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Antimicrobial Effect of Ozone and Its Application in Dentistry

A Project Submitted to
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Certification of the Supervisor

I certify that this project entitled "**Antimicrobial effect of Ozone and its application in dentistry**" was prepared by **Duaa Mohammed Ali khazal** under my Supervision at the College of Dentistry/University of Baghdad in partial fulfillment of the graduation requirements for the Bachelor Degree in Dentistry.

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Dedication

Nothing is more precious than seeing your loved ones proud of you, pushes you up and encourages you to continue dreaming and succeeding,

I would like to proudly dedicate my humble effort to my supportive family (my beloved and perfect sister kawther, brother hassoni, mom and dad) without their love and prayers I would never had been here.

To my best friends (reemy and linoo) for their continuous inspiration and support throughout my study career.

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DUAA MOH. ALI

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Table of Contents

List of Figures

List of Tables

List of Abbreviations and Symbols

CHX	Chlorhexidine
O3	Ozone gas
PPM	part per million
SRP	Scaling and root planning.
UV	Ultra-violent.
UV-B	Ultraviolet type B
UV-C	Ultraviolet type c
^{o}C	Degree Centigrade.
%	Percentage

Introduction

Ozone (triatomic oxygen or trioxygen) is the combination of three naturally occurring oxygen atoms. Ozone is present in a gas form in the concentration of 1–10 ppm in the stratosphere in nature. Molecular weight is 47.98 g/mol, and it is highly endothermic and also thermodynamically unstable as an oxygen compound (**Decro and koman, 2018).** This gas is mandatory for the survival of life on the planet because the detrimental ultraviolet radiation C and B are absorbed by ozone, thus in the absence of ozone layer, the sun is full of UV-C and UV-B rays which is intensively destructive to human DNA, furthermore, they are mutagenic and culpable for accelerating the aging of the skin and carcinogenesis. This has been demonstrated by a gradual increase in melanomas and carcinoma in the last few years and may steadily destroy the planet's life **(Bocci, 2010).**

In the past, the studies has focused on infection and treating wounds by oxygen molecules in various forms like ozone therapy and hyperbaric oxygen (HBO). HBO treatment employ O2 molecule (two-atoms) whilst the ozone therapy use O3 molecule (three-atoms). The high oxidation potential of ozone can oxidize bacteria, fungi, or viruses but it has no harmful effects on healthy human cells. In high doses, gaseous ozone, like many medicinal drugs, could be poisonous 50 part per million (PPM) for 60 minutes **(Eden, 2016).**

Medical ozone production was regulated, depending on the dosage, concentration and the frequency of administration according to the specific condition, in addition to the adoption of suitable disposable materials. Considering this substance's significant oxidizing properties the therapeutic use of ozone may appear conflicting. however, this can be demonstrated by recognize the principle of (hormesis) that include the benign effect when exposed to an agent in low doses which is detrimental effect in high doses **(Gallo and Scribante,2021).**

Ozone is an effective anti-microbial agent capable of killing bacteria, fungi, protozoa, and viruses as well. Some microorganisms survive at higher concentrations of ozone, but ozone up to 1 μg ml−1 can eliminate E. coli, Pseudomonas aeruginosa, and Serratia marcescens, as well as Candida albicans. Staphylococcae are killed with 3–5 μg ml−1. Ozone concentrations over 5 μg ml−1 will eradicate most microorganisms in a few seconds (**Limeback and Azarpazhooh, 2012).**

In dentistry, the use of ozone has been a promising type of treatment since the last few decades, as Dr. E.A. Fisch (1899–1966) was the first dentist to use ozonated water in his practice and introduced it to the German surgeon Dr. Erwin Payr (1871–1946) who used it from that time forward in surgery and reported his results at the59th Congress of the German Surgical Society in Berlin **(Bocci, 2010).**

Aims of the Study

The aims of this review are to:

1. Study the effect of ozone and its applications in different fields of dentistry.
2. Summarize ozone roles in each field (specifically, as antimicrobial agent).

