The Saudi Journal for Dental Research (2017) 8, 105–111

King Saud University

The Saudi Journal for Dental Research

www.ksu.edu.sa
www.sciencedirect.com

Dental applications of ozone therapy: A review of literature

Sansriti Tiwari [a,*], **Alok Avinash** [a], **Shashank Katiyar** [b], **A. Aarthi Iyer** [c], **Suyog Jain** [d]

[a] *Department of Pedodontics & Preventive Dentistry, Rungta College of Dental Sciences, Bhilai, C.G, India*
[b] *Department of Orthodontics and Dentofacial Orthopedics, Rungta College of Dental Sciences, Bhilai, C.G, India*
[c] *Department of Conservative Dentistry and Endodontics, Rungta College of Dental Sciences, Bhilai, C.G, India*
[d] *Department of Pedodontics & Preventive Dentistry Pedodontics & Preventive Dentistry, Rungta College of Dental Sciences, Bhilai, C.G, India*

Received 4 May 2016; revised 9 June 2016; accepted 17 June 2016
Available online 5 July 2016

KEYWORDS

Dental;
Dentistry;
Ozone therapy;
Oxygen spa

Abstract Ozone has been used successfully for the treatment of various diseases for more than a decade. Its unique properties include immunostimulant, analgesic, antihypnotic, detoxicating, antimicrobial, bioenergetic and biosynthetic actions. Its atraumatic, painless, non invasive nature, and relative absence of discomfort and side effects increase the patient's acceptability and compliance thus making it an ideal treatment choice specially for pediatric patients. This review is an attempt to highlight various treatment modalities of ozone therapy and its possible clinical applications in future.

Contents

* Corresponding author at: Department of Pedodontics & Preventive Dentistry, Rungta College of Dental Sciences, Kohka- Kurud Road, Bhilai 490024, C.G, India.
E-mail address: drsansriti@gmail.com (S. Tiwari).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjdr.2016.06.005

1. Introduction

Ozone (O_3) is a natural gaseous molecule made up of three oxygen atoms. The word ozone originates from the Greek word ozein, which means odor and was first used in 1840 by German chemist Christian Friedrich Schonbein "The father of ozone therapy."[1] The stratosphere layer of the atmosphere contains abundance of ozone[2] and it protects the living organisms from the ultraviolet rays. Ozone is heavier than air and hence it falls downward to earth from such high altitudes.[3] It cleanses the air and combines with any pollutant that it comes in contact. This is earth's natural way of self-cleansing.[4]

Since more than 100 years medical grade ozone has been used as one of the non-medication methods of treatment. The first dentist to use ozone therapy in his practice was E. A. Fisch in the 1930's, to aid in disinfection and wound healing during dental surgeries.[5] The main use of ozone in dentistry relays on its antimicrobial properties.[6]

Ozone therapy can be defined as a versatile bio-oxidative therapy in which oxygen/ozone is administered via gas or dissolved in water or oil base to obtain therapeutic benefits.[7]

2. Structure

Three oxygen atoms constitute to form a tri-atomic molecule of ozone. Equal oxygen – oxygen bonds bound them together at an obtuse angle of 116 °C. The structure of ozone has an internal steric hindrance that prevents it from forming a triangular structure.[8] As a result of this, instead of forming the expected double bonds each oxygen atom forms a single bond with the another oxygen atom resulting in a negative charge throughout the ozone molecule.[2]

3. Properties

Ozone exists as colorless gas, with a pungent odor at room temperature, detectable even at concentrations as low as 0.02–0.05 ppm.[9] Its half life varies with temperature variation. At 20 °C it has a half-life of 40 min at 0 °C about 140 min.[10]

4. Ozone production

Oxygen molecules in the air combines under the influence of factors such as ultraviolet radiation (from the sun) and electrical discharges (lightning). Intense physical stress on water (such as in areas of waterfalls and ocean waves crashing onto rocks) also results in production of ozone in nature.[11] For medical use highly specific gazettes known as Ozone Generators are used for production of ozone. Medical grade oxygen is made to flow through high voltage tubes with outputs ranging from 4000 V to 14000 V. The Ozone Generators work on one of the three principles: Ultra-violet light lamp, Corona discharge or Cold plasma.[12–14] In dentistry, there are two widely used ozone units : the heal ozone[15] and ozotop.[11]

5. Mechanism of action

Ozone therapy has a wide range of applications in treating various diseases owing to its unique properties including antimicrobial, immunostimulant, analgesic, antihypnotic, detoxicating, bioenergetic and biosynthetic actions.

