# Ozonated oil in wound healing: what has already been proven?

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## Abstract

Acute or chronic inflammatory reactions aim to control lesions, resist to pathogens attack and repair damaged tissue. The therapeutic administration of ozone known as ozone therapy appears as a possible treatment for tissue repair, as it promotes the healing of wounds. It has bactericidal, antiviral and antifungal properties and has been used as a therapeutic resource to treat inflammation. The objective was to carry out an integrative review regarding the use of ozonated oil in acute and chronic inflammations. The keywords "ozone therapy," "inflammation" and "ozone" were used in the Portuguese, Spanish and English languages. The paper selection was based on inclusion and exclusion criteria. In total, 28 articles were selected. It has been seen that ozonated oil is effective in healing cutaneous wounds. The beneficial effects are due to the healing of wounds, due to the reduction of microbial infection, debridement effect, modulation of the inflammatory phase, stimulation to angiogenesis as well as biological and enzymatic reactions that favor the oxygen metabolism, improving the wound cicatrization. In addition to promoting healing, ozonated oil reduces symptoms related to skin burns, prevents post-lesion hyperpigmentation, and reduces the pain of aphthous ulcers. Therefore, ozonated oil represents an effective and inexpensive therapeutic alternative that must be implanted in the public health system.

**Key words:** ozone; ozonated oil; chronic inflammation; acute inflammation; oxidative stress; antioxidant

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## Introduction

Ozone is a gas with high oxidizing power and has been used for different purposes. The ozone therapy is the therapeutic administration for the treatment of various pathologies. The topical route has been used to treat extensive wounds, fungal, bacterial and viral infections, ischemic lesions and other affections, showing efficiency, mainly in disinfection and wound healing.[1,2]

Topical application can be performed using ozonated oils. The ozonation of the oil promotes formulations containing ozone derivatives with adequate stability. In this context, this work aimed to carry out an integrative review of the ozonated oil by topical route in inflammations.[3]

## Search Strategy

The bibliographical survey was carried out in the Portuguese, English and Spanish languages, using the keywords: "ozone" and "ozone therapy." The descriptors were consulted in the Descriptors in Health Science (DeCS) using "ozone," since in DeCS the term ozone therapy does not appear. The inclusion criteria were original articles using ozone therapy, associated to the descriptors: "inflammation" and "patients" obtaining the result of 74 indications in PubMed with this association. The search and collection of the data were carried out in August and September, 2018. The exclusion criteria were all other types of publications (editorials, comments, reflection, and experience reports).

In the Bireme research portal, we used the filters article or thesis, all period, and the main topic ozone therapy. Thus, 14 articles were found. Subsequently, an analysis of the content was carried out in Coordination of Superior Level Staff Improvement journals, using the same search filters from the Bireme portal, resulting in 4 articles.

After analyzing the title and content of the articles, 28 papers were selected.

## Results

### Inflammatory reactions

Inflammatory reactions represent an activated action mechanism to hold the lesions' progress, to resist pathogens' attack and to repair damaged tissues. Despite physical discomforts (such as fever), inflammation is often beneficial and allows us to cope with daily stress and aggression.[4-7]

This process is complex and involves host cells and molecules such as proteins, transcription factors and chemical mediators to eliminate the initial cause of the cell lesion or necrotic cells and tissues resulting from the original lesion and then trigger the repair process.[8]

The inflammations are classified as acute and chronic. Acute is a rapid and short-lived response and it is designed to carry leukocytes and plasma proteins to the sites of the lesion. The stimuli for the acute lesions are infections, traumas, chemical and physical agents, tissue necrosis, foreign bodies and immune reactions.[9]

Chronic inflammation lasts longer and can continue for weeks, months, or years. It is characterized by infiltration into the inflamed tissue of lymphocytes and macrophages and by proliferation of blood vessels in the site. The inflammation is activated and leads to destruction of the affected tissue. Conversely to tissue destruction, the repair of the region oc-

curs, but the damaged tissue due to this type of inflammation is not fully regenerated and the result of healing is fibrosis.[8]