Chapter One: Review of Literature

1.1 Historical Background

Ozone was first discovered and named by CF Schonbein in 1840, and its use in industrial environments has an impressive history. Native Americans, for whom fishing was a central industry, recognised a correlation between a successful catch of fish and a strong odour released by the action of lightning following an electric storm. Similarly, the ancient Greeks also noticed this odour, which they termed 'ozein'. These people are recorded as preferring to fish subsequent to electric storms, a custom which prevails today. Since the upper layer of lake water is enriched with dioxygen these phenomena are explicable by an elevated level of ozone generation in this biosphere. It should be further noted that ozone in lake water arises as a consequence of air diffusion in its upper layer rather than as a product arising from chemical reactions therein **(Baysan and Lynch, 2005).**

Hansler in (1857) gave his first medical ozone generator, capable of producing an ozone/oxygen mixture at therapeutically variable dosages. Following research various publications were done by Aubourg and Wolff (1914) who subsequently introduced extracorporeal blood treatment into medical practice; by developing local treatment methods in the form of "subatmospheric ozone gas application," and Rokitansky, (1935) as a surgeon he presented the first comprehensive studies on the topical and systemic treatment of diabetic gangrene **(RG, 2015).**

1.2 Ozone Definition

Ozone is a gas molecule that is present in nature within plant's atmosphere giving the chemical formula (O3) by the union of three oxygen atoms. This structure shows that its stability is quite lower than (O_2), therefore, it is considered a highly reactive molecule. This means it is easier to formulate and breakdown over the reaction with other chemical compounds. This high reactivity is important in chemical interactions with Ozone-expending substances like: halogen compounds including chlorine or bromine which have high potential ability to break down ozone gas in the stratosphere layer. There are a considerable number of ozone depleting substances, including methylchloride, chlorofluorocarbons, bromide, halons and hydrochlorofluorocarbon **(Ritchie and Roser, 2018).** Ozone gas show a paradoxical property because of its ability to become a very potent and highly dangerous oxidant compound, whilst, in suitable concentrations, it can act as a real medicament **(Bocci and DiPaolo, 2009).**

1.3 Ozone Structure

The structure of ozone has 3 oxygen atoms, bound by equal oxygen-oxygen bonds at an obtuse angle of 116.8°. The steric hindrance prevents it from forming a triangular with each oxygen atom forming the expected 2 bonds Instead each oxygen forms only1bond, with the remaining negative charge being spread throughout the molecule as shown in figure 1 **(R.G et al., 2015).**

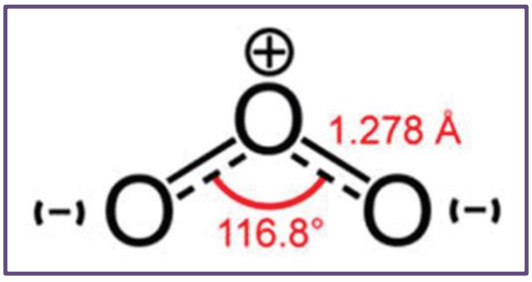

Figure 1: ozone molecule structure (R.G et al., 2015).

1.4 Ozone Properties

Ozone present as a blue gas, it has a sharp blazing odor at ambient temperature, and can be detected at concentrations also, as low as 0.02to 0.05 ppm. Its shelf life differs according to the temperature alteration. At 20 ºC it has the half-life of 40 minute and at 0 ºC around 140 minutes **(Tiwari et al., 2017).**

Ozone gas is considered heavier than the air; it moves downward whenever the gas complexes with impurities and pollutants, that is known as self-cleaning phenomenon **(Lubojanski et al., 2021).**

There are many advantages and useful properties of aqueous ozone form such as **(Nagayoshi et al., 2004):**

1. Its powerful potency
2. Easiness in handling
3. No mutagenic effect
4. Immediate antimicrobial effects
5. It is suitable for application as a disinfectant for dental and medical instruments decontamination.

1.5 Production of Ozone

Oxygen gas particles aggregates in the air under the effect of several factors such as ultraviolet radiation (sun rays) and lighting that produce electrical discharge. The Intensive natural stress on water caused in the waterfalls area and when ocean ground-swell bump up against the rocks, all may results in ozone production naturally. Specific devices known as (Ozone Generators) are hired for production of ozone for medical use from the oxygen molecules by an electrical discharge that enhanced the physical production of ozone at the lighting time **(Tiwari et al.,2017).**

The maximum dose generated, 5% ozone to 95% oxygen, is well below the explosive limit. The ozone generators generate ozone molecules by using one of the principles given below.

