

## The antimicrobial potential of ozone against microorganisms that affect oral health

O potencial antimicrobiano do ozônio contra microrganismos que acometem a saúde oral

El potencial antimicrobiano del ozono frente a microorganismos que afectan la salud bucal

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### **Abstract**

**Objective:** The objective of this narrative literature review article is to address ozone and its antimicrobial potential, which is used in ozone therapy to treat various pathogens that affect oral health, talking about which treatments we can use this therapy in, discussing its effectiveness and mechanism of action. **Methodology:** Research was carried out with the aim of obtaining the maximum amount of information on the subject, so that the article would become rich, with a scientific basis and current, thus, searches were made on the following websites: DeCs, Web of Science, Science Direct, The Cochrane Library, Scielo, PUBMED Central, BVS/BIREME, Research, society and development journal and Google Academy. **Results:** Ozone therapy has great potential to destroy viruses, bacteria, fungi and protozoa, thus being a therapy that has an effect against pathogens that invade the human body and harm health. **Conclusion:** Ozone is a substance with a strong antimicrobial effect, and is widely used in dentistry, as it is effective against pathogens that cause problems in the oral cavity. It can be used in treatments against fungi, periodontal diseases, cavities, proliferation and development of dental biofilm, peri-implant mucositis and in the disinfection of root canals. Thus, ozone therapy is a therapy that has great benefits when used by dentists, increasing the range of treatments that the dentist can perform and improving the patient's health.

**Keywords:** Pathogens; Ozone therapy; Antimicrobials; Oral cavity; Complementary therapies.

### **Resumo**

**Objetivo:** O objetivo deste artigo de revisão de literatura narrativa é abordar sobre o ozônio e seu potencial antimicrobiano, que é usado na ozonioterapia para tratar diversos patógenos que acometem a saúde bucal, falando sobre em quais tratamentos podemos utilizar dessa terapia, discutindo sua efetividade e mecanismo de ação. **Metodologia:** Foram feitas pesquisas com objetivo de obter o máximo de informações sobre o tema, para que o artigo se tornasse rico, com embasamento científico e na atualidade, dessa forma, foram feitas buscas nos sites : DeCs, Web of Science, Science Direct, The Cochrane Library, Scielo, PUBMED Central, BVS/BIREME, Research, society and development journal e Google Academy. **Resultados:** A ozonioterapia possui grande potencial de destruir vírus, bactérias, fungos e protozoários, sendo assim, uma terapia que possui efeito contra patógenos que invadem o organismo humano e

prejudicam a saúde. Conclusão: O ozônio é uma substância com grande efeito antimicrobiano, possuindo grande uso dentro da odontologia, por ter efetividade contra patógenos que provocam problemas da cavidade oral, podendo ser utilizado em tratamentos contra: fungos, doenças periodontais, cáries, proliferação e desenvolvimento do biofilme dentário, mucosite peri-implantar e na desinfecção dos canais radiculares. Assim, a ozonioterapia se trata de uma terapia que possui grandes benefícios quando utilizada pelos cirurgiões dentistas, aumentando o leque de tratamentos que o dentista pode realizar e sendo algo que eleva a saúde do paciente.

**Palavras-chave:** Patógenos; Ozonioterapia; Antimicrobianos; Cavidade oral; Terapias complementares.

### Resumen

**Objetivo:** O objetivo deste artigo de revisão de literatura narrativa é abordar sobre o ozônio e seu potencial antimicrobiano, que é usado na ozonioterapia para tratar diversos patógenos que acometem a saúde bucal, falando sobre em quais tratamentos podemos utilizar dessa terapia, discutindo sua efetividade e mecanismo de acción. **Metodología:** La investigación se realizó con el objetivo de obtener la mayor información posible sobre el tema, para que el artículo fuera rico, con base científica y actualmente por ello se realizaron búsquedas en los sitios web: DeCs, Web of Science, Science Direct, Biblioteca Cochrane, Scielo, PUBMED Central, BVS/BIREME, Revista de investigación, sociedad y desarrollo y Google Academy. **Resultados:** La ozonoterapia tiene un gran potencial para destruir virus, bacterias, hongos y protozoos, por lo que es una terapia que tiene efecto contra patógenos que invaden el cuerpo humano y dañan la salud. **Conclusión:** El ozono es una sustancia con gran efecto antimicrobiano, ampliamente utilizada en odontología, ya que es eficaz contra patógenos que causan problemas en la cavidad bucal, pudiendo ser utilizado en tratamientos contra: hongos, enfermedades periodontales, caries, proliferación y desarrollo de biopelículas. dental, mucositis periimplantaria y desinfección de conductos radiculares. Así, la ozonoterapia es una terapia que presenta grandes beneficios al ser utilizada por los cirujanos dentistas, aumentando la gama de tratamientos que el dentista puede realizar y siendo algo que mejora la salud del paciente.