## Ozone

Ozone is formed during a reversible endothermic reaction that consumes 286.19 J. In the environment, ultraviolet rays, which are absorbed, catalyze this reaction and enable the ozone to controls the irradiation, protecting the planet Earth. Within the oxidizing agents, ozone is the third most powerful, preceded in order by fluorine and persulfate.[10]

Ozone is a highly reactive and unstable molecule. This low stability of approximately 3 seconds in the gas phase avoids its storage. Therefore, *in situ* generation of ozone becomes necessary. Ozone can be produced by three different techniques: exposure of $O_2$ to ultraviolet light, perchloric acid electrolysis and electrochemical discharge.[11,12]

The lifetime of the ozone molecule is directly related to temperature. The higher the temperature, the shorter the lifetime of the ozone and, consequently, the lower its power of action. As an example, the ozone half-life is 140 minutes at 0°C, and reached only 40 minutes at 20°C.[10]

Ozone gas was discovered in 1840 in Switzerland by Christian Friedrich Schobein who, when working with high electricity in the presence of oxygen, produced an electric discharge with formation of an unpleasant odor gas. In 1854, Werner Von Siemens built the first ozone generator. At that time, it was already known that ozone was unstable, having to be produced and then used. With the construction of the ozone generators, its use was initiated in industrial applications, or even to clean water, promoting its potent microbicidal action.[10]

Ozone has been used for the treatment of drinking water in Europe since the beginning of the 20th century, without loss in the organoleptic properties, and in relation to microorganisms, its action is as effective as chlorine.[12]

Payr, an Austrian physician, Fisch, a Swiss dentist, and Wolff, a German physician are the pioneers in clinical research involving ozone,[1] though their observations were empirical. Currently there are scientific reports that prove the benefits of ozone in the treatment of wounds, dental use, disinfection, treatment of herniated disc and hepatitis C.[13]

## Ozone therapy: use of ozone in medicine

The practice of ozone began in tuberculosis treatment, followed by its use in dental clinics and gangrene treatments. The surgeon Payr was the first one to be use the ozone rectal route.[14]

However, the inappropriate use of gas via intravenous by individuals with no practice in ozone therapy or in medicine resulted in death by embolism. In this way, the use of ozone was condemned in some countries.[10]

Ozone has the ability to assist in eliminating bacteria, viruses and parasites as well as biofilms. In this context, it was assumed that the intravenous injection of a gas mixture composed of $O_2$ and $O_3$ into bacterial sepsis in patients with HIV would inactivate the pathogens and would cure the disease. In the early 1990's, technicians migrated to Africa and to West Indies to perform such procedure; indeed, it was seen that intravenous administration of $O_2$–$O_3$ caused embolism and patients' death. The ozone administration by intravenous route has been banned in Europe since 1984.[13] The use of ozone should never be used by inhalation or injection in accordance with the Madrid Declaration on Ozone Therapy.[15] However, there are publication proving the security of ozone by inhalation route in low concentrations.[16]

In countries such as Cuba, Russia and Ukraine this therapy is widely used for several treatments, in form of infusion of ozonated saline solution and rectal ozone insufflations due to the low cost and the applicability in thousands of patients.[17] In Brazil, the Brazilian Association of Ozone therapy was founded in 2006. However, the Federal Medical Council does not yet recognize the ozone therapy. The gas can be used in scientific research, according to Resolution 196/96 of the National Health Council, which includes approval of the research project by Research Ethics Committee.[18] In 2011, Federal Medical Council and the Brazilian National Sanitary Surveillance Agency argued that the ozone therapy has no scientific support to be regulated. In 2017, it was proposed the law project number 227 at senate, which provides the authorization for prescription of ozone therapy throughout the national territory. In August 2017, the Senate initiated a public consultation about of this law.[19] In 2018, ozone therapy had a historic gain, and it was incorporated into the Integrative and Complementary Practices of the Brazilian Unified Health System. The Integrative and Complementary Practices address comprehensive care for the population through practices involving diverse therapies.[20] The use of this therapy, in Brazil and in other emerging countries, could be reduce the costs of public health, so efforts should be made to regulate.

