Review Article

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Ozone and its Role in Periodontics

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ABSTRACT

Background: Dentistry is changing as we are now using modern science to practice dentistry. In comparison with classical medicine modalities such as antibiotics and disinfectants, ozone therapy is quite inexpensive, predictable and conservative. Since its introduction in 1840, ozone therapy is proving to be a new therapeutic modality with great benefits to the patients. Ozone therapy has become an adjuvant element in the control and treatment of infections in areas such as surgery, dermatology, cosmetics and in dentistry, as it has been studied for years in industry and medicine because it is an antimicrobial agent highly effective against various microorganisms. The potent antimicrobial power of ozone, along with its capacity to stimulate the circulatory system and modulate the immune response, makes it a therapeutic agent of choice in the treatment of medical pathologies and infectious oral diseases. In this review article, we will be looking at the mechanism of action of ozone, its generation using various devices and its applications in dentistry along with its toxicity.

Keywords: Ozone, periodontics, dentistry.

INTRODUCTION

Gingival and periodontal diseases are bacterial infections are a major concern in dentistry. Treatment to halt the progression of these diseases involves sub-gingival mechanical debridement and establishing an environment for bacteria compatible with gingival health. The mechanical removal of biofilm and adjunctive use of chemotherapeutic agents have shown promising results in reversing the pathological conditions affecting the periodontium to restore the periodontal health. As the periodontal researchers is looking for alternatives to antibiotic treatments (biofilm resistance), the emergence of ozone therapy seems to be a promising future.[1] It is believed that most of the causes and factors contributing in the etiology of the disease may be reduced and treated by applying ozone in its different forms (gas, water, oil). Periodontal disease (PD) is a multifactorial infectious-inflammatory disease with a cyclic evolution, characterized by the destruction of the fibers of the periodontal ligament, resulting in the formation of pockets, apical migration of the junctional epithelium, alveolar bone loss that leads to tooth mobility in the more advanced stages leading to tooth loss. Elevated levels of anaerobic bacteria in dental plaque such as Aggregatibacteractinomycetemcomitans, Porphyromonasgingivalis, Tannerella forsythia, Prevotella intermedia, Treponema denticola and Fusobacterium nucleatum, are the primary etiologic factor for the progression of periodontal disease.[2,3] 1840 Christian Friedrich Schönbein, a German Chemist [father of ozone therapy]. When he passed an electrical discharge through water, a strange smell was produced, which he called ozone, derived from the Greek wor-d “Ozein” which means odor[4]

Definition: Oxygen/ozone therapy is a term that describes a number of different practices in which

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oxygen, ozone or hydrogen peroxide are administered via gas or water to kill disease microorganisms, improve cellular function and promote the healing of damaged tissues.[5] Many centuries ago, the Greeks noticed a peculiar odor in air that followed lightning and thunderstorm and described it "ozein" meaning to "to smell".[6] Ozone (O3) is a triatomic molecule, consisting of three oxygen atoms. Its molecular weight is 47.98g/mol.[7,8] Ozone is thermodynamically highly unstable compound that depends on conditions like temperature and pressure, decomposes to pure oxygen with a short half-life.[9]Groundlevel ozone is an air pollutant with harmful effects on the respiratory system. Ozone in the upper atmosphere filters potentially damaging ultraviolet light from reaching the Earth's surface. It has many different applications in various fields; one of them is usage of ozone in medicine.[10] Ozone is normally present as a gas made of three atoms of oxygen with a cyclic structure. The medical generator of ozone produces it from pure oxygen passing through a high voltage gradient (5–13 Megavolts) according to the reaction.[11]

Ozone (O3) is naturally produced by the photo dissociation of molecular oxygen (O2) into activated oxygen atoms, which then react with further oxygen molecules. This transient radical anion rapidly becomes protonated, generating hydrogen trioxide (HO3), which, in turn, decomposes to an even more powerful oxidant, the hydroxyl radical (OH). Ozone gas has a high oxidation potential and is 1.5 times greater than chloride when used as an antimicrobial agent.[12] Ozone is 1.6-fold denser and 10-fold more soluble in water (49.0 ml in 100 ml water at 0 0C) than oxygen. Although ozone is not a radical molecule, it is the third most potent oxidant (E 0= +2.076 V)) after fluorine and persulphate. Ozone is an unstable gas that cannot be stored and that should be used at once. It has a half-life of 40 min. at 200C, that means that its concentration measured in ug/ml of the gas mixture is halved every 40 min.

