

PERIODONTAL OZONOTHERAPY: A REVIEW

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ABSTRACT

Gingival and Periodontal diseases are concern with development and formation oral biofilm. The mechanical removal of the biofilm and adjunctive use of various antibiotics have been conventional methods for periodontal therapy. Now a day's new approach for periodontal treatment is Ozone therapy. It is considered that different forms of Ozone used in periodontal treatment has beneficial biologic effects ,anti-microbial activity, immunostimulating effect ,ant hypoxic effects, oxidation of bio-molecules precursors and microbial toxins ,healing and tissue regeneration properties make the use of Ozone well indicated in all stages of gingival and periodontal diseases. The primary objective of this article is to provide general review about various aspects of ozone therapy and its clinical application in the management of periodontal diseases.

Key Words: Antimicrobial, Immunostimulating, Antihypoxic, Oxidation

INTRODUCTION

Ozone is a chemical compound consisting of three oxygen atoms , having molecular weight 41.98 g/mol .It is a powerful oxidizer.^[1] In 1840 some chemist named ' OZONE' from greek word OZEIN – to smell and was first used by German chemist Christian Friedrich Schonbein . The brief historic background of Ozone reveals that, in 1857 first ozone generator was developed by Werner Von Siemen in Germany. In 1870 in Germany there was a first report of ozone being used therapeutically to purify blood by C Lender. Wounds and other infections were treated by ozone in World War I. Before 1932 many Swiss dentists used ozone in dentistry. In 1957, a researcher patented on Ozone generator, which lead to the extensive use of ozone in medical practice. ^[2] Medical grade ozone is a mixture of pure O₂ and pure O₃ in ratio of 0.1%-5% of O₃ and 95%-99.5% of O₂ . In atmosphere it exists with highest level in stratosphere region known as the ozone layer between 10-50 km above the sea level. It absorbs dangerous ultraviolet radiation.Ozone therapy can be defined as versatile bio-oxidative therapy in which oxygen is administered via gas or dissolved

in water or oil base to obtain therapeutic benefits.^[3,4]This various forms of ozone can be generated by different methods which are widely used in periodontal treatment. Ozone Generation – Ozone is very unstable gas, in order to prepare medical grade ozone, it must be prepared immediately before use. Due to its instability even after preparation within less than an hour, only half is transformed into oxygen. To control the decomposition of O₃ into oxygen it can be associated with a vehicle with aqueous properties to promote the conversion more quickly or with a vehicle with more viscous properties to retard the conversion.^[5]

Three different systems of generating ozone gas; -
[6, 7]

- 1) Ultraviolet system produces low concentration of ozone in esthetics, air purification.
- 2) Cold plasma system used in air & water purification.
- 3) Corona discharge system produces high concentration of ozone.

It is the most commonly used system in the medical or dental field. It is easy to handle & it has a controlled ozone production rate. Commercially

available ozone generator: Cur ozone USA (Ontario, Canada) [5]

Mechanism of Action

Antimicrobial – The use of ozone is justified as a new option of irrigating agent with antimicrobial action which results from oxidation of microbial cellular component. When dissolved in water it becomes highly unstable & hydroxyl radicals are generated. It kills bacteria by two mechanism one by direct reaction of molecular ozone & other by a free radical mediated reaction. Ozone can kill bacteria by rupturing their cell membrane within few seconds. This involves chemical modification & fragmentation of monounsaturated & polyunsaturated fatty acids in the cell wall when the membrane is damaged, its permeability increases & ozone molecules can readily enter the cells. There are several potential action of ozone, which are applied in clinical practice of dentistry & medicine, such as antimicrobial, anti-inflammatory, immunostimulating, antihypoxic, detoxicating, biosynthetic, bioenergetics, hemostatic etc.

