

THE IMPLICATIONS OF USING OZONE IN GENERAL DENTAL PRACTICE

Annie V Issac, * Kiran Joseph, ** Rino Roopak Soman, ***
Anju Samuel, † Swaroop Chandy, †† Nithin Mathew Cherian †††

* Senior Lecturer, Department of Periodontology, St Gregorios Dental College, Chelad, India

** Professor & Head, Department of Periodontics, St Gregorios Dental College, Chelad, India

*** Reader, Department of Periodontology, St Gregorios Dental College, Chelad, India

† Senior Lecturer, Department of Periodontology, St Gregorios Dental College, Chelad, India

†† Senior Lecturer, Department of Community Dentistry, St Gregorios Dental College, Chelad, India

††† Senior Lecturer, Department of Oral & Maxillofacial Surgery, St Gregorios Dental College, Kothamangalam, Ernakulam, India

ABSTRACT

Ozone water is successfully used in the treatment of different diseases. Gingival and periodontal diseases represent a major concern both in dentistry and medicine. Majority of the causes and etiologic agent can be treated with ozone water. Several known action of ozone water like antihypoxic, antimicrobial, immunostimulating action can be beneficial in treating periodontal diseases and regeneration.

KEYWORDS: Ozone; cold plasma system; corona discharge system

INTRODUCTION

Ozone has been known as an accompaniment to electrical storms during all the history of mankind. Ozone is a colorless gas form of oxygen and is present in atmosphere. It is one of the most important gases in the stratosphere due to its ability to filter ultraviolet rays, which is critical for the maintenance of biological balance in the biosphere. It has been used to purify water throughout the world for many years as it is highly effective in killing bacteria present in different forms. It effectively kills bacteria, fungi, viruses and parasites at a lower concentration. Christian Friedrich Schönbein (1840), a German Chemist, is regarded as the Father of ozone therapy.^[1] When he passed an electrical discharge through water, a strange smell was produced, which he called Ozone, derived from the Greek word 'OZEIN' which means odor. In 1857 Joachim Hänsler, a German physicist and physician, along with German physician, Hans Wolff, developed the first ozone generator for medical use.^[2] In the 1920s Dr Edwin Parr, a

Swiss dentist, started to use O₃ as part of his disinfection system.^[3] The first dentist who used ozone was Edward Fisch in 1950 for treating Austrian surgeon Ernst Payr for a gangrenous pulpitis and thereby inspired him to begin a line of investigations dedicated to ozone use in health care.^[2]

MECHANISM OF ACTION^{†[4]}

1. Anti-microbial action: Ozone is responsible for cell death which is non-specific but selective for microbial cells and does not cause damage to human body cells because of its major antioxidative property. It causes local damage of cytoplasmic membrane by ozonolysis of dual bonds and ozone-induced modification of intracellular contents which includes oxidation of proteins and loss of organelle functions due to secondary oxidant effects. Ozone is very effective against antibiotic resistant strains. Moreover, in viral infections, its action lies in intolerance of the infected cells to peroxides by reacting with the unsaturated fatty acids of lipid layer in cell membranes and change of activity of reverse transcriptase that takes part in synthesis of viral proteins.

2. Immuno-stimulatory action: Ozone is responsible for influencing cellular and humoral immune system thereby stimulating the proliferation of immuno-competent cells and synthesis of immunoglobulins. It also activates function of macrophages and increases sensitivity of micro-organisms to phagocytosis. These immune cells of the body respond to the action of ozone and produce special messengers called cytokines which are actually weak cytokines. The cytokine molecules then stimulate other immune cells and ultimately resist to the diseases. Ozone causes the synthesis of biologically active

substances such as interleukins, leukotrienes and prostaglandins which helps in reduction of inflammation and finally leads to acceleration of wound healing.

3. Anti-hypoxic 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. Moreover, the flexibility of the erythrocytes is restored as well as the surface structures of the erythrocytes are changed. This leads to reduction of blood cell rolling and enables blood flow in capillary vessels

4. Bioenergetic and biosynthetic effect: Ozone activates the protein synthesis, increases the amount of ribosomes and mitochondria in cells. As a result of which the functional activity of the cells as well as the regeneration potential of tissues and organs is elevated.