Ozone is a controversial gas because, while it is very useful in the stratosphere by absorbing dangerous B and C ultraviolet radiations, it is toxic for the pulmonary tract in the troposphere, particularly mixed with carbon monoxide (CO), N2O and traces of acids as it occurs in smog. It must be clear that if we want to use ozone in medicine, we must avoid its toxicity. Ozone forms near ground level as a result of the reaction of ultraviolet light with hydrocarbons, nitrous oxide, and sulfur compounds to produce photochemical smog. Ozone is not the cause of smog, but it is a by-product. Since ozone is a powerful oxidizer, it actually helps clean the atmosphere of these dangerous compounds. Ozone is used as a measuring device for smog levels to reiterate [it is not smog, but nature's way of cleaning up the smog.[13]

OZONE THERAPY

Medical ozone, an ozone/oxygen mixture consisting of purest O2 and purest O3, is produced from medical oxygen (in accordance with pharmaceutical legislation) using a medical ozone generator. The usefulness of ozone for sterilization, as an anti-inflammatory agent, and for maintaining hemostasis are of current interest in the dental field. Several experimental studies have shown that oxygen-ozone therapy may have a beneficial effect in patients with periodontitis, mainly through dual mechanisms: inhibiting the growth of anaerobic bacteria in periodontal pockets and increasing blood flow to the gingival level thus promoting tissue healing. In addition, oxygen-ozone stimulates white blood cell function by activating host defense mechanisms and accelerating the healing process.30 In medical use the gas produced from medical grade oxygen is administered in precise therapeutic doses, and never via inhalation, and advocates that it has excellent health benefits in dental caries, decrease blood cholesterol and stimulation of anti-oxidative responses, modifies oxygenation in resting muscle and is used in complementary treatment of hypoxic and ischemic syndromes. [14-21]

1950 A German dentist, Dr. E.A. Fisch, used ozonated water for dental procedures and pioneered its use in medicine. Fisch had the first idea to use ozone as either a gas or ozonated water in his practice.[22]

MECHANISM OF ACTION

Ozone in the gaseous or aqueous phase has been shown to be a powerful and reliable antimicrobial agent against bacteria, fungi, protozoa and viruses.Ozone therapy disrupts the integrity of the bacterial cell envelope through oxidation of the phospholipids and lipoproteins. In fungi, O3 inhibits

cell growth at certain stages. With viruses, the O3 damages the viral capsid and upsets the reproductive cycle by disrupting the virus-to-cell contact with peroxidation. There are several known actions of ozone on human body, such as anti-microbial. Immunostimulating, antihypoxic, analgesic, detoxicating, bioenergetics and biosynthetic etc. Ozone can attack many biomolecules, such as the cysteine, methionine and histidine residues of proteins. By oxidizing the biomolecules featured in the dental tissues, ozone has severely disruptive effect on cariogenic bacteria, resulting in the elimination of acidogenic bacteria. Ozone decarboxylates pyruvic acid produced by acidogenic bacteria during cariogenesis to acetic acid.[23,24]

Several studies based on the mechanism of action of Ozone reinforces the concept that optimal ozone concentrations are critical for achieving a therapeutic result. Too low concentrations are practically useless (at best elicit a placebo effect), too high may elicit a negative effect (malaise, fatigue) so that ideally they must be just above the threshold level to yield an acute, absolutely transitory oxidative stress capable of triggering biological effects without toxicity. The plasma is the critical phase where ozone dissolves and produces a considerable amount of the ozone dose but, if its concentration is correct, they allow formation of ROS [Reactive Oxygen Species] and LOP [Lipid Oxidation Products]. The fundamental ROS molecule is hydrogen peroxide (H2O2) that is a non-radical oxidant able to act as an ozone messenger responsible of eliciting several biological and therapeutic effects. H2O2 diffuses easily from the plasma into the cells and its sudden appearance represents the triggering stimulus for activation of different biochemical pathways in erythrocytes, leukocytes and platelets resulting in numerous biological effects depending upon the cell type.[11,12,13]

Several known actions of therapeutic Ozone are as follows-[25,26,27]

1. Antimicrobial effect: The anti-microbial effect of Ozone because of its action on cells by damaging its cytoplasmic membrane due to ozonolysis of dual bonds and ozone-induced modification of intracellular contents because of secondary oxidants effects. This action is non-specific and selective to microbial cells. 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.