**Palabras clave:** Patógenos; Ozonoterapia; Antimicrobianos; Cavidad oral; Terapias complementarias.

## 1. Introduction

The presence of microorganisms in the oral cavity makes it prone to a large number of situations that can result in comorbidities or even death of the patient. Practically all dental specialties have to be concerned about this fact, in the same way that many substances have been used throughout the development of dentistry.

Ozone therapy has emerged as a promising approach to combat microorganisms in the oral cavity, offering a viable and safe alternative compared to traditional methods. Ozone, a triatomic allotrope of oxygen, has potent antimicrobial properties due to its ability to generate reactive oxygen species (ROS), which promote the oxidation of essential cellular components in pathogens. Recent studies have demonstrated the efficacy of ozone against a wide range of oral microorganisms, including cariogenic bacteria, periodontal pathogens and fungi.

A study conducted by Bocci et al. (2022) highlighted that topical application of ozone can significantly inhibit the growth of *Streptococcus mutans*, one of the main bacteria associated with dental caries. The authors observed a notable reduction in bacterial biofilm formation, suggesting that ozone may be a valuable tool in preventing caries. In addition, a study by Baysan and Lynch (2021) evaluated its effectiveness in disinfecting periodontal pockets. The results indicated that ozone treatment led to a substantial reduction in the count of periodontal pathogens, such as *Porphyromonas gingivalis*, improving periodontal clinical indices, highlighting its potential as an adjunct in periodontal therapy.

The use of ozone appears to have a very broad action when used in the oral cavity. For example, Noriega et al. (2023) investigated the use of ozone in oral candidiasis; experiment in which participants who received treatment with it showed a faster recovery and a significant reduction in symptoms compared to the control group, highlighting the effectiveness of ozone as an alternative with antifungal capacity. Such evidence suggests that ozone therapy can play a crucial role in modern dentistry, not only due to its antimicrobial action, but also due to its ability to improve clinical results and provide a safe and effective therapeutic option.

Therefore, the objective of this work was to conduct a literature review to investigate the use of ozone therapy and its applicability in dental specialties.

## 2. Methodology

Research was carried out with the aim of obtaining the maximum amount of information on the subject, so that the article would become rich, with a scientific basis and current, thus, searches were made on the following websites: DeCs, Web of Science, Science Direct, The Cochrane Library, Scielo, PUBMED Central, BVS/BIREME, Research, society and development journal and Google Academy. Rother's work (2007) was used as a basis for the construction of this narrative literature review article to define the approach, structure and methodology that should be used in the preparation of the article. To obtain current, rich and necessary information in the composition of this article, the following descriptors were used: Pathogens; Ozone therapy; Antimicrobials; Oral cavity; Complementary Therapies.

## 3. Results

### 3.1 History of Ozone Therapy

The ozone molecule is composed of 3 atoms and oxygen and is considered a less stable form of oxygen. The origin of the word ozone comes from the Greek word "smell", due to its strong odor (Sunnen, 1988). Its great bactericidal potential causes a direct attack on microorganisms, oxidizing their biological material, in addition to the fact that the power and effectiveness of ozone as a bactericide can be up to four thousand times faster than the effect produced by chlorine (Mehlman & Borek, 1987). After penetrating the human body, ozone triggers better cellular oxygenation and improves body metabolism (Pino et al. 1999). The use of ozone as a therapy began in 1857, through experiments carried out by the engineer Werner Von Siemens, who developed and patented the first ozone generator, which, through an electrical source, produces a silent discharge or corona and which later became a standard for modern equipment (Kogelschatz, 2003).

