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Review on role of ozone therapy in the re-mineralization of dental caries.

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INTRODUCTION:

Ozone (o_3) (healozonex4^{tm}) is an unstable gas composed of three atoms of oxygen and is the most powerful oxidant. It is one of the most important gases in the stratosphere due to its ability to filter UV rays which is critical for the maintenance of biological balance in the biosphere. Ozone is the layer of earth's stratosphere. It shields us from the harmful sunlight and prevents skin cancers. It is an effective oxidizing agent. Atmospheric air is made up of nitrogen (71%), oxygen (28%) and other gases (1%) including ozone which is altered by processes related to altitude, temperature and air pollution (Saini et al. 2010a; Saini et al. 2009a). Ozone (O3) which has a characteristic, penetrating odor is present in small amounts in atmospheric air. Ozone molecules are composed of three oxygen atoms and present naturally in the upper layer of atmosphere in abundance as long as sun is shining (Saini et al. 2010a; Saini et al. 2009b; Saini et al. 2010b). It protects living organisms by surrounding the earth at altitudes of 50,000 to 100,000 feet from the ultra-violet rays (Saini et al. 2010a; Saini et al. 2009a). As it falls downward to earth, being heavier than air, combines with any pollutant it comes in contact with and cleans the air. This is earth's natural way of self-cleansing (Saini et al. 2009b). Medical Ozone is made when medical grade oxygen is electrically activated (using an Ozone Generator) to form ozone (Saini et al. 2009b). It is a mixture of the purest oxygen and purest ozone. According to its application, the ozone concentration may vary between 1 and 100 µg/ml (0.05-5%). The ozone therapist determines the complete dosage according to the medical/dental indication and the patient's condition (Bortolaia and Sbordone 2002; Marsh, 2005). Ozone is an unstable gas and it quickly gives up nascent oxygen molecule to form oxygen gas. The release of nascent oxygen has beneficial effects on every part and organ (Saini et al. 2010a). It has been used in medical field since long due to its extremely strong oxidant property that oxidizes nearly all surfaces to the highest oxidation stage. It is used as a circulatory enhancement and stimulation of oxygen metabolism, disruption of tumor metabolism and to kill pathogens (Saini et al. 2010a; Saini et al. 2009a). O3 is a powerful oxidant capable of interacting as metabolic & immune modulator as well as anti-microbial agent. Multiple microbiological & the biochemical studies justified that there are no doubts about the effectiveness of ozone in bacterial reduction (Thomas and Nakaishi, 2006). Among other things, ozone is used to purify drinking water and water in dental equipment and for sterilizing instruments for medical use (Walker, 2000). Atmospheric air is made up of nitrogen (71%), oxygen (28%) and other gasses (1%) including ozone which is altered by processes related to altitude, temperature and air. Ashwini Maiya (2011) introduced as ozone is a colorless gas form of oxygen and is present in atmosphere. It is a powerful oxidizer. It effectively kills bacteria, fungi, viruses and parasites at a dramatically lower concentration. It is a great supplement to conventional therapeutic dental modalities. Treatment may be achieved by increasing the resistance of the tooth against the microbial activity and reducing the extent of microbial activity. In addition to the recent materials and techniques, the therapeutic actions of ozone may provide beneficial results by reducing the demineralization of the tooth. Ozone unit was developed under the name 'Heal Ozone' by Kavo – Dental Gmbh and Co. Ozone finds its place in all disciplines of dentistry. The treatment is completely painless and increases the patient's acceptability and compliance with minimal adverse effects, thus making it an ideal treatment choice for pediatric patient's where patient compliance is the key to its success. In 1839, Christian Friedrich Schonbein, first noticed the emergence of a pungent as with an electric smell. According to the Greek language, he called it ozone and presented a lecture entitled "On the smell at the positive electrode during electrolysis of water" at the Basel Natural Science Society (Stübinger and Sader 2006). Oxygen/ozone therapy has a long history of research and clinical application with humans. The first medical application was in 1870 when Dr. C. Lender purified blood in test tubes. Medical applications became widespread throughout Europe and America. As of 1929, more than 114 diseases were listed for treatment with oxygen/ozone therapy. Interestingly enough, in 1930, a German dentist, E.A. Fisch, used ozone on a regular basis in his dental practice in Zurich, Switzerland, and published numerous papers on the subject.