2. Immunostimulating action: Ozone promotes cellular and humoral immune system. It causes proliferation of immunocompetent cells as well as synthesis of immunoglobulins. It also stimulate function of macrophages and increases killing of microorganisms by phagocytosis.Ozone causes the synthesis of biologically active substances such as interleukins, leukotrienes, and prostaglandins which reduces the inflammation and helps in wound healing. Ozone in high concentrations causes immunosuppressive effect whereas in its low concentration causes immunostimulating effect. Application of medical ozone is extremely useful for immune activation in patients with a low immune status and/or immune deficit.

3. Antihypoxic action: Ozone brings about the rise of pO2 in tissues and improves transportation of oxygen in blood, which results in change of cellular metabolism – activation of aerobic processes (glycolysis, Krebs cycle, β-oxidation of fatty acids) and use of energetic resources. It also prevents formation of erythrocytes aggregates and increases their contact surface for oxygen transportation. Its ability to stimulate the circulation is used in the treatment of circulatory disorders and makes it valuable in the revitalizing organic functions. Ozone activates the Krebs cycle by enhancing oxidative carboxylation of pyruvate, stimulating production of ATP. It also causes a significant reduction in NADH and helps to oxidize cytochrome C. There is a stimulation of production of enzymes, which act as free radical scavengers and cell-wall protectors: glutathione peroxidase, catalase and superoxide dismutase. Production of prostacyclin, a vasodilator, is also induced by O3.

4. Analgesic & Anti-inflammatory action: Ozone promotes the synthesis of active substances like interleukins, leukotrienes and prostaglandins, which as a result lead to reduction of inflammation and pain. Since the infection or inflammation is acidic and ozone is basic, so the varied chemistry of

infection and inflammation lead to transportation of ozone to the area. these effects seem to be due to its way of acting on diverse targets: a. Decrease the production of mediators of the inflammation. b. The oxidation (inactivation) of metabolic mediators of pain. c. It clearly improves local blood microcirculation, with an improvement in the oxygen delivery to the tissues, essential for the generation of anatomic structures; the elimination toxins and in general to the resolution of the physiological disturbance that generated the pain.

5. Bio-energetic and bio-synthetic action: It activates mechanisms of protein synthesis, increases amount of ribosomes and mitochondria in cells. These changes on the cellular level explain elevation of functional activity and regeneration potential of tissues and organs. Ozone attacks the glycoproteins, glycolipids and other amino acids, inhibits and blocks the enzymatic control system of the cell. This results in an increase in membrane permeability, the key element of cell viability, leading to immediate functional cessation. Then ozone molecules can readily enter the cell and destroy the microorganism. Ozone can attack many biomolecules, such as the cysteine, methionine and histidine residues of proteins by oxidizing the biomolecules featured in the dental tissues.

6. Pro- and Anti-oxidant function: Ozone has a cyclical structure exists in several mesomeric states in dynamic equilibrium . Ozone having a paired number of electrons in the external orbit is not a radical molecule, but it is far more reactive than oxygen and generates some of the radical oxygen species (ROS) produced by oxygen during mitochondrial respiration. Phagocytes reacting with pathogens produce anion superoxide (O2 –), H2O2, and hypochlorous acid (HClO) catalyzed by myeloperoxidase. Moreover, H2O2 is produced by almost all cells by the nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase isoenzymes, indicating the relevance of ROS in the normal organism. Interestingly Ozone, in the presence of inorganic and/or organic compounds immediately reacts and generates a great variety of oxidized molecules, disappearing in a matter of seconds.

Ozone doses, however low they are, cause the increase in the content of free and dissolved blood oxygen with rapid intensification of enzymes that catalyze aerobic oxidation of carbohydrates, lipids and proteins with formation of ATP energy substrate.[28,29]

7. Miscellaneous actions: Ozone therapy appears to be a safe, economical, effective treatment for patients with cardiovascular disorders based on biological responses that improve blood circulation and oxygen delivery to ischemic tissue owing to the concerted effect of NO and CO and an increase of intra-erythrocytic 2,3-diphosphoglycerate level. By improving oxygen delivery, it enhances the general metabolism. It up-regulates the cellular antioxidant enzymes and induces HO-1 and HSP-70 and induces a mild activation of the immune system and enhances the release of growth factors from platelets. It also procures a surprising wellness in most of the patients, probably by stimulating the neuro-endocrine system.