ROUTES OF ADMINISTRATION^[5]

Gaseous ozone: Ozone can be used in gaseous form via an open system or via a sealing suction system to avoid inhalation and its adverse effects. Therapeutic methods of administration include injection of ozone gas and insufflations of periodontal pocket with ozone gas. Injection protocols include intraosseous injection of ozone gas mixture locally into the alveolus, subgingivally or intramuscularly. There are three different systems for generating ozone gas.^[6,7]

- i. **Ultraviolet System:** produces low concentrations of ozone, used in esthetics, saunas, and for air purification.
- ii. **Cold Plasma System:** used in air and water purification
- iii. **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.

Appliances producing ozone gas for dental use

HEALOZONE: An air based system by which gas application takes place in a closed circuit with the help of air tight cap. Concentration of gas produced =2100ppm adjacent to the tissue.

OZONYTRON: This system consisting of a glass probe uses the power of high frequency and

voltage and emits electromagnetic energy around the treated area splitting the environmental diatomic oxygen into single oxygen atom and ozone. Concentration of gas produced =10-100 μ g/ml.

Advantage

No air tight cap present in this system so gas can be applied to gingival/periodontal pockets or root canals.

PROZONE: A system producing preset tissue-compatible dosages of ozone gas in the indicated areas of periodontitis with the help of exchangeable plastic attachments (Perio tips).

Advantage

It is easy to use and safe in application.

Ozonized oil: In addition to gaseous and aqueous form, oils that are ozonized also seems extremely convenient.^[5]

Ozonated water: Ozonated water has been shown to be very effective against bacteria, fungi and viruses.

- Syed Sadatullah in his study used ozonized water which was freshly prepared using the ORM AW600 ozone gas generator machine (ORM Beauty and Health care Sdn Bhd, Petaling Jaya, Malaysia). Ozone gas produced from this ozonizer was introduced into 1 liter of sterile distilled water for 20 minutes. The concentration of dissolved ozone in the water was measured using an EcoZone EZ10W portable dissolved ozone meter (Ecosensors Inc., Newark, USA). The concentration of ozonized water used for this study was between 0.08 ppm and 0.1 ppm. The ozonized water was used within 20 minutes after its preparation.^[8]
- Huth *et al.*, used aqueous ozone in the form of ozonated phosphate buffered saline in photometrically confirmed concentration of 1.25-2.0microgram ml.^[9]
- Benjamas Thanomsub in his study used Ozone water generated with a commercial ozone generator (Brightzone) model OZ100 (Brightgreen Technology Co. Ltd., Hsin-Tien City, Taiwan) using atmospheric air as the oxygen source. The ozonated air produced at a constant flow rate by the apparatus was passed by a silicone tube to a diffuser.^[10]
- Ozonated water has a half-life of about only 20 min and will degrade back into oxygen

very quickly, so it should be used within the first 5–10 min to assure its potency. To overcome this disadvantage of ozonated water, Sae Hayakumo et al used ozone nanobubble water (NBW3) which was developed using nanobubble generating technology by Chiba et al. The procedure of generating nanobubble water has been protected by patent.¹¹

INDICATIONS OF OZONE THERAPY^[12]

In Medicine

- Rheumatic disease
- Skin Lesions
- Ulcers
- Inflammatory condition
- Viral infections
- Circulatory disorder

In Dentistry^[13]

- Dental Caries
- Denture cleaning
- Bleaching of discoloured tooth
- Adjunct to scaling and rootplaning
- Soft tissue pathosis

Contraindications of Ozone Therapy^[14]

1. Pregnancy.
2. Severe anemia
3. Recent myocardial infarction
4. Glucose-6-phosphate-dehydrogenase deficiency
5. Hyperthyroidism.
6. Severe myasthenia.
7. Acute alcohol intoxication.

OZONE IN DENTISTRY

According to German dentist Fritz Kramer, ozone, such as in the form of ozonized water can be used in the following ways.