Ozone therapy: Chemistry and apparatus: Ozone (O3) is a triatomic molecule, consisting of three oxygen atoms. Its molecular weight is 47, 98 g/mol and thermo-dynamically highly instable compound that, dependent on system conditions like temperature and pressure, decompose to pure oxygen with a short half-life (Burns, 1997). Ozone is 1.6-fold denser and 10-fold more soluble in water (49.0 mL in 100 mL water at 0° C) than oxygen. Although ozone is not a radical molecule, it is the third most potent oxidant (E_ 5 12.076 V) after fluorine and per sulfate. Ozone is an unstable gas that cannot be stored and should be used at once because it has a half-life of 40 min at 20 °C (Bocci, 2006). Ozone (O3) is naturally produced by the photo dissociation of molecular oxygen (O2) into activated oxygen atoms, which then react with further oxygen molecules. This transient radical anion rapidly becomes protonated, generating hydrogen trioxide (HO3), which, in turn, decomposes to an even more powerful oxidant, the hydroxyl radical (OH). It is the fundamental form of oxygen that occurs naturally as a result of ultraviolet energy or lightning, causing a temporary recombination of oxygen atoms into groups of three. In the clinical setting, an oxygen/ozone generator simulates lightning via an electrical discharge field. Ozone gas has a high oxidation potential and is 1.5 times greater than chloride when used as an antimicrobial agent against bacteria, viruses, fungi, and protozoa. It also has the capacity to stimulate blood circulation and the immune response. Such features justify the current interest in its application in medicine and dentistry and have been indicated for the treatment of 260 different pathologies (Nogales et al. 2008).

Ozone generation equipments : Medical grade ozone is a mixture of pure oxygen and pure ozone in the ratio of 0.05% to 5% of O_3 and 95% to 99.95% of O_2. Due to the instability of the O_3 molecule, medical grade ozone must be prepared immediately before use. After preparation with in less than an hour, only half of the mixture is still ozone while the other half is transformed into oxygen. As a result, it is impossible to store ozone over long periods of time. In order to control the decomposition of O_3 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. There are three different systems for generating ozone gas: Ultraviolet System produces low concentrations of ozone, used in aesthetic, saunas, and for air purification. Cold Plasma System: used in air and water purification. 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. Commercially available ozone generator: CurOzone^{TM} USA Inc. (Ontario, Canada) developed the HealOzone^{TM}, which is now distributed by KaVo Dental (KaVo, Biberach, Germany), for use in dentistry. HealOzone X4^{TM} unit can produce ozone from oxygen in the ambient air (Low Dosage mode) or from pure oxygen supplied by an oxygen bottle (High Dosage mode). The latter method generates higher concentrations of ozone gas. The HealOzone X4^{TM} uses high voltage to convert oxygen into ozone. The ozone generated by the machine is channeled through the hand piece to the affected tooth or root canals in order to treat the carious lesions. After treatment, the ozone gas is then suctioned off, dried and converted back into oxygen by the ozone neutralizer. The unit includes oxygen supply, air supply, dryer, differential pressure sensor, ozone generator, tooth cup, ozone, hand piece, tubing, moisture trap, ozone neutralizer and vacuum pump.