Ozone is created in nature in three ways: first, by lightening, which provides the wonderful fresh smell after a thunderstorm; second, through waterfalls and crashing surf, which accounts for the energetic feeling and calm experienced near these sites; and third by photons from the sun that split nitrous oxide, a pollutant formed by the combustion of hydrocarbons in the internal combustion engine. In 1857, Werner Von Siemens designed an ozone generator that has since greatly evolved. The cylindrical dielectric type, sometimes called the 'Siemens Type' ozone generator, makes up most of the commercially available ozone generators in use. The medical generator of ozone produces it from pure oxygen passing through a high voltage gradient (silent electrical discharge). It is not possible to use oxygen concentrators or oxygen/air mixtures due to their nitrogen component and the consequent possibility of nitrogen oxides being formed in the discharge tube.[30]

GENERATING OZONE

There are three different systems for generating Ozone gas: [22]

a. Ultraviolet System: produces low concentrations of ozone, used in esthetics, saunas, and for air purification.

b. Cold Plasma System: used in air and water purification.

c. Corona Discharge System: produces high concentrations of Ozone. It is the most common system used in the medical/ dental field. It is easy to handle and it has a controlled ozone production rate. Medical grade ozone is a mixture of pure oxygen and pure ozone in the ratio of 0.05% to 5% of O3 and 95% to 99.95% of O2. Due to the instability of the O3 molecule, medical grade ozone must be prepared immediately before use. Within less than an hour after preparation, only half of the mixture is still Ozone while the other half is transformed into OZONE GENERATORS .Ozone can be used as ozonated water, Oxygen/Ozone gas and ozonated olive oil.

The ozone unit for dental use was initially developed by CurOzone Inc. (Canada). Subsequently manufactured under license and distributed by KaVo Dental GmbH and Co. (Germany) under the name "HealOzone". Its use has been pioneered by Professor Lynch et al. at Queen's University in Belfast, Northern Ireland, and Barts and the London Queen Mary's School of Medicine and Dentistry in London, UK. It converts oxygen to Ozone. The HealOzone device comprises an air filter, vacuum pump, an ozone generator, a handpiece fitted with a sealing silicone cup and a flexible hose. The procedure usually takes between 20 and 120s per tooth. Patients are also supplied with a patient kit, which consists of toothpaste, oral rinse and oral spray, all containing fluoride, calcium, zinc, phosphate and xylitol, and aims to enhance the remineralization process. The OzoTop is a free-flow ozone delivery system using a corona discharge. It is a compact, easy to use tabletop unit. Due to the delivery system of the OzoTop, root canals and periodontal pockets can be penetrated easily. The system may be used in all cases where point of placement disinfection is needed, including periodontology, endodontology, restorative, implantology, apthous ulcers/herpes, gerodontology (dentures) and impression materials.[25]

The HealOzone works at 2100 parts per million of ozone with a flow rate of 615 ccm. Any remaining high level of ozone concentration must be destroyed after use. The destructor filter, manganese dioxide, breaks down the ozone into oxygen and returns it to the treatment area atmosphere. HealOzone creates an environmental condition within and around the tooth, which enables Nature to allow natural remineralization to occur. HealOzone has no adverse effect on healthy tissues found within the oral cavity, so it is impossible to overdose. Lower organisms such as bacteria and viruses do not possess enzyme coats. This allows Ozone to easily penetrate their cell membranes and render them sterile. Higher organisms actively produce enzyme coats, which prevent reaction with Ozone. This protects these cells from attack. Interestingly, Ozone does target target and attack diseased human cells, which possess deficient cell wall enzymes and/or disrupted DNA or RNA strands. This explains why clinical Ozone treatment is toxic to infecting organisms but not to the patient.

OZONE IN DENTISTRY[44,45,46,47,48,49,50]

Dental Unit Water Lines Disinfection: Dental unit water lines are ideal environment for the growth of microorganisms entering Dental units from the municipal water supply and from previously treated patients. Wirthlin et al (2003) concluded that controlling DUWL biofilm would have beneficial effects on nosocomial infections.[31]

Uses in Oral Medicine Herpes lesions have been studied with topical ozone administration.99, 100 Ozonated oil applied on herpes labialis and mandibular osteomyelitis demonstrated faster healing times than conventional protocols. ozone, in these cases, neutralizes herpes virions by direct action,thus inhibiting bactericidal suprainfections, and stimulating the healing of tissues through circulatory prompting. Ozone has been proven to be one of the most powerful oxidants we can use in dentistry.

