of pathogens.
3. Restoration of proper oxygen metabolism
4. Improves circulation.
5. Stimulation of the humoral anti-oxidant system.
6. Induction of a friendly ecologic environment.
7. Reduction of inflammation and pain
8. Prevents shock and stroke damage
9. It improves brain function and memory.

**METHODS OF OZONE ADMINISTRATION**

**1. Gaseous Ozone** – To prevent inhalation and adverse effects, gaseous ozone could be applied topically through an open system or a sealing suction system as a preconditioning system. It is most commonly used in restorative dentistry and endodontics as a noninvasive treatment. Dental caries can be treated with ozone, which can also be used as a disinfectant before direct restoration and to treat hypomineralized teeth. [19]

**2. Ozonated Water** - Ozonated water is very effective against bacteria, fungi and viruses as well as bacteria in dental biofilm. When compared to other chemical cleaners, it is less expensive. [32] Since aqueous ozone has a lower cytotoxicity than gaseous ozone, it has the best biocompatibility for oral administration [33]. When applied for 3 minutes, gaseous ozone is considered a more efficient microbicide than aqueous ozone as a dental disinfectant. Since ozone gas is harmful when inhaled into the respiratory tract[34], ozonated water can be used to fight oral infections and pathogens. Commercially available as ultra-pure, Tripple ozone treatment system.

**3. Ozonized Oil** – Along with the gaseous and aqueous form of ozone, ozonized oil is a competitive antimicrobial agent due to the wide accessibility of sunflower oil. Staphylococci, Streptococci, Enterococci, Pseudomonas, Escherichia coli, and particularly Mycobacteria have been found to be resistant to ozonized oil [35, 36, 37], and it is also used to treat fungal infections. Commercially available as Oleozone, Bioperoxoil.

**OZONE THERAPY IN PERIODONTICS**

Periodontitis is characterized by local hypoxia of tissues and also by complex polymicrobial florae. Increased accumulation of bacterial plaque in the gingival crevice causes dysbiosis in the oral cavity ecology leading to both gingivitis and periodontitis. [38]

Dental biofilms are ubiquitous and shield the colonizing species, making antibiotics difficult to target putative periodontal pathogens. Antibiotics at higher concentrations are required to destroy these pathogens, which are invariably associated with a toxic effect on the host's microbial flora. The use of ozone therapy in periodontics in various ways has shown positive results. Ozone is used as a mechanical debridement substitute in both gaseous and aqueous forms. Periodontal disease may be treated with ozonated water flushed under the gum line and/or ozone gas infiltrated into the periodontium.

The role of microorganisms and host response, in the etiology of periodontal disease, is well established. Ozone has an undisputed power of disinfection over other antiseptics makes the use of ozone in the treatment of periodontitis a very effective alternative to normal antiseptics and/or an additional disinfectant. Ozonated water (4 mg/l) has been found to be effective in eliminating gram positive and gram negative oral microorganisms and oral Candida albicans in pure culture and plaque biofilm bacteria. The use of ozone for decontamination of the implant surface in peri-implant therapy is currently under investigation in implant dentistry.

In 2002, Ebensberger et al. investigated the effect of ozonated water irrigation on cell proliferation in the periodontal ligament adhering to the root surfaces of 23 fully erupted third molars,

which were freshly removed. They concluded that irrigation of the avulsed teeth with non-isotonic ozonated water for 2 min could result not only in mechanical cleaning but also in decontamination of the root surface, with no negative effect on the surface of the tooth's periodontal cells[39].

In 2004 Nagayoshi et al. assessed the effect of ozonated water on the survival and permeability of oral microorganisms. The gram-negative bacteria, such as Porphyromonasgingivalis and Porphyromonasendodontalis, were substantially more responsive to ozonated water in pure culture than gram-positive oral streptococci and C.albicans. Although ozonated water had strong bactericidal activity against the bacterial biofilm, but it inhibited the accumulation of experimental dental plaque in vitro.[40]

In 2005, Ramzy et al. conducted a study in 22 patients suffering from aggressive periodontitis and irrigated the periodontal pockets with ozonized water. For a 4-week clinical trial, a blunt-tipped sterile plastic syringe was used to irrigate periodontal pockets with 150 ml of ozonized water over 5-10 min once a week. They reported significant improvement in pocket depth, plaque index, gingival index and reduction in bacterial count in sites treated with ozonized water.[41]