A. Ultra-violet light lamp

The ultraviolet method is used to break up the oxygen molecule by passing it through an ultraviolet bulb which emits light with a wavelength shorter than 220 nm, and the released oxygen atom combines with the other oxygen molecule to form the ozone gas **(Decro and koman, 2018).**

B. Corona discharge

It generates higher ozone concentrations and most popular system applied in dental and medical utility, it is simple to use and able to produce elevated concentration. These kind of generators release 3- 6% concentration of ozone, and the by-product produced in this process is nitrous oxide **(Srikanth et al., 2013).**

C. Cold plasma

This is a similar design to the corona discharge tube, but the anode and cathode are encased in glass rods filled with a noble gas. In this design, the voltage jumps between the anode and cathode rods forming an electrostatic or "plasma" field. The advantage of the cold plasma system is that no heat is imparted to the gas as it passes through the electrostatic field. It has an almost infinite lifetime due to this design **(R.G et al., 2015).**

1.6 Mode of Action

Ozone has been shown to possess a special property and has wide applications to the clinical practice of both dentistry and medicine. There are several actions of ozone, including antimicrobial action (bactericidal, viricidal, and fungicidal), immunostimulant, immune modulatory, anti- inflammatory,

biosynthetic (activation of the metabolism of carbohydrates, proteins, lipids), bioenergetic, antihypoxic, analgesic, haemostatic, etc **(Baysan and Lynch, 2005).**

1.6.1 Antimicrobial Effect:

Ozone has strong germicidal activity that has been applied broadly for refining of water. The germicidal action of O3 in water purification has been studied by many authors **(Foarde et al., 2007).** Ozone in the gaseous or aqueous phase has been shown to be a powerful and reliable antimicrobial agent against bacteria, fungi, protozoa and viruses and has got a high oxidation potential which is1.5 times greater than chloride when used as an antimicrobial agent **(Srinivasan and Chitra, 2015).** Ozone is very efficient in antibiotic resistant strains. In viral infections, ozone action lies in the intolerance of infected cells to peroxides and change of activity of reverse transcriptase, which takes part in synthesis of viral proteins. AIDS, Herpes, Hepatitis, Epstein Barr, Cytomegalovirus and other lipid envelope viruses are readily destroyed by Ozone **(Huth et al.,2009).**

The antibacterial effect of ozone occurs as a result of its oxidant action on cell by damaging its cytoplasmic membrane as a result of ozonolysis of dual bonds and also Ozone-induced modification of intracellular contents as a secondary oxidant effects. It does not damage healthy human body cells because they have free radical scavengers like superoxide dismutase, catalase, hydrolase and antioxidant nutrients like vitamin C, E, beta-carotene, selenium, methionine, glutathione which inhibit the uncontrolled activity of free radicals and thus all healthy cells are protected. Only unhealthy cells such as cancer cells which have lost this protective mechanism and organisms such as Bacteria, viruses, Fungi, Parasites which are devoid of these antioxidants and scavengers are destroyed **(Srinivasan and Chitra, 2015).**

1.6.2 Reminralization effect:

Ozone, which has come into use in dentistry, is not only a preventive application, but also a non-invasive approach for the treatment of existing initial caries. Many in vitro studies had shown the effectiveness of ozone on the pathogenic microorganisms that cause caries **(Baysan *et al.,* 2000).** Moreover, ozone has a caries-preventing effect by causing the oxidation of acidic products of carbohydrates metabolism in the saliva and caries lesions. The main philosophy in reference to ozone treatment is to change the balance of demineralization and remineralization cycles in favor of remineralization. In addition, it has been reported that remineralized tooth tissue has a more resistant structure in terms of new acid attacks **(Baysan and Beighton, 2007).**

Studies found that ozone has been used for the treatment of caries, disinfection of the cavity, reducing the levels of caries-associated microorganisms in the dental plaque, and remineralization of caries lesions with successful results. However, the clinical evidence for application of ozone is not extensive **(Almaz *et al.,* 2015).**

1.6.3 The Effect of Ozone on Microleakage:

Micro leakage is described as the movement of oral fluids between the tooth and dental restorations interface. The fluid may contain bacteria and toxic substances that could affect the tooth and the pulp **(Larson, 2005).**

It has been suggested that microleakage increases the development of carious lesions, In vitro, microleakage studies can predict the marginal sealing ability of dental sealants **(Ganesh and Shobha, 2007).** Moreover, in-vitro measurement of microleakage has been used to evaluate the effectiveness of different conditioning procedures for retention of fissure sealant **(Manhart et al, 2004).**

A recent Iraqi study on the effect of ozone on microleakage of fissure sealant concludes that the use of ozonated water pretreatment with all type of fissure sealant etching technique is preferable for minimum microleakage **(Jabbar, 2022)**.