Indications of Ozonotherapy

- 1) Plaque control
- 2) Desensitization of extremely sensitive tooth neck
- 3) Pre washing of surgical sites
- 4) Contamination control
- 5) Sterilization of cavities,root canals,periodontal infection,endodontic treatment
- 6) Bleaching of discoloured root canal treated teeth
- 7) Remineralization of pit & fissure caries ,root & smooth surface caries
- 8) Prophylaxis & prevention of dental caries
- 9) Chronic or recurrent infection in the oral cavity

Advantages

- 1) Disinfectant
- 2) Anti-inflammatory
- 3) Activation of intercellular metabolism of oral mucosa & dental wounds
- 4) Improves regional circulation
- 5) Stimulation of regenerative procedures
- 6) Hemostasis in capillary bleedings
- 7) Painless procedures

Disadvantages

- 1) Instability
- 2) Not readily available
- 3) Ozone toxicity ,if the level increases at 0.0007% per application

Contraindications

- 1) Pregnancy
- 2) Hyperthyroidism
- 3) Severe anemia
- 4) Severe active hemorrhage
- 5) Thrombocytopenia
- 6) Cardiovascular instability
- 7) Patient on ACE inhibitors

Side effects:-

- 1) Epiphora
- 2) Rhinitis
- 3) Cough
- 4) Headache
- 5) Nausea & vomiting

Goals of ozone therapy

- 1) Inactivates viruses ,bacteria ,yeast ,fungus & protozoa
- 2) Stimulates the immune system, cleans arteries ,veins & improves circulation
- 3) Purifies the blood & lymph
- 4) Normalizes hormones & enzyme production
- 5) Reduces inflammation & pain
- 6) Stops bleeding
- 7) Prevents shock & stroke damage
- 8) Reduces cardiac arrhythmia
- 9) Improves brain function & memory
- 10) Oxidizes toxins allowing their excretion chelates heavy metals

Ozone Intoxification

- 1) Patient must be place in supine position
- 2) Inhale humid oxygen
- 3) Ascorbic acid
- 4) Vitamin E
- 5) Acetylcysteine

Different forms of ozone in periodontal therapy

Different application modalities are available using ozone gas, irrigation with ozonated water & in office as well as at home use of ozonated water. [8]

A) Ozone in gaseous form

A customized suck down thermoformed hard or medium –soft dental appliance should extend 2-3mm beyond the affected gingival area, leaving a free space for gas circulation. Two ports should be attached for the gas inlet and outlet respectively at the distal and mesial of the treatment area. The edges of the appliance must be reclined with light or medium body silicon. Light cured dam can also be applied as an extra safety precaution to

completely seal the border. The ports to the generator and suction should be attached then. This procedure will treat both hard and soft tissue of the affected area. Poly vinyl chloride (PVC) or silicon cap can be used to treat individually all the indicated areas in difficult situations where such an appliance is hard to use or uncomfortable to the patients. In the study of gaseous ozone showed selective efficacy to reduce adherent bacteria on titanium or zirconia without affecting adhesion and proliferation of osteoblastic cells. *Porphyromonas gingivalis* was eliminated by ozone from all surfaces within 24 second to below the detection limit (99.94%) where *Streptococcus sanguis* was more resistant and showed the highest reduction on zirconia substrate (90%).^[9] Ozone gas has a high oxidation potential & 1.5 times greater than chloride when used as antimicrobial agent against bacteria, viruses, fungi and protozoa.