In 2007, Muller et al. correlated the effect of ozone gas with photodynamic therapy (PDT) and known antiseptic agents on a multispecies oral biofilm in vitro. They concluded that microbial communities embedded in the biofilm matrix are well protected against antimicrobial agents. Only a 5% hypochlorite solution was able to completely eliminate the bacteria.[42] Kronusova et al utilized ozone in the following cases: chronic gingivitis, periodontitis and periodontal abscesses, herpes labialis, periodontitis, etc. Almost all gingivitis patients showed a subjective and objective improvement in their condition, as well as patients with periodontal abscess, where no exudation was observed. [43]

In 2010 Kshitish and Laxman conducted a randomized, double-blind, crossover split mouth study on 16 patients with generalized chronic periodontitis. Each half of the mouth was subgingivally irrigated with either ozone or chlorhexidine at various time intervals. They concluded that the single ozone irrigation is quite effective to inactivate microorganisms.[44]

In 2011, Huth, et al. compared the efficacy of ozone against periodontal micro-with that of the known antiseptic CHX. This study indicated that high concentration of gaseous and aqueous ozone in the periodontitis therapy merit further investigation as antiseptics[45].

In 2013, Yilmaz assessed the clinical and microbiological effects of treatment with Er: YAG laser and topical application of gaseous ozone as adjuncts to initial periodontal therapy in patients with chronic periodontitis. This study shows that the antimicrobial effect of ozone is equivalent to that of the Er: YAG laser.[46]

In 2014, Shoukheba evaluated the efficacy of subgingival application of ozonated olive oil gel in severe periodontitis as an alternative to scaling and root planing.[47]

In 2014, Kaur R et al assessed the effect of chlorhexidine (CHX), povidone-iodine (PI) and ozone (OZ) on the microorganisms in dental aerosols. The study concluded that it shows similar effects of CHX, PI, and OZ in reducing aerobic and anaerobic CFUs. Ozone can be used as a pre-procedural agent.[48]

In 2015, Al Habashnehet al.[49] determined the clinical and biological effects of the adjunctive use of ozone in nonsurgical periodontal treatment. The study concluded that statistically significant improvement was observed in the study parameters in both groups at baseline and 3 months, except for GI.

A prospective randomized clinical study was conducted by Gianluca Sacco in 2017 on 113 patients with periodontal disease. Local oxygen–ozone therapy was used in combination with traditional mechanical therapy and was observed in particular that in both groups that the deepest pockets (PD > 6 mm) had the greatest decrease.[50]

In a meta-analysis conducted by R.Deepthi in 2020, out of a total of 123 studies that fit the initial inclusion criteria, 117 studies were further excluded. Only six studies were included in the meta‐analyses. It was concluded that ozone therapy can be used effectively as an adjunct to scaling and root planing in the treatment of periodontitis.[51]

**PERI-IMPLANTITIS**

The dentist and the patient are both affected by peri-implantitis. Different types of therapy are used to save the implant from complete failure after a comprehensive examination and whether a decision to save the case is rendered. With varying degrees of effectiveness, laser and/or manual debridement, as well as antiseptic solutions and topical antimicrobial medicines, are commonly used. A gaseous or aqueous source of ozone can be used to treat periimplantitis. A PVC or silicone cap of sufficient length completely covers the abutment. The gingival borders around the implant must be properly sealed. Ozone gas infiltrations can also be used in this case. Irrigation with

ozonated water may be achieved during curettage and debridement. On the treated areas, ozonized oil can be added 3–4 times a day.

Gaseous ozone (140 ppm, 33 ml/s) for 6 and 24 s was applied to saliva-coated titanium (SLA and polished) and zirconia (acid etched and polished) disks to determine the antibacterial effect on periimplantitis caused by bacteria such as streptococcus sanguinis and porphyromonasgingivalis. Gaseous ozone showed selective efficacy to reduce adherent bacteria on titanium and zirconia without affecting adhesion and proliferation of osteoblastic cells

Karapetianet al. in a study of peri-implantitis, treatment with conventional, surgical and ozone therapy methods was investigated and it was found that the most effective bacteria reduction was in the ozone-treated group.[52]

A randomized clinical study conducted by Isler et al, 2018 concluded that implant surface decontamination with the additional use of ozone therapy in surgical regenerative therapy of peri-implantitis showed clinically and radiographically significant results.[53]