5.1. Anti-microbial effect

Ozone causes inactivation of bacteria, viruses, fungi, yeast and protozoa. It disrupts the integrity of the bacterial cell envelope by oxidation of phospholipids and lipoproteins. Ozone at low concentration of 0.1 ppm, is sufficient to inactivate bacterial cells including their spores.[16] In fungi, O_3 inhibits cell growth at certain stages, budding cells being the most sensitive.[17] With viruses, the O_3 damages the viral capsid and upsets the reproductive cycle by disrupting the virus-to-cell contact with peroxidation.[18]

5.2. Stimulation of oxygen metabolism

Ozone therapy causes an increase in the red blood cell glycolysis rate. This leads to the stimulation of 2,3-diphosphoglycerate leading to an increase in the amount of oxygen released to the tissues. 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 stimulation of production of enzymes which act as free radical scavengers and cell-wall protectors: glutathione peroxidase, catalase and superoxide dismutase and prostacycline, a vasodilator.[19]

5.3. Activation of immune system

Ozone administered at a concentration of between 30 and 55 μg/cc causes the greatest increase in the production of interferon and the greatest output of tumor necrosis factor and interleukin-2 that launches an entire cascade of subsequent immunological reactions.[20]

5.4. Mechanism of action of O_3 on the human lung

Ozone exposure induces a significant mean decrement in vital capacity. It significantly increases mean airway resistance and specific airway resistance but does not change dynamic or static pulmonary compliance or viscous or elastic work. It also significantly reduces maximal transpulmonary pressure. And further more significantly increases respiratory rate and decreases tidal volume.[20]

6. Dental applications

Ozone therapy presents great advantages when used as a support for conventional treatments and is indicated for use in a wide range of dental specialties.[21]

6.1. Ozone and oral pathogens

Oral lesions are caused by various etiological factors; microorganisms playing a major role for the same.[22] Elimination of these microbial pathogens form the mainstay of an effective dental treatment. Various bacteria have been studied in relation to ozone treatment. It has been reported that an exposure of about 60 s exhibited 99.9% killing efficiency against cariogenic bacteria such as *Actinomyces naeslundii*, *Streptococcus mutans and Lactobacillus casei*. However, exposure for such a long period showed degradation of salivary proteins and hence 10 s–30 s of exposure was proven to be effective in killing a significant number of bacteria. Considering the medium for the survival rates of *S. mutans* and *L. casei* the salivary medium depicted greater survival than the saline medium.[23] Estrela et al.[24] demonstrated the effectiveness of ozone addition to an ultrasonic cleaning system against *Staphylococcus aureus* in infections of the oral cavity.

Numerous studies have observed the effect of ozone on a wide variety of microorganisms involved in the formation of biofilm : *A. naeslundii*, *Veillonella dispar*, *Fusobacterium nucleatum*, *Streptococcus sobrinus*, *Streptococcus oralis* and *Candida albicans*[25], *S. mutans*[26] and *Lactobacillus acidophilus*[27], *Streptococcus sanguis*, *Streptococcus salivarius*, *Porphyromonas gingivalis*, *Porphyromonas endodontalis* and *Aggregatibacter actinomycetemcomitans*.[26] Some investigators[26,27] reported a reduced number of bacteria and fungi after *in vivo* treatment using ozonized water. In contrast, others reported no significant success of ozone treatment in reducing the microbiota.[25] In fact, it was found that exposure to 2 mg/liter ozone for 120 s was ineffective in completely eliminating *C. albicans*.[26]

Kshitish et al.[28] evaluated the effectiveness of ozone and chlorhexidine against certain bacteria, viruses and fungi. They recorded a 25% reduction in *A. actinomycetemcomitans* and no antimicrobial effects in relation to *P. gingivalis* or *Tannerella forsythensis* after application of ozone or chlorhexidine. The antifungal effect of ozone has been found to exceed that of chlorhexidine. However, no antiviral effects against Herpes simplex virus (HSV-1 and 2), Human Cytomegalo-virus (HCMV) and Epstein Bar virus (EBV) has been reported.