Uses in Oral Surgery Ozone is known to encourage wound healing as well as control opportunistic infection. It was shown that daily treatment with ozonized water accelerates the physiological healing rate. In a study which compared the use of ozonated oil in an experimental group to a control group in which antibiotic therapy was used in the treatment of alveolitis, it was found that patients treated with ozonated oil healed more quickly.Ozone was used in the treatment of avascular osteonecrosis of the jaw (ONJ). There was complete healing of the lesions with the disappearance of symptoms.[32-37]

Uses in Periodontics 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 grampositive 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.49 One study concluded that the addition of ozone to a ultrasonic cleaning system containing different experimental solutions resulted in antibacterial activity against Staphylococcus aureus.[38]

Ozonated water has an excellent anti- inflammatory capacity.[39,40,41]

Researchers choose the NF-ƙβ system, a paradigm for inflammation associated signaling/transcription. Their results showed that that NF-kappaB activity in oral cells in periodontal ligament tissue from root surfaces of periodontally damaged teeth was inhibited following incubation with ozonized medium. 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.[42,43]

Uses in Prosthodontics The effect of ozonated water on oral microorganisms and dental plaque was assessed.49, 52 Few oral microorganisms and no viable C. albicans were detected after exposure to following ozonated water (2 or 4 mg/L) for 1 min, suggesting the application of ozonated water might be useful in reducing the number of C. albicans on denture bases.

One study compared the microbicidal effect between gaseous ozone and ozonated water on dentures and found that direct exposure to gaseous ozone was more effective.[51]

Uses in Endodontics The oxidative power of ozone characterizes it as an efficient antimicrobial. Its antimicrobial action has been demonstrated against bacterial strains, such as Micobacteria, Streptococcus, Pseudomonas aeruginosa,Escherichiacoli,Staphylococcus aureus, Peptostreptococcus, Enterococcus faecalis, and Candida albicans.

Notably, when the specimen was irrigated with sonication, ozonated water had nearly the same antimicrobial activity as 2.5% NaOCl.[45,51,52] A study evaluated the effects of intracanal medication using ozonated oil compared to a calcium hydroxide paste associated with camphorated paramonochlorophenol and glycerin (HPG) for the endodontic treatment of teeth with periapical lesions. The radiographic, histopathological, and histobacteriological analysis showed no significant differences between periradicular tissue responses to both medications. In vitro studies showed that ozone was effective over most of the bacteria found in cases of pulp necrosis. Ozone works best when there is less organic debris remaining. Therefore, the recommendation is to use either ozonated water or ozone gas at the end of the cleaning and shaping process. Ozone is effective when it is used in sufficient concentration, for an adequate time. Ozone will not be effective if too little dose of ozone is delivered or it is not delivered appropriately. Ozone therapy and Dental Caries Ozone therapy is used as an atraumatic treatment modality in dental practice. Some of the in vitro studies with short-term follow-up assessed the effect of ozone on pit and fissure cariesand primary root caries with results showing significant reductions in the number of microorganisms in the carious lesions. In small, noncavitated lesions showed a greater reduction in number of microorganisms after the application of ozone than did larger lesions, and lesions closer to the gingival margin also showed less reduction in the number of microorganisms.This suggests that the reversal of carious lesions depend on the size and localization. Noncavitated lesions were more likely to reverse than cavitated lesions. The hardness values improved significantly in the ozone-treated test lesions after 4, 6, and 8 months ($P < 0.05$) compared with baseline while the control lesions had no significant change in hardness at any recall interval. It has been shown that the infusion of ozone into noncarious dentine prevented biofilm formation in vitro from S. mutans and L. acidophilus over a 4-week period.[53-60]

Uses inPedodontics The basic actions of ozone in almost all branches of dentistry have been

discussed so far. The applications of ozone therapy in a pediatric practice rely mainly on the fact that ozone application is a very quick, effective, easy and especially a painless procedure to These aspects of the treatment not only enhance the operator efficiency but also effectively improves the patient compliance and tolerance to the treatment procedure. Attaining a positive rapport with a child patient is the key to a successful pediatric treatment which can be very effectively accompanied by using ozone therapy. Dahnhardt et al.1 treated open carious lesions with ozone in anxious children[62].

A high level of biocompatibility of aqueous Ozone on human oral epithelial cells, gingival fibroblast cells, and periodontal cells has been observed. Ozonated water is indicated in replantation of avulsed tooth without any harmful effect on periodontal cells[38,39].

Uses inOrthodontics Teeth bonded with bonding material are reported to have been affected by some form of enamel opacity after orthodontic treatment; diffuse opacity being the most common type identified. Also visible white spot lesions have been seen to develop within 4 weeks of orthodontic treatment. Although enamel bracket interface is the most susceptible area for white spot lesion formation, microleakage can invade beneath the bracket area. Thus, enamel prophylaxis is of great importance in orthodontics[63].