Mode of delivery of ozone: The route of ozone administration is topical or loco-regional in gaseous or aqueous form or as ozonated olive or sunflower oil. The management of dental caries must be of a preventive rather than just curative approach. However, the word caries is unfortunately used for both the dental caries (and cavities), which occurs in the tooth and the carious process which occurs in the biofilm. The carious lesion can be thought as a reflection of the carious process. Preventive dentistry is all about how we stop the carious process re-mineralize the initial non-cavitated white spots, alter the metabolism in plaque and control plaque itself. There are many re-mineralization products available today that can be used to reverse and arrest the carious process and promote re-mineralization of the incipient carious lesion. However there are several challenges associated with establishing the clinical effectiveness of these re-mineralization agents. They must demonstrate a benefit over and above an established and highly effective agent, namely, fluoride. They must provide a re-mineralizing benefit in addition to the natural re-mineralizing properties of saliva. (Zero, 2006; Kalra et al., 2014).The organic constituents of saliva can serve as accelerators and inhibitors of the re-mineralization process. Teeth are covered by the acquired pellicle, which has been shown to retard re-mineralization. If sugar-free chewing gum is the delivery vehicle, chewing gum has a major re-mineralizing effect in and of itself, which makes it more challenging to show an additional benefit when using gum as the delivery vehicle. Too much of a good thing could possibly disrupt the mineralization and homeostasis of the mouth and favor calculus formation (Zero, 2006). There may be ingredient compatibility issues. Products are designed to deliver a new agent i.e. calcium ions and fluoride simultaneously from single-phase products and may present formulation challenges such as long-term fluoride compatibility (Goswami et al., 2012). Pre-clinical models may not necessarily be predictive of clinical performance for these non-fluoride agents and that new agents still require direct clinical validation to ensure efficacy (Goswami et al., 2012).

Contraindications of ozone therapy: The ozone therapy in any form is contraindications in various conditions as pregnancy, glucose- 6- phoshate dehydrogenase deficiency (favism), hyper-thyroidism, severe anemia and severe myasthenia. Prolonged inhalation of ozone can be deleterious to the lungs and other organs but well calibrated doses can be therapeutically used in various conditions without any toxicity or side effects (Bocci V et al., 2009). The European Cooperation of Medical Ozone Societies warns that direct intravenous injections of ozone/oxygen gas should not be practiced due to the possible risk of air embolism.

Clinical Use in dentistry: Ozone was first applied in dentistry in 1932 by a Swiss dental surgeon Edwin Fisch. However, ozone seems to have disappeared from usage in dental care until 2001 when the first scientific studies were published examining the biomolecules found in dental caries, before and after treatment with ozone. It acts by attacking thiol groups of cysteine amino acid and annihilates the cellular membrane of carious bacteria (Baysan and Beighton, 2007). Ozone can shift microbial flora from acidogenic and aciduric microorganisms to normal commensals and helping in remineralisation (Baysan and Lynch, 2005). Ozone therapy is used to restrict the microbial growth in the treatment of periodontitis, which is a destructive inflammatory disease of the supporting tissues of the teeth and is caused either by specific microorganisms or by a group of specific microorganisms, resulting in progressive destruction of periodontal ligament and alveolar bone with periodontal pocket formation, gingival recession, or both (Saini et al. 2010b). Bacteria are the prime etiological agents in periodontal disease, and it is estimated that more than 500 different bacterial species are capable of colonizing the adult mouth and the lesions of the oral cavity have an immense impact on the quality of life of patient with complex advance diseases (Saini at al., 2009b). Periodontitis has been proposed as having an etiological or modulating role in cardiovascular and cerebro-vascular disease, diabetes, respiratory disease and adverse pregnancy outcome and several mechanisms have been proposed to explain or support such theories (Saini et al., 2010a). The oral cavity appears as an open ecosystem, with a dynamic balance between the entrance of microorganisms, colonization modalities and host defenses aimed their removal. The oral biofilm formation and development, and the inside selection of specific microorganisms have been correlated with the most common oral pathologies, such as dental caries, periodontal disease and peri-implantitis (Bortolaia and Sbordone (2002). The cooperative communal nature of a microbial community provides advantages to the participating microorganisms. These advantages include a broader habitat range for growth, an enhanced resistance to antimicrobial agents and host defence, and an enhanced ability to cause disease (Marsh 2005). Dental biofilm pathogenicity in the oral cavity is magnified by two biofilm characteristics: Increased antibiotic resistance and the inability of the community to be phagocytized by host inflammatory cells (Thomas and Nakaishi, 2006).The mechanical removal of the biofilm and adjunctive use of antibiotic disinfectants or various antibiotics have been the conventional methods for periodontal therapy (Walker, 2000., and Feres et al. 2002). As the periodontal researchers is looking for alternatives to antibiotic treatments (biofilm resistance), the emergence of ozone therapy seems

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to be a promising future. Oxygen/ozone therapy in dentistry contains a multiplicity of protocols to deal with dental infection. Three fundamental forms of application to oral tissue are applied — (1) ozonated water, (2) ozonated olive oil, and (3) oxygen/ozone gas. Ozonated water and olive oil have the capacity to entrap and then release oxygen/ozone, an ideal delivery system. These forms of application are used singly or in combination to treat dental disease. The different clinical application of ozone therapy in combating the dental disease and their treatment modalities are as follows.