Bezirtzoglou et al.[29] isolated various species of Streptococcus species, *Aerococcus viridians*, *C. albicans*, *S. aureus* and *Staphylococcus epidermidis* from toothbrushes of children and found that maximum decontamination efficacy of ozone treatment against toothbrush bristles microbiota was observed after 30 min while exposure for short time periods seems to be ineffective.

6.2. Ozone and oral tissues

Ozone application has various beneficial effects on the oral tissues including remission of various mucosal alterations, enhanced wound healing and increased turnover rate of oral cells.

Huth et al.[30] evaluated the biocompatibility of gaseous and aqueous forms of ozone in relation to the established antimicrobials. They reported that ozone is a potential antiseptic agent and the aqueous form showed less cytotoxicity than gaseous ozone or established antimicrobials (chlorhexidine digluconate 2%, 0.2%; sodium hypochlorite- 5.25%, 2.25%; hydrogen peroxide 3%) under most conditions. Therefore, aqueous ozone fulfills optimal cell biological characteristics in terms of biocompatibility for oral application.

The influence of ozonized water on the epithelial wound healing process in the oral cavity was observed by Filippi.[31] It was found that ozonized water applied on the daily basis can accelerate the healing rate in oral mucosa. This effect is more pronounced in the first two postoperative days.

In addition to these, various oral mucosal lesions such as Herpes, Aphthous ulcers, Candidiasis, Denture stomatitis also show marked resolution owing to the healing capacity of ozone.

6.3. Ozone in treatment of dental caries

Dental caries is caused by an ecological niche of caries producing organisms. Much has been reported in the literature regarding the effectiveness of ozone in the elimination of carious lesions. This is attributed not only to the marked anti microbial properties of ozone but also to the fact that ozone oxidizes the pyruvic acid produced by the cariogenic bacteria to acetate and carbon dioxide.[32]

6.3.1. Management of pit and fissure caries

Deep pits and fissures are difficult to clean and hence are highly likely to cause food lodgement resulting in bacterial growth. Ozone application in such cases has been found to be highly effective. Cleansing the fissures prior to ozone treatment is recommended. This permits the ozone to readily access the caries. After the ozone treatment, application of remineralizing agent and sealing of the clean fissures is encouraged.[33] Ozone removes the smear layer leaving behind the exposed dentin that is occluded by the remineralizing agent applied. Huth et al. concluded that ozone application significantly improved non-cavitated initial fissure caries in patients at high caries risk over a 3- month period.[34]

6.3.2. Prophylactic ozone treatment for restorative treatment

Corroborated evidence from in vitro studies indicates that the ozone can be used as a prophylactic antimicrobial agent prior to etching and placement of restorations.

6.3.3. Management of root caries

Marked reversal and arrest of shallow non-cavitated root caries lesions have been reported following the use of ozone as part of a full preventive care regimen.[35] The clinical results are enhanced when this process is applied in conjunction to the reduced frequency of consumption of fermentable carbohydrates, increased use of fluoride- containing products and improved oral hygiene. Arrest in the progression of non-cavitary root caries, without the need for its removal has been reported following regular application of ozone for 40 s, and the use of remineralizing products.[36]

Ozone is the most effective in cases of shallow lesions since it shows enhanced ability to penetrate lesions which are about 1mm deep at the maximum. The ozone unit must also be used correctly; the ozone cap must be held directly against the caries lesion allowing the ozone to penetrate the decay and biofilm. Where there is a cavitated 4mm deep root caries lesion adjacent to the gingival margin, simply using ozone treatment would probably not suffice. To manage this kind of a situation the outer caries must first be removed, leaving about 1 mm of caries over the cavity floor. Then the ozone treatment followed by routine restoration is indicated.

Ozone should be considered an adjunct to existing treatment and preventive methods rather than an isolated treatment modality.