Toxicity

Many studies show that the bronchial pulmonary system is very sensitive to ozone and this gas should never be inhaled. The respiratory tract lining is constituted by a very thin film containing a minimal amount of antioxidants that make mucosal cells extremely vulnerable to oxidation. Pulmonary embolism occurs during direct intravenous administration of O2/O3. Hence, it is prohibited by the European Society of Ozone therapy since 1983.

The known side effects of ozone therapy are epiphora, upper respiratory irritation, rhinitis, cough, headache, occasional nausea, and vomiting. Contraindications are severe anemia, hyperthyroidism, thrombocytopenia, myasthenia, acute alcohol intoxication, recent myocardial infarction, hemorrhage from any organ, glucose-6-phosphate dehydrogenase deficiency, and ozone allergy.[64]

Scientific support, as suggested by demonstrated studies, show ozone to be a potential use in dentistry. The treatment is completely painless and increases the patients' acceptability and compliance with minimal adverse effects. Although more clinical research has to be done to standardize indications and treatment procedures of ozone therapy, still many different approaches are so promising, or already established, that hopefully the use of ozone therapy becomes a standard treatment for disinfection of an operation sites in dentistry.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

REFERENCES

1. Saini R. Ozone therapy in dentistry: A strategic review. J Nat ScBiol Med 2011;2:151-3.

2. Graves DT, Jiang Y, Genco C. Periodontal disease: bacterial virulence factors, host response and impact on systemic health. CurrOpin Infect Dis 2000;13: 227-32.

3. Honda T, Domon H, Okui T, et al. Balance of inflammatory response in stable gingivitis and - progressive periodontitis lesions. ClinExpImmunol 2006;144: 35-40.

4.Kumar A, Bhagawati S et al. Current interpretations and scientific rationale of the ozone usage in dentistry: A systematic review of literature. Eur J Gen Dent 2014;3:175-80.

5. Giunta R et al. Ozonized autohemotransfusion improves hemorheological parameters and oxygen delivery to tissues in patients with peripheral occlusive arterial disease. Ann Hematol. 2001 Dec;80(12):745-8

6.Sadatullah, S & Mohamed Ozone in Dentistry: What Dentists Should Know About This Gas? King Saud University Journal of Dental Science. 2010 22. 83-87.

7. Seidler, Viktor, Linetskiy et al , Roman &Mazánek, J. Ozone and Its Usagein General Medicine and

Dentistry. A Review Article. Prague Med Rep. 2008;109(1):5-13.

8.STOPKA P.: Ozon. Progresdent 6: 8–11, 2003.

9.Stübinger S, Sader R, Filippi A The use of ozone in dentistry and maxillofacial surgery: a review. Quintessence Int. 2006 May;37(5):353-9.

10. Wikipedia. HTTP://EN.WIKIPEDIA.ORG/WIKI/OZONE_THERAPY BIBLIOGRAPHY 66

11. Lynch, Edward. "Ozone – The Revolution in Dentistry" ISBN 2003 18- 5097-08-82.

12. Naik SV, Kohli S et al. Ozone- A Biological Therapy in Dentistry- Reality or Myth?????. Open Dent J. 2016 May 11;10:196-206.

13. MollicaP.,Harris R. Integrating oxygen/ozone therapy into your practice. 2007 Dental Economics (web magazine) [http://www.oxygenhealingtherapies.com/Dental%20Ozone%20Therapy_Integrating.pdf].

14. Turˇci´c, J, J. Hancevi´c, and T. Antoljak. "Effects of Ozone on HowWellSplitThickness Skin Grafts According to Thiersch Take in War Wounds Results of Prospective Study." Langenbecks Arch Chir. 1995;380(3):144-8.

15. Akahori Y, Murakami M and Hoshi S. Sterilization effects and prevention of hospital-acquired infections by ozonated water. J JpnSoc Intensive Care Med 7: 3- 10, 2000.

16. Sakai D, Makita Y et al. Local hemostatic effect of aqueous ozone in cutting wound surface. J Hard Tissue Biol 2014 23: 245-248

17. Elvis A. & Ekta, J. Ozone therapy: A clinical review. Journal of Natural Science, Biology and Medicine. Jan-2011 2. 66-70.

18. Holmes J. Clinical reversal of root caries using ozone, doubleblind, randomised, controlled 18-month trial. Gerodontology 2003;20:106-14. BIBLIOGRAPHY 67

19. Bocci V. Is it true that ozone is always toxic? The end of a dogma. ToxicolApplPharmacol. 2006 Nov 1;216(3):493-504.

20. Hernández F, Menéndez S, Wong R. Decrease of blood cholesterol and stimulation of antioxidative response in cardiopathy patients treated with endovenous ozone therapy. Free Radical Biol Med 1995;19:115-9.