**OZONE TOXICITY**

Inhaling ozone can be harmful to the organs (more sensitive to the pulmonary system). Because of the possibility of pulmonary embolism, the European Society of Ozone Therapy has banned intravenous injections of ozone gas since 1983.Ozone therapy complications are extremely rare at 0.0007 per application. Epiphora, upper respiratory pain, rhinitis, cough, headache, nausea, vomiting, shortness of breath, blood vessel swelling, impaired breathing, heart attacks, and even stroke are some of the reported side effects. [54] Because of ozone's high oxidative strength, all materials that come into contact with it, such as glass, silicon, and Teflon, must be ozone resistant.

The patient must be seated in supine position. The patient should be provided with Vitamin E, ascorbic acid, and n-acetylcysteine when breathing humid oxygen as a treatment for ozone intoxication.

**CONTRAINDICATIONS**

The following are contraindications for the use of ozone therapy: [**30**]

- Pregnancy
- Glucose-6-phosphate-dehydrogenase deficiency (favism)
- Hyperthyroidism
- Severe anemia
- Severe myasthenia
- Active hemorrhage
- Acute alcohol intoxication
- Recent Myocardial infarction

## II. CONCLUSION

Ozone is used in most of the dental fields. The benefit of ozone therapy is that it is a non-invasive treatment process. When inhaled and delivered intravenously, it is poisonous. It is commonly used in dentistry in three forms: gaseous, aqueous, and liquid. Ozone's biocompatibility and efficacy in eliminating microorganisms from dental unit waterlines, the oral cavity, and dentures has been demonstrated. In pit and fissure caries, it can be used as a preventive agent, and in primary root caries, it can be used as a therapeutic agent. Often used as an endodontic irrigating agent and as a periodontal surgical and maintenance adjuvant. When using ozone, we must use an accurate ozone generator and collect a precise gas volume with a defined ozone concentration to prevent toxicity. The future of ozone therapy must rely on the establishment of safe and well-defined criteria, as determined by randomised controlled trials, in order to determine specific indications and recommendations for treating different dental pathologies with this promising medical agents.

## REFERENCES

[1]. Saini R, Saini S, Sharma S. Periodontal disease linked to cardiovascular disease. J Cardiovasc Dis Res 2010;1:161-2.
[2]. Bortolaia C, Sbordone L. Biofilms of the oral cavity. Formation, development and involvement in the onset of diseases related to bacterial plaque increase. Minerva Stomatol 2002;51:187-92.
[3]. Walker CB. The acquisition of antibiotic resistance in the periodontal microflora. Periodontol 2000 1996;10:79-88.
[4]. Feres M, Haffajee AD, Allard K, Som S, Goodson JM, Socransky SS. Antibiotic resistance of subgingival species during and after antibiotic therapy. J ClinPeriodontol 2002;29:724-35.
[5]. Krammer F. Ozone in the dental practice. Medical applications of ozone. Norwalk, CT: International Ozone Association, Pan American Committee; 1983. p. 258-65.
[6]. Bocci V. Autohaemotherapy after treatment of blood with ozone. A reappraisal. Journal of international medical research. 1994 May;22(3):131-44.
[7]. Garg R, Tandon S. Ozone: A new face of dentistry. Int J Dent Sci. 2009;7(2).