6.4. Restorative dentistry

Evidence gathered from studies testing the efficacy of ozone on dental materials justifies the use of ozone prior to the placement of etchant and sealant. There was no alteration observed on the physical properties of enamel, dentin including knoop surface microhardness or contact angle and adhesive restorative materials.[37] When applied for prolonged duration; ozone gas has a strong bactericidal effect on microorganisms within the dentinal tubules of deep cavities, consequently improving the clinical success of restorations.[38] Crown discoloration of non-vital teeth is treated after placing bleaching paste in the pulp chamber followed by ozone exposure for 3–4 min by the virtue of its oxidation property.[32]

6.5. Endodontics

Ozone has immense potential to be used as an antimicrobial in endodontics.[39] Ozone is effective when it is prescribed in adequate concentration, time and delivered correctly into root canals after the traditional cleaning, shaping and irrigation has been completed. The potential use of ozone gas, ozonated water and ozonized oil in endodontic therapy has been repeatedly reported in the literature.[40,41] Intra canal gas circulation of ozone at a flow rate of 0.5–1 l/min with net volume of 5 gm/ml for 2–3 min showed encouraging results against pathogenic microbes in the root canal. Ozonated water can be used as an intracanal irrigant and in infected necrotic canals, ozonized oils can be used as an intra-canal dressing reducing the marked anaerobic odor emanating from infected teeth. When used as an irrigant, ozone encourages tissue regeneration and bone healing. Also when a root canal was disinfected by ozone water with sonification, the antimicrobial efficacy was comparable to 2.5% NaOCl.[33] Hence in periapical infections, ozone therapy can increase the scope of non-surgical management of these lesions.

6.6. Hypersensitive teeth

Tooth structure loss occurring due to multiple factors like attrition, abrasion, erosion, trauma from occlusion may cause wearing away of enamel and dentin thereby causing hypersensitivity. Ozone application has been found to effectively reduce sensitivity of not only exposed enamel and dentin but also in cases of root sensitivity. 40–60 s application of ozone is found to instantly reduce pain in these sensitive teeth. Ozone initiates removal of the smear layer, opens the dentinal tubules and widens them. On applying remineralizing agent; calcium & fluoride ions enters the dentinal tubules easily, readily and completely, preventing the fluid exchange from these tubules. Hence, termination of sensitivity occurs following ozone application within seconds and also lasts longer than those by conventional methods.[42]

6.7. Ozone in periodontics

Ebensberger et al.[43] evaluated the effect of irrigation with ozonated water on the proliferation of cells in the periodontal ligament adhering to the root surfaces of avulsed teeth. They concluded that avulsed teeth when irrigated with ozonized water for 2 min showed effective mechanical cleansing and root surface decontamination with no adverse effects on the periodontal cells on the tooth surface.

In a study by Nagayoshi et al.[44] dental plaque samples were treated with 4 mL of ozonated water for 10 s. They observed that ozonated water was effective in killing gram-positive and gram negative oral microorganisms and oral *C. albicans*. This reflects its potential to control infectious microorganisms in dental plaque.

Nagayoshi et al.[45] also tested the efficacy of three different concentrations of ozone water (0.5, 2, and 4 mg/ml in distilled water) on the time-dependent inactivation of cariogenic, periodontopathogenic and endodontopathogenic microbes (Streptococcus, *P. gingivalis* and Endodontalis, Actinomyces actinomycetemcomitans, *C. albicans*) in culture and in biofilms. They confirmed that ozonated water was highly effective

in killing both gram positive and gram negative micro-organisms. Gram negative being more sensitive as compared to gram positive bacteria.

Ramzy et al.[46] used 150 ml of ozonized water to irrigate the periodontal pockets for a duration of 5–10 min once weekly for four weeks in patients suffering from aggressive periodontitis. He achieved highly significant improvement regarding pocket depth, plaque index, gingival index and bacterial count.

Ozone has also been successfully used as a pretreatment rinse to irrigate periodontal pockets prior to performing scaling and root planning. Also ozonated oils have showed improvement in patients with acute necrotizing ulcerative gingivitis.