The use of ozone therapy began in Australia and Germany, however, it was only in 1980 that this therapy spread throughout the planet. Ozone began to be used in Brazil in 1975 by the São Paulo physician Dr. Henz Konrad. Thus, ozone begins to act when it comes into contact with organic fluids, triggering the activation of reactive oxygen molecules, which influence and occur in biochemical events of cellular metabolism, providing tissue repair, together with bactericidal, fungicidal and antimicrobial effects. Ozone therapy is used to treat abscesses, diabetic foot, osteomyelitis, dysfunction of various organs and burns. In the treatment of chronic ulcers, ozone therapy has influenced neoangiogenesis and the formation of granulation tissue, due to its antiseptic effect (Santiago et al., 2016). Since their ancestors, humans have been searching for new therapies that are efficient and viable in treatments. This therapy uses ozone as a therapeutic for treating wounds, and has been used since the 19th century, and is a practice approved in several countries today. During the First World War, ozone was used for the first time to treat German soldiers who were infected with gas gangrene due to anaerobic infections caused by *Clostridium*, which is highly sensitive to ozone. Its application is low cost and it is an alternative application to treat wounds, motivated by its high healing effect and which is commonly used in the United States in many treatments (Borges, 2005).

### 3.2 Antimicrobial potential

Ozone therapy is a technique that has been increasingly investigated and is gaining new followers and supporters in several countries around the world, motivated by its many advantages, such as its low cost, easy application and minimally invasive action (Gulafsha & Anuroopa, 2019). The use of ozone has gained therapeutic popularity due to its biological properties, which exist due to its ability to modulate biological oxidative stress. Ozone applied in treatments has an antimicrobial function, through its bacterial property against gram- and gram+, being highly effective against fungi and destroying cells contaminated by viruses (Case et al., 2012).

The antimicrobial action performed by ozone therapy occurs due to the damage to cell membranes and the oxidation of intracellular proteins, which causes the loss of organelle function. The effect performed by this therapy does not interfere with the cells of the human body, due to the fact that it is a selective action of microbial cells (Reddy et al., 2013).

### **3.3 Ozone therapy applied in dentistry**

#### **3.3.1 Disinfection of root canals**

In recent years, the dental specialty known as endodontics has evolved significantly, thanks to new materials and technologies invented in recent years, which facilitate the work of the endodontist by reducing the time that endodontic treatment takes. Even though new equipment has been created in recent times, most of the failures or failures associated with endodontic treatments are related to the persistence of microorganisms that were not destroyed after intracanal medication or during chemical and mechanical preparation (Siqueira & Rôças, 2008).

Ozonated water has an effect against microorganisms, which combats bacteria that invade the dentinal tubules (Nagayoshi et al., 2004). In endodontics, most studies indicate that ozone can be used as an intracanal medication and as a good irrigant, due to the fact that endodontic treatment seeks to destroy microorganisms that exist in the root canal system before endodontic filling occurs. The application of ozone in the canal systems represents a biological treatment, which does not cause pain to the patient, improving the asepsis of the root canals, being an adjuvant to conventional treatment, with biocompatibility and antimicrobial effect (Almeida et al., 2019).

Studies have evaluated the role of ozone therapy in endodontics and obtained results that indicate the effectiveness of ozone when applied to polymicrobial infections, such as in cases of necrotic canals, as it demonstrates action against a range of bacteria such as: *Candida albicans*, *Peptostreptococcus*, *Staphylococcus*, *Pseudomonas*, *Enterococcus*, *Escherichia coli* and mycobacteria, bacteria that have been studied in in vitro research. Thus, ozone is an important agent in the treatment of periapical infections, mainly because it is a way of treating without performing surgical procedures. Another study, however, indicated that a root canal disinfected with ozonated water has an effect against microorganisms, but that it is inferior to the effect performed by sodium hypochlorite (Reddy et al., 2013).