Dental treatment modalities of ozone therapy: Removal of bacterial pathogens, oral cavity disinfection including periodontal and osseous tissues, prevention of caries, endodontic treatment in RCT, tooth extraction, tooth sensitivity, temporo-mandibular joint, gum recession treatment, tissue regeneration, controlling halitosis, pain control, teeth bleaching, re-mineralization of tooth surfaces. Ozone is an energy-rich and highly unstable form of oxygen (Johansson et al., 2009; Bocci, 2006). It is one of the most important gases in the stratosphere because of its ability to filter ultraviolet (UV) rays, which are crucial for the biological balance in the biosphere (Nogales et al., 2008). It has proved to be effective against gram negative and gram-positive bacteria, viruses, and fungi (Greene et al., 1993). Ozone's generates direct molecular-level reactions in the medium in which it is released and indirectly destroys bacteria by the production of free radicals (Castillo et al., 2008). Currently Healozone^{TM} (KaVo^{TM} GmbH, Germany) re-mineralizing solution consisting of xylitol, fluoride, calcium, phosphate and zinc, is approved for the treatment of caries. It can be used as 2100 ppm of ozone ± 5% at a flow rate of 615cc/min for 40 s (Brazzelli et al., 2006). The mechanism of Heal Ozone's action is linked to ozone's strong antimicrobial properties and its capability to oxidize proteins associated with the dental caries process. Ozone has the potential to replace or combine with fissure sealants for caries prevention. The effect of ozone on plaque bio-molecules has been recently discovered. It has a powerful oxidative ability that consumed plaque and saliva bio-molecules essential for the process of de-mineralization of lesions to occur. For caries high risk patients, ozone can be combined with fissure sealants to increase the preventive outcome. Ozone treatment provides short application time and reduction of dental anxiety with minimal cooperation requirements and high satisfaction rates. Ozone minimal running cost (ozone being produced from air) and minimal treatment time could be recommended for larger preventive schemes run at lower costs (George, 2016). The antimicrobial effect of ozone is a result of its action on cells by damaging its cytoplasmic membrane due to ozonolysis of dual bonds and also ozone-induced modification of intracellular contents because of secondary oxidant effects. This action is selective to microbial cells but does not damage human body cells because of their major anti-oxidative ability. Ozone has been used not only for management of caries but also for management of hypersensitivity, treatment of endodontics, periimplantitis, decontamination of avulsed teeth before replantation, as dental cleaners, in restorative dentistry, in oral and maxillofacial surgery etc. (Das, 2011). Leathery non-cavitated primary root caries can be arrested non operatively using ozone and remineralizing products. It is suggested that this treatment regime is an effective alternative to conventional "drilling and filling." Earlier studies compared the effectiveness of gaseous ozone as a disinfectant and chlorhexidine gel in the reduction of microorganisms in occlusal caries lesions of small children with and without excavation. The authors concluded that ozone treatment either alone or combined with a remineralizing solution was found to be effective for the remineralisation of initial fissure caries lesions. Most of the clinical studies reported that ozone is a promising alternative to conventional methods for the management of caries. Baysan and Brighten (2007) have evaluated the antimicrobial effect of ozone on primary root carious lesions and the efficacy on Streptococcus mutans and Streptococcus sobrinus. A significant reduction in microorganisms was observed furthermore, the application of ozone was able to reduce the count of S. mutans and S. sobrinus on saliva-coated glass beads. The authors concluded that ozone may be an effective alternative to traditional drilling and filling for the treatment of primary root caries. Mu¨ller et al. (2007) assessed the antimicrobial potential of ozone gas and photodynamic therapy on oral biofilm. Their results suggest that well-established biofilms are resistant to ozone application. Both ozone and photodynamic therapy had a minimal effect on the viability of micro-organisms organized in a cariogenic biofilm. It is suggested that application of ozone in addition to the use of other antibacterial methods after caries excavation might be more successful in order to eliminate the remaining bacteria under the restorations. Ozone might be a useful tool to reduce and control oral infectious microorganisms in dental plaque and dental cavity (Azarpazooh