6.8. Oral medicine

Several soft tissue lesions i.e. aphthous ulcers, herpes labialis[47] have been reported to be effectively treated using ozone therapy. This applicability is attributed to the accelerated healing properties of the ozone. Holmes et al treated patients with oral lichen planus by tissue insufflation, injection, cupping and ozonated oil applications. The therapy outcomes were encouraging in these patients with no markable adverse effects unlike more traditional pharmacological approaches.[48]

In patients suffering from carinomatous lesions, chemotherapy and radiotherapy are routinely administered and it invariably causes mucositis. Ozone therapy applied in both aqueous and gaseous forms in cases of mucositis has shown positive results, enabling the patient to eat normally, and improves the quality of life during oncological therapeutic interventions.[49]

6.9. Prosthodontics

Dentures are commonly inhabited by several micro-organisms especially *C. albicans*. Denture stomatitis is routinely encountered in clinical practice which is a manifestation of plaque accumulation on the surface of the denture and hence effective denture plaque control should be initiated to prevent such outcomes. One successful method to do so is the use of ozone as denture cleaner. It is effective against various microbes adhering on the denture surface including *C. albicans*[50] *methicillin-resistant S. Aureus* and viruses.[51] It has also been reported that ozone can be applied for cleaning the surface of removable partial denture alloys without compromising the physical properties of the alloy such as reflectance, surface roughness, and weight.[52]

Patients suffering from periimplantitis were investigated by Karapetian et al.[53] They compared the effectiveness of conventional, surgical and ozone therapy methods to cure periimplantitis. They reported that the main challenge seems to be the decontamination of the implant surface, its surrounding tissue and the prevention of recolonization with periodontal pathogenic bacteria. And the most effective bacterial reduction was recorded in the ozone-treated patient group.

6.10. Oral surgery

Ozone therapy has a vast range of applications in oral surgery; be it a simple extraction procedure or a severe jaw infection or osteotomy procedures. Ozone enhances wound healing, improves several properties of erythrocytes and facilitates oxygen release in the tissues. This causes vasodilatation and hence improves the blood supply to the ischemic zones. Therefore, it can be successfully used in cases of wound healing impairments following surgical interventions like tooth extractions or implant dentistry.[54] Kazancioglu et al. evaluated the effect of ozone therapy on pain, swelling and trismus following third molar surgery and concluded that ozone application effectively reduced postoperative pain; however it had no effect on swelling and trismus.[55]

Exposure to medical grade ozone promotes more complete and rapid normalization of nonspecific resistance and T-cellular immunity.[56] Hence it shows rapid clinical cure and reduced incidence of complications in patients when used in patients with chronic mandibular osteomyelitis. When surgical removal of bone sequestra is carried out, ozone can be used as an alternative to hyperbaric oxygen therapy owing to its extensive oxidation property and on the fact that ozone contacted bacteria can be more easily recognized and destroyed by granulocytes and the complement system. It also increases the phagocytosis performance of polymorphonuclear cells.[57] Also after performing osteotomies against infections ozonated water has shown better clinical outcomes when used for prophylactic applications.

Hammuda et al have documented successful use of ozone in cases of temporomandibular joint arthrocentesis and concluded that it is an effective conservative procedure in the treatment of internal derangement.[58] Ozone has also been recommended as a therapeutic treatment in cases of bisphosphonate related osteonecrosis of jaw.[59]

6.11. Pedodontics

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 perform. 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.[60] treated open carious lesions with ozone in anxious children. There was near total reduction (93%) of dental anxiety. The frequently encountered cases in a pediatric practice are those of trauma to the teeth. A high level of biocompatibility of aqueous ozone on human oral epithelial cells, gingival fibroblast cells, and periodontal cells has been observed.[30,43] Ozonated water is indicated in replantation of avulsed tooth without any harmful effect on periodontal cells.

6.12. Orthodontics

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.

Ghobashy et al.[61] evaluate the effects of ozonized olive oil gel in reducing enamel demineralization around orthodontic bracket during orthodontic treatment. The use of ozonized olive oil gel in addition to the standard oral hygiene regimen was found to show significantly less decalcification of teeth among orthodontic patients.