1. As a powerful disinfectant
2. Ability to control bleeding
3. Ability to cleanse wounds in bones and soft tissues.
4. By increasing the local supply of oxygen to the wound area, ozone can improve healing.
5. Ozonized water can increase temperature in the area of the wound, and this increase the metabolic processes related to wound healing

Dr. Kramer points out that ozonized water can be used in a number of different ways^[15]

1. As a mouth rinse (especially in cases of Gingivitis, Periodontitis, Thrush or Stomatitis);

2. As a spray to cleanse the affected area and to disinfect oral mucosa, cavities and in general dental surgery.

3. As an ozone/water jet to clean cavities of teeth being capped, receiving root canal therapy, and in treating painful gingivitis and stomatitis

Ozone has been applied for treatment of early carious lesions, sterilization of cavities, root canals,^[16] periodontal pockets, enhancing epithelial wound healing such as ulcerations and herpetic lesions, bleaching of discoloured root canal treated teeth, desensitization of extremely sensitive teeth, and as a rinse for the avulsed teeth or as a denture cleaner and decontamination of used tooth brush.^[17] In implant dentistry, the use of ozone is currently being investigated for the decontamination of the implant surface in peri-implant therapy.^[18]

CLINICAL APPLICATIONS OF OZONE IN DENTISTRY

Treatment of Dental Caries

Aqueous as well as gaseous ozone were used in the treatment of dental caries. Even though aqueous ozone has less viability on various microorganisms^[19] it was found to be less cytotoxic than the gaseous form.^[9] Ozone is applied through a hand piece with a silicon cup that fit tightly to the application site. The bactericidal effect of ozone on carious bacteria is by oxidizing the bacterial cell wall^[20,21] and by oxidizing the pyruvic acid that cause progression of caries to acetic acid and carbon dioxide.^[22]

Numerous studies proved its effectiveness in the treatment of pit and fissure caries, root caries and interproximal caries. In vitro studies showed that there was significant reduction in number of microorganism after Ozone therapy on pit and fissure caries and occlusal caries.^[16,23] Ozone therapy was found to be effective against Lactobacilli and some strains of streptococci.^[24]

Some authors suggested that since ozone is a potent oxidizer, it might promote remineralization of demineralized dentin^[25,26] but Zvara *et al.*, concluded that there was no remineralization of dentin and there was subsequent demineralization of remineralized dentin.^[27]

Endodontics

Ozone therapy in endodontic centres on its antimicrobial activity and its biocompatibility to periapical tissues.^[16] In vitro studies showed that ozone was effective over most of the bacteria

found in cases of pulp necrosis.^[28,31] It is used in the form of ozonized water, ozonized sunflower oil, and ozone gas. Using in-vitro research model it was established that ozone therapy was effective against bacterial strains such as Streptococci, staphylococci Enterococcus, Escherichia coli and Candida albicans.^[29,30] Efficacy of ozonized water on Enterococcus faecalis and Streptococcus mutans was studied by Nagayoshi *et al.*, and confirmed that ozonized water was highly effective in killing intratubular bacteria.^[31] Estrela *et al.*, concluded that irrigation of infected human root canals with ozonated water, 2.5% NaOCl, 2% chlorhexidine and the application of gaseous ozone for 20 min was not sufficient to inactivate *E. faecalis*^[32] but studies by Chang *et al.*, suggested that Enterococcus faecalis the prominent bacteria in root canal can be eliminated by ozone therapy.^[33] Crown discoloration is treated placing the bleaching agent into the inner of the tooth then the crown is irradiated with ozone for minimum of 3-4 min. This ozone treatment bleaches the tooth within minutes.^[13] Ozone can effectively terminate the root sensitivity. Swift relief from root sensitivity has been documented after ozone spray for 60 s followed by mineral wash onto the exposed dentine in a repetitive manner. Ozone removes this smear layer, opens up the dentinal tubules, broadens their diameter and then Calcium and Fluoride ions flow into the tubules easily, deeply and effectively to plug the dentinal tubules, preventing the fluid exchange through these tubules.^[13]

Prosthodontics

Denture stomatitis can be prevented by denture disinfection. *Candida albicans* the major causative microorganism for stomatitis is susceptible to antibacterial action of ozone. *C. albicans* were detected after exposure to flowing 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.^[29] Even though both gaseous ozone and ozonated water are used for denture cleansing, gaseous ozone was found to be more effective.^[34]