21. Clavo B, Pérez JL, López L, Suárez G, Lloret M, Rodríguez V, et al.Effect of ozone therapy on muscle oxygenation. J Altern Complement Med 2003;9:251-6.

22.Gupta, G, and B Mansi. "Ozone therapy in periodontics." Journal of medicine and life vol. 5,1 (2012): 59-67. BIBLIOGRAPHY 68

23. Bhateja, Sumit. The miraculous healing therapy – "Ozone therapy" in dentistry. Indian Journal of Dentistry 2012. 3. 150–155.

24. Maiya A. Applications of Ozone in Dentistry. Int. Journal of Clinical Dental Science. 2011;2(3):23-27

25. Taşdemir, Z' Alkan, B, The Effects of Ozone Therapy on the Early Healing Period of De-epithelialized Gingival Grafts: A Randomized Placebo-controlled Clinical Trial. J Periodontol. 2016 Jun;87(6):663-71.

26. Kumar T, Khan M. Ozone therapy in oral diseases: a reply to antibiotic resistance. World J Pharm Res. 2017;6(4):1476-1481. BIBLIOGRAPHY 73

27. Mani A, Shukla P, et al. Ozone therapy in Periodontics- A review. JMSCR 2014; 2(12): 3352-3358.

28. Bocci, Velio&Borrelli, et al. The Ozone Paradox: Ozone Is a Strong Oxidant as Well as a Medical Drug. Med Res Rev. 2009 Jul;29(4):646-82

29. Vipin Thakkar et al Ozone (O3): an excellent adjunctive tool in medical and surgical management of patientInt J Res Med Sci. 2014 Nov;2(4):1257-1261t

30. Fathi, A.M.; Mawsouf, M.N.; Viebahn-Hänsler, R. Ozone Therapy in Diabetic Foot and Chronic, NonhealingWounds. Ozone Sci. Eng. 2012, 34, 438–450.

31.Estrela C, Estrela CRA, et al. Antimicrobial efficacy of ozonated water, gaseous ozone, sodium hypochlorite and chlorhexidine in infected human root canals. Int Endod J. 2007 Feb;40(2):85-93.

32.Grootveld M, Silwood CJ, Lynch E. High resolution 1H NMR investigations of the oxidative consumption of salivary biomolecules by ozone: Relevance to the therapeutic applications of this agent in clinical dentistry. Biofactors 2006;27:5-18.

33.Nogales CG, Ferrari PH, Kantorovich EO, Lage-Marques JL. Ozone therapy in medicine and dentistry. J Contemp Dent Pract 2008;9:75-84. BIBLIOGRAPHY 76

34.Agrillo A, Petrucci MT, Tedaldi M, Mustazza MC, Marino SM, GallucciC, et al. New therapeutic protocol in the treatment of avascularnecrosis of the jaws. J CraniofacSurg 2006;17:1080-3.

35.Agrillo A, Sassano P, Rinna C, Priore P, Iannetti G. Ozone therapyin extractive surgery on patients treated with bisphosphonates. J CraniofacSurg 2007;18: 1068-70.

36.Agrillo A, Ungari C, Filiaci F, Priore P, Iannetti G. Ozone therapy in the treatment of avascular bisphosphonate-related jaw osteonecrosis. J CraniofacSurg 2007;18:1071-5.

37.Petrucci MT, Gallucci C, Agrillo A, Mustazza MC, Foà R. Role of ozonetherapy in the treatment of osteonecrosis of the jaws in multiplemyeloma patients. Haematologica 2007;92:1289-90.

38.Estrela C, Estrela CR, et al. Antimicrobial potential of ozone in an ultrasonic cleaning system against Staphylococcus aureus. Braz Dent J. 2006;17(2):134-8.

39. Huth KC, Jakob FM, et al. Effect of ozone on oral cells compared with established antimicrobials. Eur J Oral Sci 2006;114:435-40.

40. Huth KC, Saugel B, et al. Effect of aqueous ozone on the NFkappaB system. J Dent Res. 2007. BIBLIOGRAPHY 70

41.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.

42.Low SP, Williams KA, Canham LT, Voelcker NH. Generation ofreactive oxygen species from porous silicon microparticles in cellculture medium. J Biomed Mater Res A 2010;93:1124-31.