Another study also showed that the application of ozonated water in the disinfection of root canals is inferior to the antimicrobial potency of hypochlorite. However, it showed that when hypochlorite was used in conjunction with ozone, the antimicrobial effect was enhanced. However, the study has some risks of bias (Bastos et al., 2022). Other studies also evaluated the use of ozone in root canal treatment and reported that most studies indicate extremely positive results regarding the antimicrobial effect, placing ozone in a position of being suitable for endodontic treatments. Therefore, ozone therapy is shown to be an extremely important and beneficial therapy in dentistry, due to the bacteria located in root canals, which are highly sensitive to ozone (Souza & Krukoski, 2020).

#### **3.3.2 Caries prevention**

One of the infectious and transmissible diseases that has existed since the beginning of human civilization is dental caries, which has plagued humanity to this day, resulting from the colonization of microorganisms on the surface of the enamel, mainly by *Streptococcus mutans*, which metabolizes different types of fermentable carbohydrates such as sucrose, producing acids. The availability of sugar triggers an acidity that leads to the dissolution of the calcium phosphate that makes up the layers of the surface of the enamel structure, causing the phosphate and calcium to be released into the oral environment. After a certain point, mineral loss reaches a level at which the formation of a cavity can be identified, which may or may not evolve, and in extreme cases, it leads to the destruction of the entire dental crown. Scientific evidence is extremely clear regarding the role of sugar in the process of caries formation (Stephan, 1940; Gustafsson et al., 1954).

After many studies on the use of ozone as a treatment for caries, research has revealed that caries can be prevented and treated through the application of ozone (Kumar et al., 2004). Proximal, root, pit and fissure caries, and 14 activated lesions that were not cavitated, responded positively to the application of this treatment (Bocci, 2004; Kumar et al., 2004). Ozone has the function of killing microorganisms and removing the acid derivatives that are produced by them, in addition to stimulating the remineralization of the tooth structure by allowing the diffusion of phosphorus and calcium ions to the inner surface of the tooth with caries, through the dentinal tubules (Bocci, 2004; Kumar et al., 2004; Domb, 2004). Among the various factors that are related to the reversal of lesions caused by caries, location and size are the most related factors. Studies state that the application of ozone to non-cavitated lesions caused them to have fewer microorganisms present than in larger lesions closer to the gingival margin (Balan & Narayanan, 2015; Nogales et al., 2008). Other researchers have carried out tests that have shown that non-cavitated caries can be reversed and have their progression stopped if ozone is used as treatment. However, there is no pre-established protocol. However, some scholars claim the opposite. After research, they concluded that applying ozone for 10 to 20 seconds would be enough, while others say that 40 to 60 seconds would be enough, but that it would have less chance of working if applied to cavitated lesions (Azarpazhooh & Limeback, 2008).

### **3.3.3 Gingivitis and Periodontitis**

A healthy periodontium is characterized by gums that are pink in color and show no signs of bleeding or inflammation, and there is no exposed root due to the good health of the gums. Gingivitis is a type of nonspecific inflammation caused by the accumulation of biofilm in the region of the gum margin, without underlying destruction of the insertion apparatus (Lang & Bartold, 2018; American Academy of Periodontology, 2000). Although not all patients with gingivitis end up developing the disease, treating gingivitis is a strategic point to prevent the development of periodontitis (Tonetti et al., 2015; Chapple et al., 2015). The inflammation called periodontitis is defined as: chronic inflammatory disease, infectious in origin, which results in the destruction of the periodontal tissues that serve as support formed by the alveolar bone, cementum and periodontal ligament (Hajishengallis, 2015).

The CFO allows ozone to be used in periodontics, with the aim of preventing diseases and in the treatment of infection and inflammation processes. The use of ozone in periodontics is due to its chemical and physical properties, which have beneficial actions for the human body such as anti-hypoxic, antimicrobial, analgesic, detoxifying, immunostimulant, in addition to serving as a subgingival irrigant due to its bactericidal factor (Srikanth et al., 2013; Kumar et al., 2014). The application of ozone eliminates the pathogens of periodontal diseases, causing the metabolism to be restored and bringing the adequacy of oxygen levels, normalizing the periodontal microbiota, activating the immune system and improving blood circulation (Ferreira et al., 2014). Those responsible for periodontal infections are pathological agents, most of which are bacteria, which are sensitive to ozone and which weaken or die after its application (Belegote et al., 2018). Ozone therapy has become an alternative and complementary option when it comes to periodontal prevention and treatment today and has been increasingly addressed during treatments for periodontitis with disease (Uraz et al., 2019). A study showed that ozonated water has a great effect when used as an irrigator, with efficacy of up to 8 months after treatment, triggering improvements in symptoms of: probing depth, bleeding and bacterial flora (Ferreira et al., 2014).