Oral Medicine and Radiology

All virus are susceptible to ozone but differ in their susceptibility. Research had suggested that antiviral action of ozone may be due to damage to protein capsid or lipid envelop. Virus do not have

enzymatic protection against oxidative conformation so lipid envelop virus like Herpes Virus, Epsilon Bar Virus are sensitive to ozone therapy.^[35] Ozonated oil applied on herpes labialis and mandibular osteomyelitis demonstrated faster healing times than conventional treatment.^[36] Macedo and Cardoso reported that application of ozonated oil on herpes labialis and mandibular osteomyelitis demonstrated faster healing time.^[37] Clavo *et al* in their study concluded that the ozone therapy can produce an improvement in blood flow and oxygenation in some tissues & appears to have had some positive effect during the treatment of patients with advanced head & neck tumors.^[38]

Periodontics

The use of ozone in periodontics utilizes antimicrobial action against the gram positive and gram negative microflora, viruses and fungi. Ebersberger *et al* evaluated the effect of irrigation with ozonized water on the proliferation of cells in the periodontal ligament adhering to the root surfaces of 23 freshly extracted completely erupted third molars and concluded that the 2 min irrigation of the avulsed teeth with non-isotonic ozonized water might lead not only to a mechanical cleansing, but also decontaminate the root surface.^[39] Nagayoshi *et al.*, examined the effect of ozonized water on oral microorganisms and dental plaque. Results showed that, almost no microorganisms were detected after being treated with ozonized water (4 mg/l) for 10 seconds.^[40] Kshitish and Laxman conducted a randomized, double-blind, crossover split-mouth study on the effect of ozonized water on generalized chronic periodontitis and they concluded that despite the substantivity of chlorhexidine, the single irrigation of Ozonized water was quite effective to inactivate microorganisms.^[41]

Oral Surgery

Since ozone improves the rheological properties of erythrocytes and facilitates oxygen release in the tissues, it leads to vasodilatation of vessels and improved supply of blood to ischemic zones. Hence, it is useful in wound healing impairments post radiotherapy treatment, after surgical interventions like tooth extractions or implant dentistry.^[42] After a tooth is extracted or any surgical procedure the area is irrigated and insufflated which promotes faster healing without complications.^[43] Ozone therapy is found to be

beneficial for the treatment of the refractory osteomyelitis in the head and neck in addition to treatment with antibiotic, surgery and hyperbaric oxygen.^[44]

Periimplantitis

Adequate and steady plaque control regimen must be ensured for the prevention of periimplantitis. 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.^[18] In a study gaseous Ozone showed selective efficacy to reduce adherent bacteria on Titanium and Zirconia without affecting adhesion and proliferation of osteoblastic cells. *Porphyromonas gingivalis* was eliminated by Ozone from all surfaces within 24 sec to below the detection limit (99.94%), while *Streptococcus sanguis* was more resistant and showed the highest reduction on zirconia substrates (90%).^[45]

Effect of ozone in Dental Unit Water Line

In the dental clinic, bioaerosols are an important consideration for infection control and occupational health, since infectious agents could be transmitted via aerosols to patients or staff in the confines of the dental unit. Dental unit water line can be purified with ozone. Chlorhexidine and Povidone iodine showed similar effects in reducing the aerobic and anaerobic CFU's at all the three levels. Though Ozone showed lesser CFU reductions in both aerobic and anaerobic colonies, taking into account the disadvantages of CHX and PI; OZ can be used as preprocedural rinse. Preprocedural rinsing may also be of value in protecting patients and dental professionals from the transmissible diseases. In model dental unit water lines, Ozone achieved 57% reduction in biofilms and 65% reduction in viable bacteria in spite of being used in a very low dose and with a short time of application.

OZONE TOXICITY

The known side effects are epiphora, upper respiratory tract irritation, rhinitis, cough, headache, occasional nausea and vomiting.

CONCLUSION

Dentistry is changing as we are now using modern science to practice dentistry. In comparison with classic medicine modalities such

as antibiotics and disinfectants, ozonized water therapy is quite inexpensive, predictable, conservative and beneficial. The treatment is completely painless and increases the patients compliance with minimal adverse effect. Although more clinical research has to be done to standardize treatment procedure of ozone therapy still many different approaches are so promising.

CONFLICT OF INTEREST & SOURCE OF FUNDING

The author declares that there is no source of funding and there is no conflict of interest among all authors.