43.Low SP, Williams KA, Canham LT, Voelcker NH. Evaluation ofmammalian cell adhesion on surface-modified porous silicon.Biomaterials 2006;27:4538-46. BIBLIOGRAPHY 77

44. Ramzy MI, Gomaa HE, Mostafa MI. Management of aggressive periodontitis using ozonized water. Egypt Med J NRC 2005;6:229-45.

45.Nagayoshi M, Fukuizumi T et al. Efficacy of ozone on survival and permeability of oral microorganisms. Oral Microbiol Immunol 2004;19:240-6.

46. Arita M, Nagayoshi M, et al. Microbicidal efficacy of ozonated water against Candida albicans adhering to acrylic denture plates. Oral Microbiol Immunol. 2005 Aug;20(4):206-10

47. Huth KC, Quirling M, Maier S, Kamereck K, Alkhayer M, Paschos E, et al . Effectiveness of ozone against endodontopathogenic microorganisms in a root canal biofilm model. Int Endod J 2009;42:3-13.

48. Velano HE, do Nascimento LC, de Barros LM, Panzeri H. In vitroassessment of antibacterial activity of ozonized water againstStaphylococcus aureus. PesquiOdontol Bras 2001;15:18-22.

49. Cardoso MG, de Oliveira LD, Koga-Ito CY, Jorge AO. Effectivenessof ozonated water on Candida albicans, Enterococcus faecalis, andendotoxins in root canals. Oral Surg Oral Med Oral Pathol Oral RadiolEndod 2008;105:e85-91.

50. Silveira AM, Lopes HP, Siqueira JF Jr, Macedo SB, Consolaro A. Periradicular repair after two-visit endodontic treatment using twodifferent intracanal medications compared to single-visit endodontictreatment. Braz Dent J 2007;18:299-304.

51.Oizumi M, Suzuki T, Uchida M et al. In vitro testing of a denture cleaning method using ozone. J Med Dent Sci. 1998 Jun;45(2):135-9.

52.Huth KC, Quirling M, Maier S, Kamereck K, Alkhayer M, Paschos E, Welsch U, Miethke T, Brand K, Hickel R. Effectiveness of ozone against endodontopathogenic microorganisms in a root

canal biofilm model. Int Endod J. 2009 Jan;42(1):3-13.

53. Huth KC, Paschos E, Brand K, Hickel R. Effect of ozone on noncavitatedfissure carious lesions in permanent molars—a controlledprospective clinical study. Am J Dent 2005;18:223-8.

54. Baysan A, Whiley RA, Lynch E. Anti microbial effects of a novelozone generating device on microorganisms associated with primaryroot carious lesion in vitro. Caries Res 2000;34:498-501. BIBLIOGRAPHY 78

55. Baysan A, Lynch E. The use of ozone in dentistry and medicine. PrimDent Care 2005;12:47-52.

56.Baysan A, Lynch E. The use of ozone in dentistry and medicine. Part2. Ozone and root caries. Prim Dent Care 2006;13:37-41.

57. Polydorou O, Pelz K, Hahn P. Antibacterial effect of an ozone device and its comparison with two dentin bonding systems. Eur J Oral Sci 2006;114:349-53.

58. Baysan A, Lynch E. Clinical reversal of root caries using ozone:6-month results. Am J Dent 2007;20:203-8.

59. Dähnhardt JE, Jaeggi T, Lussi A. Treating open carious lesions inanxious children with ozone. A prospective controlled clinical study.Am J Dent 2006;19:267-70.

60. Knight GM, McIntyre JM, Craig GG, Mulyani, Zilm PS. The inability of Streptococcus mutans and Lactobacillus acidophilus to forma biofilm in vitro on dentine pretreated with ozone. Aust Dent J2008;53:349-53.

61. Baysan A, Beighton D. Assessment of the ozone-mediated killing ofbacteria in infected dentine associated with non-cavitated occlusalcarious lesions. Caries Res 2007;41:337-41

62. Dähnhardt JE, Jaeggi T, Lussi A. Treating open carious lesions inanxious children with ozone. A prospective controlled clinical study. Am J Dent 2006;19:267-70.

63. Ghobashy, Safaa& El-Tokhey, H.M. In vivo study of the effectiveness of ozonized olive oil gel on inhibiting enamel demineralization during orthodontic treatment. J. Am. Sci. 2012; 8(10):657-666.

64.Bocci, Velio. Tropospheric Ozone Toxicity vs. Usefulness of Ozone Therapy. Arch Med Res. 2007 Feb;38(2):265-7.