B) Ozonated water

Periodontal disease is multifactorial disease. The sulci and pockets are irrigated with ozonated water to reduce the initial microbial load and to reduce the level of inflammatory mediators such as interleukin-1 β , prostaglandin E₂, interleukin -10 and interleukin gamma in GCF. In accordance with the study by Baysan et al (2000) and Azarparhooch et al (2008) it is observed that ozonized water 4mg /lit was highly effective in reducing gram positive and gram negative organisms like *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*. This could be because, rapid inactivation of microorganisms by rupture of cell wall membrane of organisms associated with the lesion. It was also observed that *Porphyromonas gingivalis*, *Prevotella intermedia* were more sensitive to ozonized water compared to *Fusobacterium nucleatum* in pure culture. Subgingival irrigation with ozonized water is beneficial adjunct treatment modality to enhance periodontal health and has significant role in periodontal therapy. Subgingival invasive and non invasive technique with ozonized water reduces gingival inflammation and inhibits attachment loss. It is considered that the gingival index is improved at 30th day due to accelerated healing of oral mucosa associating haemostatic action and bactericidal effects of ozonized water. Ramzy et al (2005) stated that the significant reduction in plaque index at specific site in ozonized water group at 30th day. They also found that treatment of ozonized water inhibited strongly the formation of dental plaque biofilm on decalcified human

tooth. This may be due to the reduction of live bacteria in their invitro study. These findings suggest that ozonized water with antiplaque activity might be effective as disinfectant solution. Brauner has demonstrated that the combination of professional tooth cleaning and daily rinsing of the mouth with ozone water can improve clinical findings in cases of gingivitis and periodontitis. Plaque indices and a tendency to bleed, however, quickly return if the professional measures are interrupted. Rinsing the mouth with ozone water without any mechanical procedures for plaque reduction were unsuccessful.^[12] Dodwad et al (2011) compared the effect of oral irrigation with ozonated water, 0.2% Chlorhexidine and 10% Povidone iodine in patients with chronic periodontitis. The study concluded that local ozone application can serve as potent atraumatic, antimicrobial agent to treat periodontal disease non-surgically both for home care and professional practice. It may also serve as good tool during supportive periodontal therapy. Nagayoshi et al (2004) studied the effect of ozone water on oral microorganisms and dental plaque. Dental plaque samples are treated with 4ml of ozone water for 10 second and was observed that gram positive and gram negative oral microorganisms and candida albicans in pure culture as well as bacteria in plaque biofilm were killed, hence it was used to control oral microorganisms in dental plaque. Huth et al (2006) in their study declared that the aqueous form of ozone, as a potential antiseptic agent, showed less cytotoxicity than gaseous ozone or established anti microbials under most conditions. Therefore, aqueous ozone fulfils optimal cell biological characteristics in terms of biocompatibility for oral application. Ebensberger et al (2002) concluded that the effect of ozonated water on proliferation of cells in periodontal ligament has resulted in the decontamination of root surface, without negative effect on the remaining periodontal cells on root surface.^[10]

C) Ozonated Oil

Ozonated oils are pure plant extracts, through which pure oxygen and ozone are passed. The plant extracts undergoes chemical reaction to form a thick, viscous oil, or in some cases, a petroleum jelly-like product. The final product contains ozonides. In addition to gaseous and aqueous form, oils that are ozonized also seem extremely convenient. Though gaseous ozone was shown to have more effective microbial properties than aqueous form, due to its toxic effects if inhaled,

ozonated water is the most preferred form for use in dentistry. Therefore a safe system for applying gaseous ozone into the periodontal pockets that avoids inhalation still needs to be developed. ^[11]

Effect of ozone on periodontal treatments

i) Scaling and Root planning

As previously enlightened the use of ozonated water is used as irrigant during scaling and root planing; there is solid evidence that the greatest change in probing depth reduction and gain in clinical attachment occurs within 1 to 3 months post scaling and root planing.

ii) Implant

Ozone showed selective efficacy to lower down the bacterial adhesion on titanium and zirconia without affecting adhesion and proliferation of osteoblastic cells. *P.gingivalis* eliminated by ozone from all surfaces within 24 seconds to below the detection limits while *S.sanguis* showed high reduction on zirconia substrates. It helps in bone regeneration. In conventionally prepared socket, ozone is bubbled for 40 seconds and implants are placed. In cases of peri-implantitis, ozone therapy showed elimination.