and Limeback, 2008). However, the results of in vitro studies are controversial, although some researchers reported that ozone therapy had a minimal or no effect on the viability of microorganisms (Muller et al., 2007 and Baysan, et al., 2000). In-vitro application of gaseous ozone has been indicated to be useful prophylactic antimicrobial treatment prior to placement of dental sealants and helpful for initial caries prevention (Unal and Oztas, 2015). The results of some studies indicated that ozone alone has minimal effect but this can be enhanced when fluoride containing products were used in combination (Tahmasssebi et al., 2014). Ozone should be considered an adjunct to existing treatment and preventive methods rather than an isolated treatment modality. The vast majority of the dentists that are using ozone therapy treatments today use the treatment in conjunction with plaque control and diet moderation, chemotherapeutic approaches such as fluoride or chlorhexidine, sealants, and stepwise excavation. It is thus clear that clinical dentistry has adopted ozone to be used in conjunction with other clinical approaches, not as an alternative (Khullar et al., 2012). From a dental public health point of view, with dental caries being such a problem in many countries across the globe, the ozone therapy has potentially a major part to play in the prevention and treatment of dental caries. The use of ozone for managing caries is considered a breakthrough that is expected to be a cornerstone of dental care in the near future. Ozone has been used for the treatment of caries, disinfection of the cavity, reducing the levels of caries-associated microorganisms in the dental plaque, and re-mineralization of caries lesions with successful results. However there are numerous statements pertaining to hazardous effect of Ozone therapy. On the other hand, the clinical evidence for application of ozone is not extensive. During the past decades, contrary to all negative remarks, it has been shown that judicious use of ozone in chronic infectious diseases, orthopedics and dental caries has seen remarkable results. Consequently more evidence is required before ozone can be acknowledged as an alternative to present methods for the management and prevention of caries (Almaz et al., 2015). Rajiv Saini (2011) evaluated that the ozone therapy has been more beneficial than present conventional therapeutic modalities that follow a minimally invasive and conservative application to dental treatment. The exposition of molecular mechanisms of ozone further benefits practical function in dentistry. Treating patients with ozone therapy lessens the treatment time with an immense deal of variation and it eradicates the bacterial count more specifically. In future, the focus should be on well designed double blind randomized clinical trial and establishment of safe and well defined parameters to determine the precise indications and guidelines for routine use of ozone in the treatment of various dental pathologies. In contrast with traditional medicine modalities such as antibiotics and disinfectants, ozone therapy is quite economical; it will markedly reduce both medical cost and invalidity. Dentistry is varying with induction of modern science to practice dentistry. The ozone therapy has been more beneficial than present conventional therapeutic modalities that follow a minimally invasive and conservative application to dental treatment. The exposition of molecular mechanisms of ozone further benefits practical function in dentistry. 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 painless and increases the patients' tolerability and fulfillment with minimal adverse effects. Contraindications of this controversial method should not be forgotten. Gojanur and Sushma (2015) have reported the successful use of ozone in the medical field for treatment of various diseases for more than 100 years. Introduction of ozone therapy has truly revolutionized dentistry. Ozone therapy is completely painless, noninvasive and has advantages of lack of side effects or adverse reactions, increased patient's acceptability and compliance thus making it an ideal treatment option for pediatric patients where patient compliance is of prime importance. This article reviews the clinical application of ozone in pediatric dentistry. Sansriti Tiwaria et al. (2017) reviewed as Ozone has been used successfully for the treatment of various diseases for more than a decade. Its unique properties include immuno-stimulant, 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 especially for pediatric patients. This review is an attempt to highlight various treatment modalities of ozone therapy and its possible clinical applications in future. Osama Safwat et