On the other hand, due to its strong oxidizing effect, ozone might have negative effects on resin tooth adhesion related to the oxygen inhibition of polymerization. In a recent study, Cehreli SV et al.[62] studied the effects of prophylactic ozone pretreatment of enamel on shear bond strength of orthodontic brackets bonded with total or self-etch adhesive systems. They concluded that enamel pretreatment with ozone did not affect the shear bond strength of adhesive systems used for bracket bonding. Moreover, shear bond strength values of the ozone pretreated specimens were somewhat higher.

7. Conclusion

Ozone therapy has a wide range of applications in almost every field of dentistry. Its unique properties include immunostimulant, analgesic, antihypnotic, detoxicating, antimicrobial, bioenergetic and biosynthetic actions. Its atraumatic, painless, non invasive nature and relative absence of discomfort increase patient's acceptability and compliance thus making it an ideal treatment choice specially for pediatric patients.

Conflict of interest

The author declares no competing financial interests.

References

1. Seidler V, Linetskiy I, Hubalkova H, Stankova H, Smucler R, Mazanek J. Ozone and its usage in general medicine and dentistry. *Prague Med Rep* 2008;**109**(1):5–13.
2. Gujjari GK, Gujjari AK, Patel PV, Shubhashini PV. Comparative evaluation of ultraviolet and microwave techniques for toothbrush decontamination. *J Int Soc Prev Community Dent* 2011;**1**:20.
3. Garg R, Tandon S. Ozone: a new face of dentistry. *Int J Dent Sci* 2009;**7**:2.
4. Kagan J. Are You Ready For This – Ozone Therapy. Ozone Information for Clinicians [online], 2003 [cited 2010 January 13]. Available from: URL: <http://www.theozone.cc/HTMLOzoneF/ch37>.
5. Bayson A, Lynch E. Antimicrobial effects of ozone on caries. In: Lynch E, editor. *Ozone: the revolution in dentistry*. London Quintessence Publishing Co.; 2004. p. 165–72.
6. Greene AK, Few BK, Serafini JC. A comparison of ozonation and chlorination for the disinfection of stainless steel surfaces. *J Dairy Sci* 1993;**76**(11):3617–20.
7. Bocci V. Ozone as Janus: this controversial gas can be either toxic or medically useful. *Mediators Inflamm* 2004;**13**(1):3–11.
8. Patel, Kumar V, Gujjari S, Vidya GD, Patel Amrita. Therapeutic effect of topical ozonated oil on epithelial healing of palatal wound site: a planimetrical and cytological study. *J. Clin. Investig. Dent*. 2011;**2**:248–58.
9. Iliadis D, Brian J, Millar. Ozone and its use in periodontal treatment. *Open J. Stomatology* 2013;**3**(2):197–202.
10. Mandhare et al. Miracle of ozone therapy as an alternative medicine. *Int J Pharm Chem Biol Sci* 2012;**2**(1):63–71.
11. Holland Alan. Ozone in endodontics. *Endod Pract* 2010;6–10.
12. Schonbein C. Notice of C Sch., the discoverer of ozone. *Annual Report of the Board of Regents of the Smithsonian Inst., 1868*. Washington, DC: US Government Printing Office; 1869. p. 185–92.
13. Wolff H. *Das Medizinische Ozon*. Heidelberg: VFM Publications; 1979.
14. Payr E. Uber ozonbehandlung in der chirurgie. *Munch med Wschr* 1935;**82**:220–91.