Healing

According to Andreas Filippi et al ozonized water applied on daily basis on oral mucosa can increase the healing rate .It should be noted that the influence of ozone leads to a higher expression of cytokines that are important for wound healing especially TGF-B1.^[13] It is an important substance for regulation and co-ordination in the initial wound healing phase. TGF-B1 has marked influence on cell proliferation, chemotaxis, angiogenesis, synthesis of extracellular matrix & collagen synthesis. ^[14, 15, 16]

Studies addressing the use of ozone in bone regeneration in dentistry are necessary because its effectiveness still unknown.

Exophytic growth

Ozone therapy was used in the form of topical ozonated oil on exophytic enlarged gingival lesion and therapeutic benefits which were obtained after short term application .After application of ozonated oil patient reported less pain, discomfort ,improved clinical inflammation sign and reduced ulceration on overlying epithelium of the lesion with an improved sign of healing.Few authors^[17,18,19] have proposed that the base oils (olive,sunflower ,coconut oil etc)during their

ozonation process trap O₃ in the form of stable ozonide. More over the phenomenon of an improved wound healing after ozone therapy has been related to rapidly changing cell types and to the release of cytokines that modulate the complex healing process. ^[20-22]

The reduced inflammatory sign can be attributed to the oxidizing potential of ozone. ^[23]Ozone has been shown to oxidize prostaglandins. ^[24] Prostaglandin is a biologically active compound that participates in inflammatory reactions and thus local application of ozonides may promote the removal of inflammatory phenomenon.

Recurrent infection

Ozone can be topically used in recurrent Aphous ulceration (minor and major aphthae) ,Herpetiform variant,Behcets disease,Coleic disease.Apparent beneficial use of topical ozone application using the Healozone appliance.

On Blood cells

Ozone by changing the protein of erythrocyte and cytoskeleton increases the elasticity of the cell membrane which reduces adhesion between cells and allows their flow even through the smallest capillaries thus improving tissue oxygenation.The whole process is associated with the stimulation of erythrocytes metabolism and increased ATP production with some involvement of H₂O₂ Researchers put forward the hypothesis that reinfusion of ozonated blood serum can cause increased release of nitric oxide responsible for vasodilation .There is a stimulation of the production of glutathione peroxidase, catalase and superoxide dismutase which act as free radical scavengers .^[25,26]

On leukocytes

Ozone behaves as a weak cytokine such as TNF α , IL-2, IL-6, and TGF- β inducers. H₂O₂ is one of the most significant cytokines inducer, which is formed when ozone reacts with the unsaturated fatty acids of the lipid layer in cellular membranes. ^[27, 28]

On platelets

H₂O₂ generated by blood ozonation activate phospholipase C, phospholipase A₂ , cyclooxygenates and lipo-oxygenase and thromboxan synthetase , allowing a step increase of intracellular Ca₂ , release of prostaglandin E₂ , prostaglandins F_{2a} and Thromboxane A₂ with irreversible platelet aggregation. ^[29, 30]

On Pain

Reduced pain can be related to the membrane stabilizing effect of ozone on the pain receptors cells. The excitability threshold of the pain receptor cell membrane can be increased with ozone. The direct oxidation of protein (algopeptides) is another mechanism which may be responsible for the reduction in the pain.^[31] Algopeptides are proteins which help in the transportation of the pain impulses to the brain and are formed in the place of tissue damage.

Bacteria, Virus and Protozoa

Bacteria

Ozone acts by oxidation of their lipid and lipoproteins components on bacterial cell membranes. There is evidence for interaction with proteins as well.^[32, 33] Ozone seems to render the spores defective in germination, perhaps because of damage to the spores of inner membrane.^[34]

Virus

Viruses are susceptible to ozone, yet differ widely in their susceptibility. Lipid-enveloped viruses are sensitive to ozone. Analysis of viral component showed damage to polypeptide chains and envelope proteins impairing the viral attachment capability and breakage of viral RNA.^[35, 36]

Protozoa and Fungal

Ozone inhibits cell growth at certain stages.^[37]

Hs-CRP

CRP is an acute phase reactant that is produced in the liver and circulates in the blood. It has been suggested that pro-inflammatory cytokines are released into the blood stream, causing a systemic inflammatory response, measurable as an increase in CRP levels treatment with ozonated water (SRP) showed significant reduction in hs-CRP.