1.7 Routs of Administration in Dentistry

1.7.1 Ozonated Water: -

Ozonated water has been shown to be a very powerful antimicrobial agent against bacteria, fungi and viruses and is also less expensive than other chemical cleaners **(Nagayoshi et al., 2004).** The ozonated water may be helpful in controlling oral lesions and various infections because gaseous ozone has been shown to have some side (toxic) effects if inhaled into the respiratory system **(Holmes, 2003).**

1.7.2 Gaseous Ozone: -

In restorative dentistry and endodontics gaseous ozone is mostly used. Topical application of the gaseous ozone can be either by an open system or a sealing suction system to prevent inhalation and adverse effects. Ozonated gas was proved to be effective more than ozonated water **(Azarpazhooh and Limeback, 2008).** Ozone appears to be a part of noninvasive therapy as a disinfectant agent before the placing of direct restoration and act as a therapy for hypo-mineralized teeth **(Nogales et al.,2008).**

1.7.3 Ozonized Oil: -

It is competitive antimicrobial agent due to wide accessibility of sunflower oil. It is found efficacious against *Streptococci, Enterococci, Staphylococci, Pseudomonas, Escherichia coli* and especially *Mycobacteria* and is used for the cure of fungal infections **(Gupta and Deepa, 2016).**

1.8 Ozone systems for dental use

There are many systems that can be applied in dental practice **(Gupta and Mansi, 2012; Gupta and Deepa, 2016), include:**

- **OzonyTron by MYMED Gmb H.** :Oxygen activator system (OzonytronX—Biozonix, München, Germany) applied the power of voltage and great rhythm. It does not contain closed circle here, thus ozone could be used in the areas that are hard to be reached, such as root canals and gingival pockets (Figure 2) **(Gopalakrishnan and Parthiban, 2012).**

Figure 2: ozonytronX (Gopalakrishnan and Parthiban, 2012).

- **Product photo (Prozone) by W & H:** this system is simple to handle and reliable to be applied to live tissue because of the compatible concentrations which can be adjusted according to the places indicted for treatment e.g. endodontics and periodontitis. the sterilization process is achieved through the gassing of the pockets by changing the plastic (tip) attachments in endo or perio treatments **(Gupta and Mansi, 2012).**
- **Customized thermoforming dental machine:** For ozone gas application thermoforming dental system could be intended as medium, soft or hard which extend 2-3 mm over the infected gingival surface, the gas circulation needs a free area to be left. Intended tissue places whereas the

appliance is difficult to be managed or annoying to the patient, silicone cap or polyvinylchloride could be used (**Gupta and Deepa, 2016).**

- **Ozotop:** It is a solid, simple ,compact, table top system that contain a free unrestricted flow from distribution device that uses corona discharge. endodontic canals and gingival pockets could be efficiently accessed (Figure 3**) (Shiraguppi et al., 2018)**

Figure 3 : Ozotop generator (Shiraguppi et al., 2018)

- **HealOzone by KaVo:** It is a device based on air in which gas is used in a locked circuit as shown in (Figure 4) (**Baysan and Lynch, 2005)**

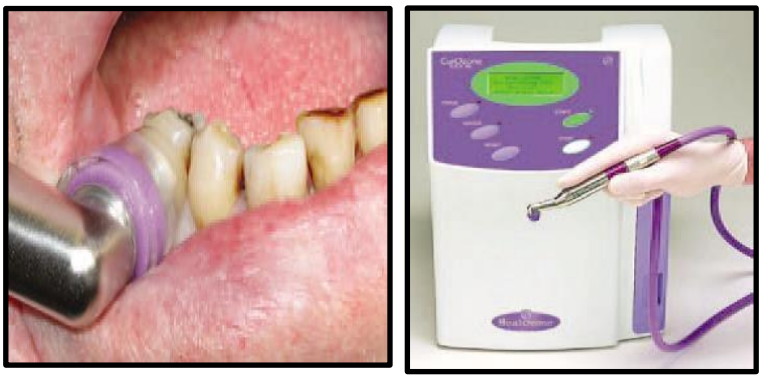

Figure 4 : Ozone delivery system and its application (Baysan and Lynch, 2005)

- **Ozonized olive oil in dental and home use:** A blunt needle with 10ml syringe or other suitable tips (Figure 5) may be used to induce ozonized

olive oil in gingival pockets. It is possible to do this process once a week **(Shoukheba *et al,* 2014)**.