15. Mudd JB. Freeman BA Reaction of ozone with biological membranes. In: Lee SD, editor. *Biochemical effects of environmental pollutants*. Ann Arbor, MI: Ann Arbor Science Publishers; 1977. p. 91–133.
16. Broadwater WT, Hoehn RC. Sensitivity of three selected bacterial species to ozone. *Appl Microbiol* 1973;**26**(3):391–3.
17. Bocci V. Autohaemotherapy after treatment of blood with ozone. A reappraisal. *J Int Med Res* 1994;**22**(3):131–44.
18. Elvis AM, Ekta JS. Ozone therapy: a clinical review. *J Nat Sci Biol Med* 2011;**2**(1):66–70.
19. Chang Hubert et al. Oxidative consumption of oral biomolecules by therapeutically-relevant doses of ozone. *Adv Chem Eng Sci* 2012;**2**:238–45.
20. Werkmeister H. Subatmospheric 02/03 treatment of therapy-resistant wounds and ulcerations. *OzoNachrichten* 1985;**4**:53–9.
21. Pattanaik B, Jetwa D, Pattanaik S, Manglekar S, Naitam DN, Dani A. Ozone therapy in dentistry: a literature review. *J Interdiscip Dent* 2011;**1**(2):87–92.
22. Gonzalez-Munoz L, Flichy-Fernandez AJ, Ata-Ali J, Pascual-Moscardo A, Penarrocha-Diago MA. Effect of ozone therapy upon clinical and bacteriological parameters of the oral cavity: an update. *J Clin Exp Dent* 2011;**3**(4):e325–7.
23. Johansson E, Claesson R, van Dijken JWV. Antibacterial effect of ozone on cariogenic bacterial species. *J Dent* 2009;**37**:449–53.
24. Estrela C, Estrela CR, Decurcio Dde A, Silva JA, Bammann LL. Antimicrobial potential of ozone in an ultrasonic cleaning system against *Staphylococcus aureus*. *Braz Dent J* 2006;**17**:134–8.
25. Muller P, Guggenheim B, Schmidlin PR. Efficacy of gasiform ozone and photodynamic therapy on a multispecies oral biofilm in vitro. *Eur J Oral Sci* 2007;**115**:77–80.
26. 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**:240–6.
27. Knight GM, McIntyre JM, Craig GG, Mulyani, Zilm PS. The inability of *Streptococcus mutans* and *Lactobacillus acidophilus* to form a biofilm in vitro on dentine pretreated with ozone. *Aust Dent J* 2008;**53**:349–53.
28. 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.
29. Bezirtzoglou E, Cretoiu SM, Moldoveanu M, Alexopoulos A, Lazar V, Nakou M. A quantitative approach to the effectiveness of ozone against microbiota organisms colonizing toothbrushes. *J Dent* 2008;**36**(8):600–5.
30. 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.
31. Filippi A. *The influence of ozonised water on the epithelial wound healing process in the oral cavity. Clinic of oral surgery, radiology and oral medicine*. Switzerldand: University of Basel; 2011. Available at: <www.oxyplus.net>.
32. Kumar A, Bhagwati S, Tyagi P, Kumar P. Current interpretation and rationale of the ozone usage in dentistry: a systemic review of literature. *Eur J Gen Dent* 2014;**3**(3):175–80.
33. Reddy SA, Reddy N, Dinapadu S, Reddy M, Pasari S. Role of ozone therapy in minimal intervention dentistry and endodontics – a review. *J Int Oral Health* 2013 Jun;**5**(3):102–8.
34. Huth KC, Paschos E, Brand K, Hickel R. Effect of ozone on non-cavitated fissure carious lesions in permanent molars -a controlled prospective clinical study. *Am J Dent* 2005;**18**:223–8.