al. (2017) evaluated the clinical changes in dentin of deep carious lesions in young permanent molars, following ozone application with and without the use of a re-mineralizing solution, using the stepwise excavation. Regarding dentin color and consistency, no significant differences were observed following ozone application, with and without a re-mineralizing solution. There were no significant differences between ozone treatment, and calcium hydroxide during the different evaluation periods concerning the dentin color. Burcak et al. (2010) investigated the effect of ozone pre-treatment on the micro-leakage and marginal integrity of pit and fissure sealants placed with or without a self-etch 6th generation adhesive. Janavathi et al. (2015) reviewed the ozone effects and found consistent, safe and with minimal and preventable side effects. Medical O3 is used to disinfect and treat disease. Mechanism of actions is by inactivation of bacteria, viruses, fungi, yeast and protozoa, stimulation of oxygen metabolism, activation of the immune system. Medication forms in a gaseous state are somewhat unusual, and it is for this reason that special application techniques have had to be developed for the safe use of O3. In local applications as in the treatment of external wounds, its application in the form of a transcutaneous O3 gas bath has established itself as being the most practical and useful method. Ozonized water used in dental medicine as spray or compress with encouraging results. Diseases treated are infected wounds, circulatory disorders, geriatric conditions, macular degeneration, viral diseases, rheumatism/arthritis, cancer, SARS and AIDS. Jelena et al. (2019) evaluated local effect of gaseous ozone on bacteria in deep carious lesions after incomplete caries removal, using chlorhexidine as control, and to investigate its effect on pulp vascular endothelial growth factor (VEGF), neuronal nitric oxide synthase (nNOS), and superoxide dismutase (SOD). Antibacterial effect of ozone on residual bacteria after incomplete caries removal was similar to that of 2% chlorhexidine. Effect of ozone on pulp VEGF, nNOS, and SOD indicated its biocompatibility. Noetzel, et al. (2009) evaluated the efficacy of Ca(OH)2, Er:YAG laser or gaseous ozone (either alone or combined with instrumentation and various irrigants) against Enterococcus faecalis in root canals and reported encouraging results**.** Thaman, and Sood (2012) used ozone in everyday dental practice. In the era of minimal invasive dentistry, use of ozone in treating early tooth caries without cavitations is very promising and showed that it is now possible to arrest and reverse these lesions in a predictable and repeatable way without invasive intervention. Ozone can really replace the drilling and filling technique? Is it really an effective method for painless removal of caries. Nazlı et al. (2019) find out the effectiveness of the ozone application in two-visit indirect pulp therapy of permanent molars with deep carious lesion. The aim of this randomized, three-arm parallel, single-blinded clinical trial was to evaluate the clinical and microbiological effectiveness of the ozone application in two-visit indirect pulp therapy. The two-visit indirect pulp therapy yielded successful results for all study groups. However, CHX would be conveniently preferable due to improving the treatment success. Kollmuss et al. (2014) evaluated the anti-microbial effect of gaseous and aqueous ozone on biofilms of caries pathogen microorganisms with regard to concentration and time dependency. Biofilm cultures of Actinomyces naeslundii, Streptococcus mutans and Lactobacillus paracasei were grown on nitro-cellulose-membranes for 48 hours. The membranes were cut into equal-sized pieces and exposed to chlorhexidine digluconate (CHX) (positive control; 0.1-20%), gaseous (1-64 g m3) and aqueous ozone (1.25-20 microg ml(-1)) for 60 seconds. The influence of exposure time (30-120 seconds) was tested for exemplary concentrations of the three agents and observed major effects for concentration/contact time of the agent on bacterial survival. High concentrated gaseous and aqueous ozone seem to be potential alternatives to CHX with equivalent antimicrobial activity. Tricarico et al. (2020) find out the therapeutic application of ozone and its derivatives in the dental field has been used for many purposes. However, there has yet to be a consistent evaluation of the outcomes, due to the lack of standardization of the treatment operating procedures. Clinical trials and case reports of good, neutral, as well as negative results related to ozone treatment specifications were evaluated. A better understanding of the mechanisms of action of this bio-oxidative therapy could open new horizons