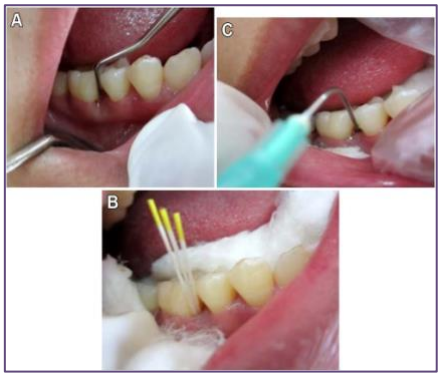

Figure 5 : Ozone oil application (Shoukheba et al, 2014)

- **Ozone water for irrigation**: As ozonated water is extremely efficient against both gram positive and gram negative bacteria,in the affected areas through and after professional scaling, non-invasive surgical pockets curettage and root planning could use ozone water for irrigation. Also the antimicrobial activity of ozonated water in dental plaque against different bacterial species is high **(Gupta and Mansi, 2012).**

1.9 Advantages/Disadvantages of Ozone in Dental Use:

1.9.1 Advantages (Deepa and Pooja, 2012):

1. Non-invasive / minimal intervention technique.

2. Induction of a friendly ecologic environment.

3. Improves metabolism of infected tissues by means of its oxidizing effect**.**

1.9.2 Disadvantages (Nogales et al., 2008):

1. Ozone toxicity.

2. Instability.

3. Not readily available.

1.10 Indications/ Contraindications of Ozone in Dental Use:

1.10.1 Indications (Baysan and Lynch, 2005):

1) Oral cavity infections (chronic or recurrent).

2) Control and prevention of dental caries.

3) Calcification of teeth.

4) Bleaching and root canal treatment.

5) Sterilization of cavities, periodontal pockets and herpetic lesions.

6) Desensitization of sensitive tooth necks.

7) Surgical sites pre-washing.

8) Control of the plaque.

9) Control of contamination.

1.10.2 Contraindications (Decro and koman, 2018):

1) Pregnancy

2) Favism (Glucose-6-phosphate-dehydrogenase deficiency).

3) Hyperthyroidism

4) Severe anemia

5) Severe myasthenia

6) Active hemorrhage

7) Acute alcohol intoxication

8) Recent Myocardial infarction.

1.11 Ozone Application in Dentistry

Dentistry is changing as has been used modern science to practice dentistry. Various research shows that in comparison with classical medicine modalities such as antibiotics and disinfectants, ozone therapy is quite inexpensive, and very promising **(Gulafsha et al. 2019).**

Ozone has been shown to have properties of healing wounds, antimicrobial and disinfectant action so that it's used in dentistry. It also, has been helpful for treatment of early carious lesions, sterilization of cavities, root canals, periodontal pockets, enhancing epithelial wound healing such as ulcerations and herpetic lesions, bleaching, desensitization of sensitive teeth, treatment of peri-implantitis, and as a rinse for the avulsed teeth or as a denture cleaner and decontamination of used toothbrush. **(Hickel and Huth., 2004).**

Ozonated water had almost the same antimicrobial effectiveness as 2.5% sodium hypochlorite and also the cells when treated with ozonated water show a high metabolic activity of fibroblasts **(Ahmed *et al*., 2016).** The aqueous type of ozone, as a potential antiseptic agent, showed less cytotoxicity than gaseous ozone. Therefore, ozonated water has a biocompatibility for oral application **(Kshitish and Laxman, 2010).**

1.11.1 Ozone in Periodontology:

Periodontal disease is one affecting the supporting structures of teeth. It usually begins as gingivitis and in a small proportion of cases progresses to periodontitis. However, it is ultimately the host response and bacterial, genetic variance which decides the transition and severity of the disease. In a clinical study, periodontal pockets in patients with aggressive periodontitis were irrigated once a week for 4 weeks with 150 ml of ozonated water for 5–10 min post scaling and root planing (SRP). The following therapy; pocket depth, plaque, gingival indices, and bacterial count were reduced significantly in the quadrant treated **(Ramzy MI *et al*., 2005).**