[8]. Mollica P, Harris R. Integrating oxygen/ ozone therapy into your practice. [Online]. [Cited 2010 January 13].
[9]. Nagayoshi M, Fukuizumi T, Kitamura C, Yano J, Terashita M, Nishihara T. Efficacy of ozone on survival and permeability of oral microorganisms. Oral MicrobiolImmunol 2004;19:240-6.
[10]. Low SP, Williams KA, Canham LT, Voelcker NH. Generation of reactive oxygen species from porous silicon microparticles in cell culture medium. J Biomed Mater Res A 2010;93:1124-31.
[11]. Low SP, Williams KA, Canham LT, Voelcker NH. Evaluation of mammalian cell adhesion on surface-modified porous silicon. Biomaterials 2006;27:4538-46
[12]. Seidler V, Linetskiy I, Hubálková H, Stanková H, Smucler R, Mazánek J. Ozone and its usage in general medicine and dentistry. A review article. Prague Med Rep. 2008 Jan 1;109(1):5-13.
[13]. Teresa B, Wolanska E, Cieszko-Buk M, Orlowski M, Chalas R. Practical use of ozone in dentistry-comments. Ann Universitalis Maria Curie-SklodowskaLubin-Polonia. 2008;63(28).
[14]. Sawadaishi K, Miura K, Ohtsuka E, Ueda T, Ishizaki K, Shinriki N. Ozonolysis of supercoiled pBR322 DNA resulting in strand scission to open circular DNA. Nucleic Acids Res 1985;13:7183-94.
[15]. Mudd JB, Leavitt R, Ongun A, McManus TT. Reaction of ozone with amino acids and proteins. Atmos Environ 1969;3:669-82.
[16]. Young SB, Setlow P. Mechanisms of killing of Bacillus subtilis spores by Decon and Oxone, two general decontaminants for biological agents. J ApplMicrobiol 2004;96:289-301.
[17]. Roy D, Wong PK, Engelbrecht RS, Chian ES. Mechanism of enteroviral inactivation by ozone. Appl Environ Microbiol 1981;41:718-23.
[18]. Burleson GR, Murray TM, Pollard M. Inactivation of viruses and bacteria by ozone, with and without sonication. ApplMicrobiol 1975;29:340-4.
[19]. Katzenelson E, Koerner G, Biedermann N, Peleg M, Shuval HI. Measurement of the inactivation kinetics of poliovirus by ozone in a fast-flow mixer. Appl Environ Microbiol 1979;37:715-8.
[20]. Mudd JB, Dawson PJ, Santrock J. Ozone does not react with human erythrocyte membrane lipids. Arch BiochemBiophys 1997;341:251-8
[21]. Bocci V. The case for oxygen-ozone therapy. Br J Biomed Sci 2007;64:44-9.
[22]. Arsalane K, Gosset P, Vanhee D, Voisin C, Hamid Q, Tonnel AB, et al. Ozone stimulates synthesis of inflammatory cytokines by alveolar macrophages in vitro. Am J Respir Cell MolBiol 1995;13:60-8.
[23]. Van Hoof HJ, Zijlstra FJ, Voss HP, Garrelds IM, Dormans JA, Van Bree L, et al. The effect of ozone exposure on the release of eicosanoids in guinea-pig BAL fluid in relation to cellular damage and inflammation. Mediators Inflamm 1997;6:355-61.
[24]. Bayram H, Sapsford RJ, Abdelaziz MM, Khair OA. Effect of ozone and nitrogen dioxide on the release of proinflammatory mediators from bronchial epithelial cells of nonatopicnonasthmatic subjects and atopic asthmatic patients in vitro. J Allergy ClinImmunol 2001;107:287-94.
[25]. Cho HY, Zhang LY, Kleeberger SR. Ozone-induced lung inflammation and hyperreactivity are mediated via tumor necrosis factor-alpha receptors. Am J Physiol Lung Cell MolPhysiol 2001;280:L537-46
[26]. Reth M. Hydrogen peroxide as second messenger in lymphocyte activation. Nat Immunol 2002;3:1129-34
[27]. Maresca M, Colao C, Leoncini G. Generation of hydrogen peroxide in resting and activated platelets. Cell BiochemFunct 1992;10:79-85.
[28]. Iuliano L, Colavita AR, Leo R, Praticò D, Violi F. Oxygen free radicals and platelet activation. Free RadicBiol Med 1997;22:999-1006.
[29]. Valacchi G, Bocci V. Studies on the biological effects of ozone: 10. Release of factors from ozonated human platelets. Mediators Inflamm 1999;8:205-9.
[30]. Nogales CG, Ferrari PH, Kantorovich EO, Lego-Marques J. Ozone therapy in medicine and dentistry. J. Contemp Dental Pract. 2008, 9:1-9
[31]. Gupta G, Mansi B. Ozone therapy in periodontics. Journal of medicine and life. 2012 Feb 22;5(1):59.
[32]. Nagayoshi M, Kitamura C, Fukuizumi T. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. J Endod. 2004;30:778–781.