BIBLIOGRAPHY

1. Seaverson K, Tschetter D, Kaur T. Patient guide to oxygen/ ozone therapy. Health centered cosmetic dentistry. [Online]. [Cited 2010 January 13]. Available from: URL:<http://www.toothbythelake.net/ozone_therapy.html>
2. Chitnis P, Bhusari BM. Ozone Therapy : A New Revolution In Periodontics International Journal of Innovative Research & Development. 2013;5:1173-80.
3. Bocci VA. Scientific and medical aspects of ozone therapy. State of the art. Arch Med Res 2006;37:425-35.
4. Adalja C. Ozone: A Future In Periodontal Therapy. Indian Journal of Applied Research 2013;3:295-7.
5. Gupta G, Mansi B. Ozone therapy in periodontics. J Med Life 2012;5:59-67.
6. Nogales CG, Ferrari PA, Kantorovich EO, Lage-Marques JL. Ozone therapy in medicine and dentistry. J Contemp Dent Pract 2008;4:75-84.
7. Garg R, Tandon S. Ozone: a new face of dentistry. Int J Dent Sci 2009;7:2.
8. Sadatullah S. The antimicrobial effect of 0.1 ppm ozonated water on 24-hour plaque microorganisms in situ. Braz Oral Res 2012;26:126-31.
9. Huth KC, Jakob FM, Saugel B, Cappello C, Paschos E, Hollweck R, *et al.* Effect of ozone on oral cells compared with established antimicrobials. Eur J Oral Sci 2006;114:435-40.
10. Thanomsut B, Anupunpisit V, Chanphetch S, Watcharachaipong T, Poonkhum R, Srisukonth C. Effects of ozone treatment on

- cell growth and ultra structural changes in bacteria. *J Gen Appl Microbiol.* 2002;48:193-9.
11. Hayakumo S. Clinical and microbiological effects of ozone nano-bubble water irrigation as an adjunct to mechanical subgingival debridement in periodontitis patients in a randomized controlled trial. *Clin Oral Investig* 2013;17:379-388.
 12. Seidler V, Linetskiy I, Hubalkova H. Ozone and its usage in general medicine and dentistry-a review article. *Prag Med Rep* 2008;109:5-13.
 13. Bhateja S. The miraculous healing therapy "Ozone therapy" in dentistry. *Indian Journal of Dentistry* 2012;3:150-5.
 14. Johnson RD. Oxygen/ozone therapy. Alternative approach to dental health. [Online] [Cited 2010 January 13]. Available from:URL:<Dr.runarjohnson.Com/ozone.Htm>.
 15. Krammer F. Ozone in the dental practice. Medical applications of ozone. Norwalk, CT: International ozone association. Pan American committee 1983;258-65.
 16. Baysan A, Beighton D. Assessment of the ozone-mediated killing of bacteria in infected dentine associated with noncavitated occlusal carious lesions. *Caries Res* 2007;41:337-41.
 17. Gopalakrishnan S, Parthiban S. Ozone a new revolution in dentistry. *J Bio Innov* 2012;3:58-69.
 18. Karapetian VE, Neugebauer J, Clausnitzer CE, Zoller JE. Comparison of different periimplantitis treatment methods. http://www.helbo.at/datasheets/poster_karapetian_0304.pdf
 19. Müller P, Guggenheim B, Schmidlin PR. Efficacy of gasiform ozone and photodynamic therapy on a multispecies oral biofilm in vitro. *Eur J Oral Sci* 2007;115: 77-80.
 20. Sagai M, Bocci V. Mechanisms of Action Involved in Ozone Therapy: Is healing induced via a mild oxidative stress? *Med Gas Res* 2011;1:29.
 21. Magni E, Hickel R, Nicoletalli. Influence of gasiform ozone on the micromechanical properties of dentin. *International Dentistry SA* 11: 5.
 22. Komali G. Ozone Therapy-A Revolutionary Noninvasive Therapy in Dentistry. *Open Access Scientific Reports* 2012;1:473.
 23. Baysan A, Whiley RA, Lynch E. Antimicrobial effect of novel ozone - generating device on micro-organisms associated with primary root carious lesions in-vitro. *Caries Res* 2000;34:498-501.
 24. Fagrell TG, Dietz W, Lingstrom P, Steiniger F, Noren JG. Effect of ozone treatment on different cariogenic microorganisms in vitro. *Swed Dent J* 2008;32(3):139-47.
 25. Gupta M, Abhishek. Ozone: An Emerging Prospect In Dentistry. *Indian Journal of Dental Sciences* 2012;4:47-50.
 26. Atabeka D, Oztas N. Effectiveness of Ozone with or without the additional use of remineralizing solution on non cavitated fissure carious lesions in permanent molars. *Eur J Dent* 2011;5:393-9.
 27. Zaura E, Buijs MJ, Ten Cate JM. Effect of ozone and sodium hypochlorite on caries-like lesions in dentin. *Caries Res* 2007;41:489-92.
 28. Velano HE, do Nascimento LC, de Barros LM, Panzeri H. In vitro assessment of antibacterial activity of ozonized water against *Staphylococcus aureus*. *Pesqui Odontol Bras* 2001;15:18-22.
 29. Arita M, Nagayoshi M, Fukuizumi T, Okinaga T, Masumi S, Morikawa M, *et al*. Microbicidal efficacy of ozonated water against *Candida albicans* adhering to acrylic denture plates. *Oral Microbiol Immunol* 2005;20:206-10.
 30. Hems RS, Gulabivala K, Ng YL, Ready D, Spratt DA. An in vitro evaluation of the ability of ozone to kill a strain of *Enterococcus faecalis*. *Int Endod J* 2005;38(1):22-9.
 31. Nagayoshi M, Kitamura C, Fukuizumi T, Nishihara T, Terashita M. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. *J Endod* 2004;30:778-81.
 32. Estrela C, Estrela CRA, Decurcio DA, Hollanda ACB, Silva JA. Antimicrobial efficacy of ozonated water, gaseous ozone,