Antihypoxic effect

Ozone brings about the rise of pO_2 in tissues and improves transportation of oxygen in blood, which results in change of cellular metabolism- activation of aerobic processes (glycolysis, Krebs cycle, beta-oxidation of fatty acids) and use of energetic resources. Repeating low doses of ozone activate enzymes: super- oxide dismutase, catalases, dehydrogenase, and glutatione peroxidise. They are part of complex enzymatic systems which protects organisms against the action of oxygen-free radicals. 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 revitalizing organic functions.^[38] Ozone improves the metabolism of inflamed tissue by increasing their oxygenation and reduced local inflammatory processes. By changing the cell membrane structures of erythrocytes and causing the increase of its negative charge it influences the structure change as well as blood cell elasticity. This is consequence reduces blood cell rolling and enables blood flow in capillary vessels. By increasing the concentration of 2, 3 Diphosphoglycerate (2, 3-DPG), ozone changes the configuration of erythrocytes, which enable them to return oxygen in the inflamed tissues^[39].

Immune stimulation

Cellular and humoral immune system is influenced by ozone. It helps in synthesis of immunoglobulin and also stimulates the proliferation of immunocompetent cells. It increases sensitivity of micro-organisms to phagocytosis and also activates the function of macrophages. Ozone causes the synthesis of biologically active substances such as interleukins, leukotrienes and prostaglandins which helps in reducing inflammation and wound healing^[38]. Huth et al (2007) examined the effect of ozone on the influence on host immune response. These researchers chose the NF-KappaB system, a paradigm for inflammation-associated signalling /transcription. Their results showed 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 Huth study establishes a condition under which aqueous ozone exerts inhibitory effects on the NF-kappaB system, suggesting that it has an anti-inflammatory capacity.

Biosynthetic Effect

It activates mechanism of protein synthesis, increase amount of ribosomes and mitochondria in cells. These changes on the cellular level explain elevation of functional activity and regeneration potential of tissue and organs.^[38] Ozone causes secretion of vasodilators such as NO, which is responsible for dilatation of arterioles and venules.^[38] It also activates angiogenesis.^[39] Ozone, when acting on the organic substance of mineralized tooth tissue intensifies their remineralization potential. At the same time, it is capable of “opening” dentinal tubules, which enables the

diffusion of calcium and phosphorus ions to the deeper layer of carious cavities. [40]

MMP

The effect of ozonotherapy on salivary levels of extracellular matrix metalloproteinases was assessed in patients with periodontitis. The MMP transcription requires the presence of certain inflammatory mediators IL-1 β , TNF- α , TGF- β , PGE2 or growth factors. Bocci emphasizes that ozonotherapy can progressively inhibit MMP secretion through a gradual decrease in plasma levels of PAF, LTB4, PGE2 & TXA2. [41] The author also claims that only long term at least 6 months ozone application can effectively delimit chronic periodontitis. Ozone therapy combined with SRP leads to decrease in salivary MMP concentration in patient with aggressive periodontitis.

On Haemorrhage

In vitro study indicated that arrest of bleeding under the influence of ozone is due to the formation of fibrin membrane on the surface of flowing blood, leading to rapid and effective haemostasis. These haemostatic changes have been attributed to increased release of growth factors after the ozone treatment consequently leading to vasoconstriction and platelet aggregation.