[33]. Azarpazhooh A, Limeback H. The application of ozone in dentistry: a systematic review of the literature. J Dentist. 2008;36:104–116.
[34]. Huth KC, Jakob FM, Saugel B, Cappello C, Paschos E, Hollweck R, et al. Effect of ozone on oral cells compared with established antimicrobials. Eur J Oral Sci 2006;114:435-40.
[35]. Sechi LA, Lezcano I, Nunez N, Espim M, Duprè I, Pinna A, et al. Antibacterial activity of ozonized sunflower oil (Oleozon). J ApplMicrobiol 2001;90:279-84.
[36]. Rodrigues KL, Cardoso CC, Caputo LR, Carvalho JC, Fiorini JE, Schneedorf JM. Cicatrizing and antimicrobial properties of an ozonized oil from sunflower seeds. Inflammopharmacology. 2004; 12:261-270.
[37]. Antimicrobial properties of an ozonized oil from sunflower seeds. Inflammopharmacology. 2004;12:261 -270.
[38]. Bocci VA. Tropospheric ozone toxicity vs. usefulness of ozone therapy. Arch Med Res 2007;38:265-7
[39]. Ebensberger U, Pohl Y, Filippi A. PCNA-expression of cementoblasts and fibroblasts on the root surface after extraoral rinsing for decontamination. Dental Traumatology. 2002;18:262.
[40]. Nagayoshi M, Fukuizumi T, Kitamura C. Efficacy of ozone on survival and permeability of oral microorganisms. Oral Microbiology & Immunology. 2004;19:240.
[41]. Ramzy MI, Gomaa HE, Mostafa MI. Management of Aggressive Periodontitis Using Ozonized Water. Egypt. Med. J. N R C. 2005;6(1):229–245
[42]. Muller P, Guggenheim B, Schmidlin PR. Efficacy of gasiform ozone and photodynamic therapy on a multispecies oral biofilm in vitro. Eur. J. Oral. Sci. 2007;115:77–80.
[43]. Kronusova M. Aplikaceatomarnihokysliku v ordinacipraktickehozubniholekaře. Progresd ent . 2007;3:34–36.
[44]. Kshitish D, Laxman VK. The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: A clinical and microbiologic study. Indian J Dent Res . 2010;21:341–348.
[45]. Huth ,Saugel , Jakob Effect of aqueous ozone on the NF-kappaB system. J Dent Res. 2007
[46]. Yılmaz S, Algan S, Gursoy H, Noyan U, Kuru BE, Kadir T. Evaluation of the clinical and antimicrobial effects of the Er: YAG laser or topical gaseous ozone as adjuncts to initial periodontal therapy. Photomedicine and laser surgery. 2013 Jun 1;31(6):293-8.
[47]. Shoukheba MY, Ali SA. The effects of subgingival application of ozonated olive oil gel in patient with localized aggressive periodontitis. A clinical and bacteriological study. Tanta Dental Journal. 2014 Apr 1;11(1):63-73.
[48]. Kaur R, Singh I, Vandana KL, Desai R. Effect of chlorhexidine, povidone iodine, and ozone on microorganisms in dental aerosols: Randomized double-blind clinical trial. Indian Journal of Dental Research. 2014 Mar 1;25(2):160.
[49]. Al Habashneh R, Alsalman W, Khader Y. Ozone as an adjunct to conventional nonsurgical therapy in chronic periodontitis: a randomized controlled clinical trial. J Periodontal Res2015;50:37‐ 43.
[50]. Sacco G, Campus G. The treatment of periodontal disease using local oxygen‐ ozone. Ozone Ther 2017;1:45‐ 52
[51]. Deepthi R, Bilichodmath S. Ozone therapy in periodontics: A meta-analysis. Contemporary Clinical Dentistry. 2020 Apr;11(2):108.
[52]. Karapetian VE, Neugebauer J, Clausnitzer CE, Zoller JE. Comparison of different periimplantitis treatment methods. In Poster presentation 19th Annual Meeting Academy of Osseointegration March 2004 (pp. 18-20).
[53]. Isler SC, Unsal B, Soysal F, Ozcan G, Peker E, Karaca IR. The effects of ozone therapy as an adjunct to the surgical treatment of peri-implantitis. Journal of periodontal & implant science. 2018 Jun;48(3):136.
[54]. Matsumura K, Hyon SH, Nakajima N. Surface modification of polyethylene-co-vinyl alcohol hydroxyapatite immobilization and control of periodontal ligament cells differentiation. Biomaterials. 2004;25:4817–4824.