### **3.3.4 Bacterial biofilm control**

Bacterial biofilm is more likely to establish itself on the tooth surface because the tooth surface does not undergo renewal, creating good conditions for bacteria (Fejerskov & Thylstrup, 1994). Thus, bacteria that are not dissolved by saliva end up attaching themselves to the tooth surface naturally. However, if mechanical or chemical measures are applied correctly, preventing bacterial accumulation and the organization of these bacteria on the tooth surface, biofilm plaque will be controlled.

However, failures in controlling these measures can result in the formation of a pathogenic bacterial biofilm, which can cause an imbalance in the health of hard and soft tissues, triggering or not some types of problems in the oral cavity (Cury20 1999; Oppermann & Rösing55 1999).

### 3.3.5 Peri-implant mucositis treatment

The pathological modification of the tissues surrounding the implant integrated with the bone is called peri-implantitis, which is caused by the association of occlusal trauma associated with microbiota. Thus, the ways of treating inflammatory processes are, for example: application of medication to stimulate regeneration, use of antimicrobials, mechanical debridement and oral hygiene guidance (Oliveira et al., 2015). The application of ionized water and ozone gas are examples of ozone administration that can be used as an indication for the treatment of peri-implantitis. The way of applying this treatment must follow some steps: seal the gum around the tooth, wrap the abutment with a material chosen by the dentist so that there is no contact with the ozone. When performing curettage, a wash is performed with ozonated water, where the professional can prescribe the use of ozonated oil 3 to 4 times a day so that healing occurs more quickly (Grupta & Deepa, 2016).

### 3.3.6 Antifungal function

The oral cavity has one of the most vast and complex microbiota within the human body. Approximately 500 different species are found in this cavity, including bacteria, fungi and viruses, most of which belong to the periodontium and the rest located in the microenvironment region composed of the tongue, smooth mucosa and the surface of the teeth (Paster et al., 2006). The microorganisms found in the mouth are mostly commensal, although they can become pathogenic under certain circumstances, which is what happens in oral fungal infections caused by yeasts of the genus *Candida* (Hahnel et al., 2012). *Candida* yeasts are microorganisms that are normally found in regions of the skin, genitourinary system, oropharynx, gastrointestinal tract and upper respiratory tract without causing harm to individuals who are in good health (Yapar, 2014). A common pathogen that is an opportunistic being is *C. Albicans*, which is isolated from 30% to 40% of adults who are in good health (Vanden Abbeele et al., 2008; Abaci et al., 2010). It is a yeast that can be developed in a human being even if it does not cause any symptoms. However, a range of chronic or acute infections can occur. Oral candida has two classifications, primary or secondary, with or without lesions. The manifestation of primary candida occurs acutely and is known as "pseudomembranous candidosis and erythematous candidosis", when it is chronic, it is plaque candidosis and nodular or hyperplastic (Sherman et al., 2002).

Ozone can be used to treat various fungi that affect oral health, and can be administered in its gaseous or aqueous phase through various routes. Based on this fact, an in vivo study was conducted using ozonated water, and it was found that rinsing with this substance is highly effective against the microbial load of plaque, as it is a potent microbial agent against fungi, protozoa, bacteria and viruses. It can be combined with brushing and dental floss during oral hygiene, in addition to being economical and easy to prepare. However, the degree of its activation on microorganisms in the oral cavity requires further research, as it does not destroy the entire microbial population of dental plaque (Sadatullah et al., 2012).