35. Lynch E. Ozone Caries Management. [online], 2008. Available from: URL: <http://www.oralhealthgroup.com/news/ozone-caries-management/1000223303>.
36. Holmes J. Clinical reversal of root caries using ozone, double blind, randomized control 18 month trial. UKSmiles Dental Practice. *Gerodontology* 2003;**20**:106–14.
37. Schmidlin PR, Zimmermann J, Bindl A. Effect of ozone on enamel and dentin bond strength. *J Adhes Dent* 2005;**7**(1):29–32.
38. 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**(4):349–53.
39. Virtej A, MacKenzie CR, Raab WH, Pfeffer K, Barthel CR. Determination of the performance of various root canal disinfection methods after in situ carriage. *J Endod* 2007;**33**:926–9.
40. Siqueira Jr JF, Rocas IN, Cardoso CC, Macedo SB, Lopes HP. Antibacterial effects of a new medicament- the ozonized oil compared to calcium hydroxide pastes. *Rev Bras Odontol* 2000;**57**:252–6.
41. Silveira AM, Lopes HP, Siqueira Jr JF, Macedo SB, Consolaro A. Periradicular repair after two-visit endodontic treatment using two different intracanal medications compared to single-visit endodontic treatment. *Braz Dent J* 2007;**18**:299–304.
42. Garg R, Tandon S. Ozone: a new face of dentistry. *Internet J Dent Sci* 2009;**7**:2.
43. 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.
44. Nagayoshi M, Fukuizumi T, Kitamura C. Efficacy of ozone on survival and permeability of oral microorganisms. *Oral Microbiol Immunol* 2004;**19**:240.
45. Nagayoshi M, Kitamura C, Fukuizumi T. Antimicrobial effect of ozonated water on bacteria invading dentinal tubules. *J Endod* 2004;**30**:778–81.
46. Ramzy MI, Gomaa HE, Mostafa MI. Management of aggressive periodontitis using ozonized water. *Egypt Med J N R C* 2005;**6**(1):229–45.
47. Nagarakanti S, Athuluru D. Ozone: a new revolution in dentistry. *Webmed Cent Dent* 2011;**2**(12), WMC002685.
48. Holmes J, Domb W, Zaremski E, Jaeger R. Lichen planus: a review and alternative treatment modality in dentistry. Available from : URL: <http://www.prodentalcpd.com/members/articles/oral_medicine/oral_medicine/lichen_planus_a_review_and_alternative_treatment_modality_in_dentistry>.
49. Shenberg JE. Gaseous and aqueous ozone therapy for treatment of mucositis secondary to chemotherapy/radiotherapy: a case report. *Pain Pract* 2011;**21**(3):69–73.
50. 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.
51. Murakami H, Mizuguchi M, Hattori M, Ito Y, Kawai T, Hasegawa J. Effect of denture cleaner using ozone against methicillinresistant *Staphylococcus aureus* and *E. coli* T1 phage. *Dent Mater* 2002;**21**:53–60.
52. Suzuki T, Oizumi M, Furuya J, Okamoto Y, Rosenstiel SF. Influence of ozone on oxidation of dental alloys. *Int J Prosthodont* 1999;**12**:179–83.
53. Karapetian VE, Lowden E, Zöller JE. Die Verwendung von Ozon in der Periimplantitistherapie - Klinische Studie. ZWR (The Use of Ozone in Periimplantitis-Treatment). *Das Deutsche Zahnärzteblatt* 2007;**116**:214–8.
54. Das S. Application of ozone therapy in dentistry. *Indian J Dent Adv* 2011;**3**(2):538–42.
55. Kazancioglu HO, Kurklu E, Ezirganli S. Effects of ozone therapy on pain, swelling, and trismus following third molar surgery. *Int J Oral Maxillofac Surg* 2014;**43**(5):644.
56. Agapov VS, Shulakov VV, Fomchenkov NA. Ozone therapy of chronic mandibular osteomyelitis. *Stomatologiia (Mosk.)* 2001;**80**:14–7.
57. Gupta M. Abhishek. Ozone: an emerging prospect in dentistry. Indian. *J Dent Sci* 2012;**4**(1):47–50.
58. Hammuda A, Hamid MS, Elsharrawy EA, Elsholkamy MA, Iskandar A. Use of ozone in temporomandibular joint arthrocentesis, clinical study. *J Am Sci* 2013;**9**(7).
59. Agrillo A, Petrucci MT, Tedaldi M, Mustazza MC, Marino SM, Gallucci C, et al. New therapeutic protocol in the treatment of avascular necrosis of the jaws. *J Craniofac Surg* 2006;**17**:1080–3.
60. Dahnhardt JE, Jaeggi T, Lussi A. Treating open carious lesions in anxious children with ozone. A prospective controlled clinical study. *Am J Dent* 2006;**19**:267–70.
61. Ghobashy Safaa Ali, El-Tokhey Heba Mohamed. In vivo study of the effectiveness of ozonized olive oil gel on inhibiting enamel demineralization during orthodontic treatment. *J Am Sci* 2012;**8**(10):657–66.
62. Cehreli Sevi Burcak, Guzey Asli, Arhun Neslihan, Cetinsahin Bahtiyar, Unver Bahtiyar. The effects of prophylactic ozone pretreatment of enamel on shear bond strength of orthodontic brackets bonded with total or self-etch adhesive systems. *Eur J Dent* 2010;**4**:367–73.