Ozonated water, has been shown to be efficacious against Gram-positive and Gram-negative oral microorganisms as well as bacteria in plaque biofilm. It may be used as a mouth rinse in cases of gingivitis, oral thrush or stomatitis, as a spray to cleanse the affected area and to disinfect oral mucosa, as a water jet in treatment of painful gingivitis and stomatitis **(Gupta and deepa, 2016).** In patients suffering from aggressive periodontitis. Statistically significant decreases in terms of pocket depth, plaque index, gingival index, and bacterial count were observed. In a study, authors compared the effect of oral irrigation with ozone water, 0.2% chlorhexidine and 10% povidone iodine, in chronic periodontitis patients and concluded that local ozone application could be used as a powerful atraumatic and antimicrobial agent in the nonsurgical treatment of periodontal disease for both home care and professional practice **(Dodwad *et al.*, 2011).**

A study concluded that addition of ozone to an ultrasonic cleaning system containing various experimental solutions had antibacterial activity against *Staphylococcus aureus* **(Low SP *et al.*, 2010).**

Ozone causes vasodilatation and increases perfusion in tissues. Ozone

therapy on de-epithelialized gingival graft improved the perfusion in graft site during the 1st week when evaluated using laser Doppler flowmetry. Furthermore, ozone-treated group reported less pain and better quality of life compared to graft alone, post-surgery **(Taşdemir *et al*., 2016).**

1.11.2 Ozone in Endodontics:

The reliability of the use of ozone in endodontics is dependent primarily on its antimicrobial action. A randomized trial investigated the effectiveness of ozone gas and sodium hypochlorite/CHX in treating apical periodontitis and found no significant difference among the groups with regard to success rate, bactericidal action, periapical lesion size, and periapical index score at 6–12 months. Thus, ozone was considered an effective root canal disinfecting agent in treating apical periodontitis **(Kist *et al*., 2017).**

The effect of ozone has been synergistic when used in combination with other agents to disinfect root canals. Sodium hypochlorite, 2% CHX and ozone gas individually had no efficacy against *Enterococcus faecalis* and *C. albicans* in infected canals; but a combination of 2% CHX irrigation followed by a 24s exposure to ozone gas completely eliminated them and this synergistic combination can be used in the treatment of infected root canals **(Noites *et al.,* 2014)**

In a study, gaseous ozone was found to be less effective in reducing *E. faecalis* as compared to hypochlorite in an organized biofilm and was recommended to be used as an adjuvant in canal irrigation when hypochlorite is contraindicated **(Boch *et al.,* 2016).**

Ozone acts well in the absence of remaining organic debris. therefore, it is better to use ozone gas or ozone water after the complete preparation and shaping of the root canals **(Pattanaik et al., 2011).** The device's principle is

simple: the machine generates ozone, which is subsequently delivered to the necrotic root canals through the handpiece. The gas leakage is prevented by the silicone cover. Deep ozone penetration has been demonstrated to provide the highest disinfection efficacy. At the end of the procedure, the O3 gas is returned back into O2 gas by the ozone intoxicator (Figure 6). Ozone was studied in a variety of disinfection procedure combinations, the highest effectiveness was achieved when ozone gas was administrated at a dose of (16) ppm with an ultrasonic agitation system **(Lubojanski *et al.*, 2021).**

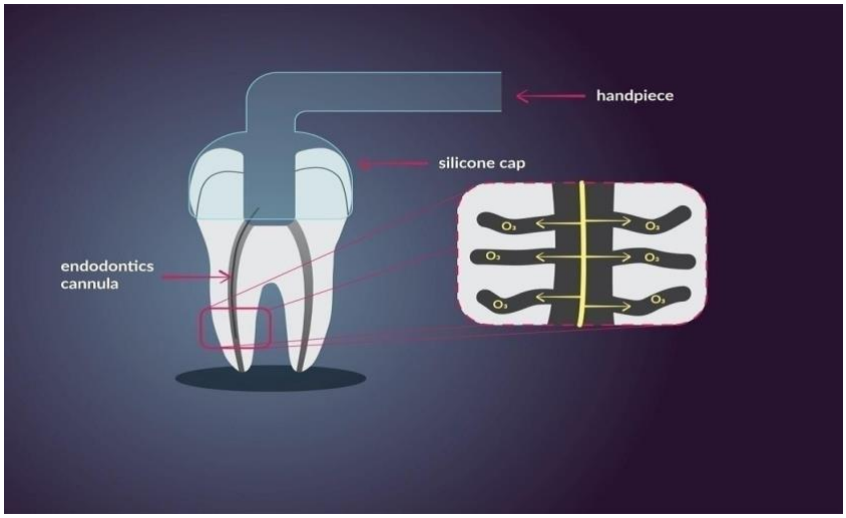

Figure 6 : Application of ozone in endodontics (Lubojanski et al.,2021)