Ozone toxicity

Gaseous form of ozone can be toxic to bronchial-pulmonary system, so this gas should not be

REFERENCES

1. Stopka P. Ozon Progredent 2003; 6:8-11.
2. Sameer M, Monica M. Ozone treating dental infections. Ind J Stomatol 2011; 2:256-9.
3. Baysan A, Whiley R. Antimicrobial effects of a novel ozone generating device on microorganisms associated with primary root carious lesion in vitro caries Res 2000;34:498-501.
4. Baysan A, Beighton D. Assessment of the Ozone-Mediated Killing of bacteria in infected dentine associated with non-cavitated occlusal carious lesions caries Res 2007;41:337-341.
5. Gopalakrishnan S, Parthiban S. Ozone – A New Revolution in Dentistry. J BioInnov. 2012; 1(3):58-59.
6. Holmes J. Ozone and Ozone Therapy: An historical perspective 2006;
7. Komali G. Ozone Therapy. A Revolutionary Non - invasive Therapy in Dentistry. Open Access Scientific Reports 2012; 1(10):473.
8. Gupta G, Mansi B. Ozone therapy in Periodontics. JMedLife.2012; 5:59-67.
9. Hauser-Gerspach ,Vadasz J, Dernojic I, Gass C, Meyer J, et al (2012) .Influence of gaseous ozone in peri-implantitis : bactericidal efficacy and cellular response. An in vitro study using titanium and zirconia. Clin Oral. Investig 16:1049-1059.
10. Ebensberger U, Pohl Y, Filippi A. PCNA-expression of Cementoblasts and Fibroblasts on the root surface after extraoral rinsing for decontamination. Dent Traumatol 2002; 18:262-266.
11. Thanomsub B, Anupunpisit V , Chanphetch S, Watcharachaipong T, Poonkhum R, Srisukonth C. Effects of ozone treatment on cell growth and

inhaled. Pulmonary embolism is occurred when direct intravenous administration of O₂/O₃. Known side effects are epiphora, upper respiratory tract irritation, rhinitis, cough, headache, occasional nausea, vomiting, shortness of breath, blood vessel swelling, poor circulation, heart problems and at times stroke [42]. In the event of ozone intoxication the patient must be placed in supine position, and treated with vitamin E and n-acetylcysteine [43].

CONCLUSION

In contrast with traditional medicines and modalities such as antibiotics and disinfectants, ozone therapy is quite inexpensive, predictable and conservative. The ozone therapy has been more beneficial than present conventional therapeutics modalities. Treating patients with ozone therapy lessens the treatment time with an immense deal of variation and it eradicates the bacterial count more specifically .The treatment is completely painless and increases the patient's 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.

ultrastructural changes in bacteria. *J Gen Appl Microbiol* 2002; 48: 193-9.

12. 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; 21:341-8.

13. Bocci V, Luzzi E, Corradeschi F, Silvestri S, "Studies on the biological effects of ozone: 6. Production of transforming growth factor 1 by human blood after ozone treatment", *J Biol Regul Homeost Agents* 1994; 8:108.

14. Cromack D.T, Porras-Reyes B, Purdy J.A, Pierce G.F, Mustoe T.A, "Acceleration of tissue repair by transforming growth factors β : identification of in vivo mechanism of action with radiotherapy-induced specific healing deficits", *Surgery* 1993;113:36

15. Pierce G.F, Mustoe T.A, Dever T.F." Transforming growth factors β induces increased directed cellular Migration and tissue repair in rats ",*Prog Clin Biol Res* 1988; 266:93

16. Roberts A.B, Sporn M.B "physiological actions and clinical applications of transforming growth factors β (TGF- β)", *Growth factors* 1993;8:1

17. Tranagli V, Zanardi I, Valacchi G, Bocci V. Ozone and Ozonated oils in Skin Disease: A Review *Mediators Inflamm* 2010; 6:10-41.

18. Travagli V, Zanardi I, Bocci V. Topical application of ozone and ozonated oils as anti-infective agent's .an insight into the patent claims .Recent patents on anti-infective drug discovery.2009:4130-42.