- sodium hypochlorite and chlorhexidine in infected human root canals. *Int Endod J* 2007;40:85-93.
33. Chang H, Fulton, Lynch E. Antimicrobial efficacy of ozone on *Enterococcus faecalis*. IADR Abstract 2003
 34. Oizumi M, Suzuki T, Uchida M, Furuya J, Okamoto Y. In vitro testing of a denture cleaning method using ozone. *J Med Dent Sci* 1998;45:135-9.
 35. Riesser V, Perrich J. Possible mechanism of poliovirus inactivation by ozone. In: Forum on Ozone Disinfection. Proceedings of the International Ozone Institute. Syracuse, NY, 1977:186-92.
 36. Nogales CG, Ferrari PH, Kantorovich EO, Lage-Marques JL. Ozone therapy in medicine and dentistry. *J Contemp Dent Pract* 2008;9:75-84.
 37. Macedo SB, Cardoso C. The use of ozone in Dentistry. 160 Campinas International Conclave 2005;115. Retrieved from <<http://www.articlesbase.com/health-articles/ozone-therapyin-dentalpractice-a-new-face-2360543.html>>.
 38. Clavo B, Ruiz A, Lloret M. Adjuvant ozone therapy in advanced head and neck tumors: a comparative study. *eCAM* 2004:1-5.
 39. 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.
 40. Nagayoshi M, Fukuizumi T, Kitamura C, Yano J, Terashita M, Nishihara T. Efficacy of ozone on survival and permeability of oral microorganisms. *Oral Microbiol Immunol* 2004;19:240.
 41. Kshitish D, Laxman VK. The use of ozonated water and 0.2% chlorhexidine in the treatment of periodontitis patients: A clinical and microbiologic study. *Indian J Dent Res* 2010;21:341-348.
 42. Stubinger S, Sader R, Filippi A. The use of ozone in dentistry and maxillofacial surgery: A review. *Quintessence Int* 2006;37:5:353-9.
 43. Filippi A. The influence of ozonised water on the epithelial wound healing process in the oral cavity. *Deutsche Zahnärztliche Zeitschrift* 1997;56:104-8.
 44. Walker JT, Bradshaw DJ, Fulford MR, Marsh PD. Microbiological evaluation of a range of disinfectant products to control mixed- species biofilm contamination in a laboratory model of a dental unit water system. *Appl Environ Microbiol* 2003;69:3327-32.
 45. Hauser-Gerspach I, Vadaszan J, Deronjic I, Gass C, Meyer J. Influence of gaseous ozone in peri-implantitis: bactericidal efficacy and cellular response. An in vitro study using titanium and zirconia. *Clin Oral Investig* 2012;16:1049-59.