1.11.3 Ozone for Treatment of Periimplantitis:

For the prevention of periimplantitis an adequate and steady plaque control regimen must be ensured. Ozone, a powerful antimicrobial kills the microorganisms causing periimplantitis. In addition ozone shows a positive wound healing effect due to the increase of tissue circulation. Gasiform ozone or ozonized water shows an increased healing compared to wound healing without ozone therapy **(Karapetian *et al.*, 2007).**

In an in vitro study, 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 *Porphyromonas gingivalis*. Gaseous ozone showed selective efficacy to reduce adherent bacteria on titanium and zirconia without affecting adhesion and proliferation of osteoblastic cells **(Hauser-Gerspach *et al.*, 2012*).***

When treating plaque biofilm specimen with about (4) ml of Ozonated water for 10seconds it was noticed that gram positive and gram negative oral bacteria and Candida-albicans in the pure culture in addition to microorganisms in dental plaque would be eliminated, besides that there was lowering in the plaque, bleeding and gingival indices. When using ozone as an irrigant in comparison to chlorhexidine appear that was significantly inactivate microbial pathogens which are responsible for periodontal infections, also fungicidal effects was recorded, in peri-implant inflammations aqueous or gaseous ozone forms may be applied, as in (Figure 7) **(Komali, 2012 ; Gulafsha and Anuroopa, 2019)**

Figure 7: Application of ozone in peri-implantitis (Gopalakrishnan and Parthiban, 2012)

1.11.4 Ozone in Oral Surgery:

A study evaluated the efficacy of ozone gas on the reduction of dry socket, which occurred after surgical extraction of lower jaw third molars. Two groups were evaluated: in the control group, saline solution was used for irrigation of extraction sockets and, intra-alveolar ozone was applied at 12 s (Prozone, W&H, UK) in the experimental group. They concluded that the ozone gas has a positive effect on reducing the development of dry socket and pain following third molar surgery depending on metabolic capabilities of ozone for promoting hemostasis, increasing the supply of oxygen, and inhibiting bacterial proliferation **(Ahmed *et al*., 2016).**

Application of ozone in combination with antibiotic therapy over a 15 day period was used to treat osteoradionecrosis of jaw following bisphosphonate treatment for multiple myeloma. Out of the twelve treated eight had complete resolution and four had partial resolution **(Petrucci *et al*., 2007).**

The use of ozone to prevent dry socket was investigated among 30 patients requiring bilateral surgical extraction of impacted lower third molars. Subjects were exposed to either intra-alveolar ozone for 12s or saline irrigation and evaluated at 48 h post-extraction and after 1 week. Ozone therapy following extraction significantly reduced the incidence (3.3%) of dry socket compared to controls (16.7%). Hence, ozone can reduce the incidence of dry socket and improve healing following surgical removal of third molars **(Ahmed *et al*., 2016).**

1.11.5 Antimicrobial Efficacy of Ozone as Denture Cleaners:

Microbial plaque accumulating on the dentures is composed of several oral microorganisms, mainly *C. albicans*. Denture plaque control is essential for the prevention of denture stomatitis. The application of ozonated water may be

useful in reducing the number of *C. albicans* ondenture plates **(Arita *et al*., 2005).** The use of ozone as denture cleaner is effective against methicillin-resistant S. aureus and viruses **(Murakami *et al*.,2002)** Ozone can be applied for cleaning the surface of removable partial denture alloys with little impact on the quality of alloy in terms of reflectance, surface roughness, and weight **(Azarpazhooh *et al*., 2007).**

Direct exposure to gaseous ozone was a more effective microbicide compared with ozonated water. Therefore, gaseous ozone can be clinically useful for disinfection of removable prosthesis**. (Gopalakrishnan *et al*., 2012).** There is also some evidence on the effectiveness of aqueous ozone applicationin adjunct to aminoalcohol for decontamination of the implant surfaces **(Nagayoshi M, *et al*., 2004).**