The cytotoxic activity of the ozonated water compound flow on *C. albicans* adhering to the resin plate was also analyzed. The ozone treatment was effective in reducing the number of viable strains, through the use of 0.5 mg/L of ozonated water with a flow rate of 2 L/min for one minute (Arita et al., 2005). Another study obtained results through the use of ozonated water (0.9–0.12 ppm) in a 30-second flow over four hours in a sink with a colony of *Candida auris*, collecting the culture every four hours after disinfection. The swabs were subsequently vortexed in 1 mL of DeyEngley neutralizer that underwent several dilution steps and was seeded in Sabouraud Dextrose. Obtaining the result that the pathogen concentration decreases gradually after disinfection with ozonated water, reaching undetectable levels in the filter after 2 days. Other results indicated that there was a

reduction of *C. auris* in colonized sinks after the application of ozonated water, with the pathogens having been reduced to levels that are not detectable in the filters, showing that water with ozonation can be used to reduce the risk of fungal contamination in similar situations (Livingston et al., 2018).

#### **4. Discussion**

Ozone therapy is a non-invasive therapy used in medicine since the First World War, which later spread throughout Europe and the entire planet, and was later implemented in dentistry, where it is still used today. It is a therapy used in several dental specialties, such as: Endodontics, Periodontics, Dentistry, Surgery, Oral and Maxillofacial Traumatology and Stomatology.

A positive point of this therapy is its few side effects (contraindications), which are associated with the oxidative capacity of ozone, which causes materials that come into contact with this gas to be oxidized, highlighting the importance of using ozone-resistant materials. Another way in which ozone can become harmful is if a patient undergoing this treatment becomes poisoned by ozone, which can cause a series of symptoms and problems that impact the quality of life and health of the human being. This shows that it is extremely important for the dentist to be trained in Ozone Therapy, so that he or she knows how much gas to use in each specific treatment and what to do if an incident occurs during its use.

Ozone is a great antimicrobial agent, which in endodontics is widely used in the disinfection of root canals, being an additional option in addition to the common and traditional hypochlorite, but when combined with each other, they trigger a greater antimicrobial effect than when used individually, becoming a great tool for preventing new problems in the tooth such as necrosis and cavities. In addition to controlling bacterial biofilm, preventing bad odors and excessive bacterial growth, ozone can be a weapon in the fight against tooth decay in dentistry, reversing the process of non-cavitated tooth decay and allowing the diffusion of calcium and phosphorus ions towards the inner surface of the decayed tooth by opening the dentinal tubules. Periodontitis and gingivitis are oral health problems that have existed for decades and persist to this day. Even with advances in technology, many people suffer from the consequences of these diseases, and ozone therapy is a complementary option in the treatment of these diseases, killing the bacteria that cause bacterial growth in teeth and soft tissues. Fungi develop in different environments when their conditions are favorable, changes in pH, humidity and a large presence of bacteria can end up influencing fungal growth and development, however, ozone has factors that hinder and destroy the development of specific fungi, and can also be used in day-to-day clinical dental treatments.

Thus, studies point to the great usefulness of ozone therapy in dentistry due to its antimicrobial activity, but another factor that benefits it even more is that it is a non-drug and comfortable treatment model, unlike corticosteroids that can cause side effects such as allergic reactions and gastrointestinal irritation.

#### **5. Conclusion**

Ozone Therapy is a therapy that has a lot of scientific evidence that proves its application and effectiveness, studies that have been carried out by different authors over the years, which complement each other by pointing out the benefits and harms of ozone in dentistry. This therapy presents a range of benefits when applied to different dental treatments, due to the fact that it is a mechanism that has a great antimicrobial factor, which destroys viruses, bacteria and fungi that harm the oral cavity, being a great ally in periodontal treatments, preventing cavities through the control of biofilm, fungi such as candida, disinfection of root canals and in the treatment of peri-implantitis mucositis.

Thus, it is seen that Ozone Therapy is a great ally for dentists because it is something non-invasive and has many uses within dental treatments. However, despite having many benefits, it is something that is little talked about both during graduation and throughout clinical life, which highlights the importance of more studies that show the importance of ozone applied in

dentistry, so that this can be publicized through magazines and newspapers until more dentists know about the existence of this therapy and start using it, which will consequently benefit and improve human health.

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