19. Rae Ian D .ozonised oils as disinfectants. *Ambix* 2006; 53:3-20.

20. Patel PV, Kumar S, Vidya GD, Patel A, Holmes JC, Kumar V. Cytological Assessment of Healing Palatal Donor Site wound and Grafted Gingival wound after Application of Ozonated Oil: An Eighteen Months Randomized Controlled Clinical Trial. *Acta Cytol*.2012; 56:277-84.

21. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. *Physiol Rev* .2003; 83:835-70.

22. Patel PV, Kumar V, Kumar S, Patel GD. Therapeutic Effect of Topical Ozonated Oil on Epithelial Healing of Palatal Wound Site: A Planimetric and Cytological Study. *J Investig Clin Dent* .2011; 2:248-58.

23. Klichowska –Palonka M, Bachanck T.Ozone therapy for labial and oral inflammations. *Polish Journal of Environmental Studies* 2006; 15:211-13.

24. Friedman M, Madden MC, Samet JM, Koren HS. Effects of ozone exposure on lipid metabolism in human alveolar macrophages. *Environ Health Perspect* 1992; 97:95-101.

25. Mudd JB, Dawson PJ, Santrock J. Ozone does not react with human erythrocyte membrane lipids. *Arch BiochemBiophys*.1997; 341:251-8.

26. Bocci V. The case for oxygen-ozone therapy. *Br J Biomed Sci*.2007; 64:44-9.

27. 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. *AMJ Respir cell Mol Biol*. 1995; 13:60-8.

28. Reth M. Hydrogen peroxide as second messenger in lymphocytes activation. *Nat Immunol* 2002; 3:1129-34.

29. Iuliano L, Colavita AR, Leo R, Pratico D, Violi F. Oxygen free radicals and platelet activation. *Free RadicBiol Med*. 1997; 22:999-1006.

30. Valacchi G, Bocci V. Studies on the biological effects of ozone. Release of factors from ozonated human platelets. *Mediators Inflamm*. 1999; 8:205-9.

31. Steppan JC, Boxley Murphy K, Muto M, Balagurunathan K, Meaders T. Ozone's mechanism of action for relieving pain associated with herniated intervertebral disc. *J vasc Interv Radiol*.2009; 20:S16-S17.

32. Sawadaishi K, Miura K, Otsuka 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.

33. Mudd JB, Leavitt R, Ongun A, Mc Manus TT. Reaction of ozone with amino acids and proteins. *Atmos Environ* 1969; 3:669-82.

34. Young SB, Setlow P. Mechanism of killing of *Bacillus Subtilis* Spores by Deon and Oxone, two general decontaminants for biological agents. *J ApplMicrobiol*.2004; 96:289-301.

35. Roy D, Wong PK, Engelbrecht RS, Chian ES. Mechanism of enteroviral inactivation by ozone. *Appl Environ Microbiol*.1981; 41:718-23.

36. Burleson GR, Murray TM, Pollard M. Inactivation of viruses and bacteria by ozone, with and without sonication. *Applmicrobiol*.1975; 29:340-4.

37. Bocci VA. Scientific and medical aspects of ozone therapy .State of the art. *Arch Med Res*.2006; 37:425-35.

38. Seidler V, Linetskiy I, Hubalkova H .Ozone and its usage in General Medicine and Dentistry. A Review Article. *Prague Medical Report*.

39. Teresa B, Wolanska E, Cieszko –Buk M, editors. Annales universitatis Maria Curie; Skłodowska. Lubin-Polonia: 2008.
40. Lynch E. Leczenie Prochnicy Z Wykorzystaniem systemu Healozon .e Dentico .2004; 134:3.
41. Bocci V. Ozone: A new medical drug 1st ed Dordrecht. Springer; 2005, p162.
42. 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.
43. Nogales CG, Ferrari PH, Kantorovich EO. Ozone therapy in medicine and dentistry .*J Contemp Dental Pract*.2008; 9:1-9.