1.11.6 Role of Ozone in Pedodontics:

Throughout the global population there is a high incidence and prevalence of early childhood caries and as consequence there is high complication of behavior management in many children when having their first treatment, so it was necessary to find a compatible treatment modality that is efficient in preventing and managing the progressive carious lesions in primary teeth **(Ximenes et al., 2017).** Ozone therapy is becoming common in dental practice every day, as it shown to be beneficial in many application procedures and treatments in pediatric dentistry **(Derco and Koman, 2018).**

Its unique properties include immune stimulant, pain reduction, anti-hypnotic, anti-oxidative, bio-energetic and biosynthetic and antimicrobial activities. Besides its non-traumatic, non-invasive in nature, and had significant biological compatibility and relative absence of toxic effects, this had leaded the patient more compliant and cooperative, all of these features not only encourage

the patient tolerance and compliance but also provide a better dental performance during treatment procedure **(Tiwari et al.,2017).**

In cavitated lesions, especially ozone gas application provides an antibacterial effect in the cavity surface and satisfies intended to stop progression of the lesion. Although there is not enough clinical experience about the interval and dose of ozone gas to prevent the lesion becoming active after a certain period of cavitated caries lesions, it is thought that promising results can be obtained especially in children who have difficulties in cooperativeness, and this technique may be developed widely and used in routine clinical practice **(Derco and Koman, 2018).**

Traumatic injury of the teeth is one of the most common instances encountered in a pediatric dentistry. Aqueous ozone has been found to have a significant level of biological compatibility with human mucosal epithelial cells, periodontal fibroblast cells, and gingival connective tissue cells. The use of ozonized water in the replantation of avulsed teeth is recommended as it has no adverse effects on periodontal ligament cells **(Tiwari *et al.,* 2017).**

Ozone has a wide antibacterial effect in water and because of these characteristics it can be utilized for disinfection of the avulsed tooth; irrigation for 2 minutes with ozonized water promotes a better mechanical cleaning and decontamination. No harmful effects were seen on the attached tissues or other tooth structure **(Malik *et al.,* 2020).**

1.12 Ozone Toxicity:

Despite proponents for ozone use and the potential applications, toxicity can occur even at environmental levels and may be related to cardiac, respiratory, and neurologic events **(Gulafsha and Anuroopa, 2019).**

Ozone is a strong oxidizing agent and when inhaled is extremely toxic to the bronchio-pulmonary system. It can cause sore throat, dryness of mouth, coughing, potentiate asthma, and lung damage when inhaled **(Srinivasan1 *et al.,* 2019)**

In high doses 50 ppm for 60 minutes, gaseous ozone, like many therapeutic drugs, is hazardous. For this reason, safety concentration levels of 0.3 ppm for about 15 min., when ozone is used in dentistry, it should be cautiously administered up to 0.1 ppm inhalation **(Eden, 2016).**

The use of Ozone as a therapeutic agent has minimal or no negative consequences **(Komali, 2012).** Yet inhaling ozone inadvertently may induce eye irritation, nausea, vomiting, coughing, and in extremely sensitive people, a slight headache. Ozone rectal application may cause pain, gurgling, or minor cramping. Patients may have flu like symptoms as a result of the Herxheimer response in certain instances **(Hattur and Shrisel, 2017).** Certain adverse effects have been observed as a result of inconsistent Ozone usage, including rhinitis, blood vessel enlargement, poor circulation, and cardiac problems like stroke as assembled in (Table 1) **(Komali, 2012).**

Table 1 : Potential adverse reactions of ozone (Baysan, and Lynch, 2005).

Body site	Negative side effects
Pulmonary	Reversible adverse reactions in respiratory function, lung inflammation and toxic changes.
Blood	produces toxic cytological chemical products
Immune system	Induce allergic reactions
Brain	In high concentration may lead to brain damage
Cells	Elevate the count of neutrophils and the fluid levels of circular prostaglandins
Skin	Reduction in alpha tocopherlol including stress related Responses

Conclusion

The use of ozone for treatment of oral and dental diseases indeed, is a pattern shift in the clinical practice, and authors believe it can be a promising agent in the practice of dentistry. Ozone has shown to be impressively useful by the virtue of being minimally invasive, conservative and more tolerable by the patients. It could be used to treat a variety of dental diseases and the oxidative effect of ozone on the microbiota has been shown in several studies. The researches concerning the antimicrobial properties of ozone has continued over the last 20 years and has conclusively shown the ability of this gas to kill a wide range of bacteria and other microorganism. Concerning the studies done so far, it can be said that ozone can have further applications other than as antiseptics and antibiotics, which given an addition to dental treatment.

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