related to the personalization of treatments and the quality of dental care. The critical condition to achieve these goals is an improved knowledge of the qualitative/quantitative characteristics of ozone and its derivatives. Unlike other drugs, ozone does not act directly through traditional drug-receptor interactions. When administered in the gaseous form, it is a gaseous mixture where ozone represents at most 5% of the total, while the remaining part is generally made up of oxygen, acting as a gas transmitter. On the other hand, ozone, due to its extreme reactivity, cannot be used for the transmission of chemical signals to induce physiological or biochemical changes. Moreover, ozone cannot be considered a pro-drug in the common sense of the term. A pro-drug is a biologically inactive molecule that, once introduced into the body, requires chemical transformations, generally of enzymatic nature, for its activation. Ultimately, ozone can be classified as an effector molecule generator. Depending on method of administration, administration site, dosage and derivative formulations, different hydrophilic (mainly hydrogen peroxide). Maryam et. al. (2018) studied the mineralization of sulfolane in aqueous systems by $CaO2/O3$ and $CaO/O3$. If 1.6 g/L of oxidants ($CaO2$ and CaO) were used along with 5 L/min of $O3$ in a batch reactor, degradation of sulfolane followed a pseudo-first order kinetics model. Both sulfolane and TOC were totally removed in less than 40 and 150 min respectively. For these treatments, the pH of the aqueous solutions was above 11, which made $O3$ more effective in removing sulfolane. However, the high pH of the solution didn't improve TOC removal. For TOC removal the presence of $CaO2$ and CaO was necessary. Once these conditions were optimised in the lab, field experiments were designed and evaluated to treat contaminated ground water samples. The field tests were successful in mineralization of sulfolane within a reasonable time (4 h). Sulfolane degradation took 150 min in these experiments. The pH of the water samples was brought to near neutral (pH = 6.5) by bubbling $CO2$. Mohammed S Bin-Shuwaish (2016) studied the effectiveness of ozone in Cavity disinfectants in operative dentistry. The degree of success in the elimination of bacteria during cavity preparation and prior to the insertion of a restoration may increase the longevity of the restoration and therefore the success of the restorative procedure. The complete eradication of bacteria in a caries-affected tooth, during cavity preparation, is considered a difficult clinical task. In addition to weakening the tooth structure, attempts to excavate extensive carious tissue completely, by only mechanical procedures, may affect the vitality of the pulp. Therefore, disinfection of the cavity preparation after caries excavation can aid in the elimination of bacterial remnants that can be responsible for recurrent caries, postoperative sensitivity, and failure of the restoration. However, the effects of disinfectants on the restorative treatment have been a major concern for dental clinicians and researchers. This review aims to explore existing literature and provide information about different materials and techniques that have been used for disinfecting cavity preparations and their effects and effectiveness in operative dentistry and, therefore, helps dental practitioners with clinical decision to use cavity disinfectants during restorative procedures. Zekeriya et al (2016) evaluated the effects of ozone therapy (OT) on the early healing period of deep eithelialized gingival grafts (DGG) placed for non- root coverage gingival augmentation by laser Doppler flowmetry (LDF). It can be concluded that ozone therapy enhanced blood perfusion units in the first postoperative week. This outcome is also consistent with improvement in wound healing, accompanied by an increase in quality of life and decrease in postoperative pain in the test group. Giovanna Marcílio et al. (2020) reviewed the randomized controlled trials (RCTs) in 8 databases. Primary outcome measures were antimicrobial effect and adverse events. We used the Cochrane risk of bias tool to evaluate methodological quality of included RCTs and GRADE approach to evaluate the certainty of the evidence. They used the Review Manager software to conduct meta-analyses. Based on a very low certainty of evidence, there is not enough support from published RCTs to recommend the use of ozone for the treatment of dental caries. Well-conducted studies should be encouraged, measuring mainly the antimicrobial effects of ozone therapy at long term and following the recommendations of the CONSORT statement for the reporting of RCTs.

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