Considering the therapeutic standards, there are differences in the concentrations used of ozone. Acceptable patterns come from recommendations and adaptations according to the symptoms and illnesses of the patients. The safe therapeutic concentrations have been formerly defined as 10–40 µg ozone/mL blood.[21] Until 2002, it was believed that low doses of ozone were stimulatory and high doses were inhibitory; however, this information is incorrect, because the ozone in complex form or in high doses is also effective and the possibility of adverse effects should be noted.[17]

Ozone therapy increases tissue oxygenation, stimulates the production of endogenous antioxidants, and causes an immunosuppressive effect (with the stimulation of oxytocin release) in the circulatory system.[22] Ozone therapy acts by direct oxidation causing, for example, inactivation of microorganism or pain mediator and by activation of a nuclear effectors (Nrf2 or nuclear factor kappa B (NF-κB)) inducing a pharmacological response.[15] In low doses ozone stimulates cell protective pathways without altering cell viability[23] and ozone in high doses can be genotoxic.[8]

In tissue repair, the ozone therapy promotes the healing of the wounds[24] and, by the topical route, can be used in the gaseous and oil forms.

## Ozone action mechanism

Ozone acts as a bio-regulator releasing endothelial cell factors and normalizing cellular redox balance when in contact with

a biological fluid.[25] It may also alter the levels of cytokines (interleukin-8, tumor necrosis factor-α, transforming grown factor beta, platelet-derived growth factor).[26]

Ozone by intravenous route dissolve in biological fluids (plasma, urine and lymph) and reacts with polyunsaturated fatty acids, antioxidants, reduced glutathione and albumin.[27] These compounds act as electron donors and undergo oxidation, resulting in formation of hydrogen peroxide ($H_2O_2$) and lipid oxidation products.

$H_2O_2$ operates as an ozone messenger to initiate therapeutic and biological effects,[27] acting as a signal transduction regulator, activating immune defense.[28] The process helps the cells to survive the injury. However, in excessive amount, it can be harmful to cells.

Ozone stimulates the production of interferon, interleukins and antioxidant enzymes.[29] The immune system can also be stimulated by the activation of neutrophils and release of cytokines. It has the ability to reestablish cellular redox homeostasis.[30]

Ozone is a bio-regulator that protects against damage caused by chronic oxidative stress, pre/ post-conditioning mechanism,[31] it regulates the levels of nitric oxide, the p65 subunit of NF-κB and the tumor necrosis factor-α.[32]

The controlled administration of ozone promotes an adaptation to oxidative stress, stimulating endogenous antioxidant, protecting against tissue damage.[33]

For cutaneous wounds ozone is used in high frequency generators, producing heat that results in local peripheral vasodilation, increased blood flow, oxygenation and cellular metabolism, accelerating the healing process.[34]

The effect on the skin is due to its reaction with the polyunsaturated fatty acids and traces of water present in the upper layer of the dermis, generating reactive oxygen species (ROS) and lipo-oligopeptides, among which is $H_2O_2$. Only ROS and lipo-oligopeptides formed from this reaction can be partially reduced by the enzymatic antioxidants of the skin (glutathione oxidase, superoxide dismutase, catalase) and non-enzymatic low molecular weight molecules (isoforms of vitamin E, vitamin C, glutathione, uric acid and ubiquinol) or to be partially absorbed via intravenous and lymphatic capillaries. ROS are the most effective natural agents against antibiotic-resistant pathogens. In addition, it improves metabolism and immune functions, contributing to a satisfactory recovery.[35]

In patients with immunosuppression, ozone therapy is able in the activation and synthesis of ILs, leukotrienes and prostaglandins that will reduce inflammation and improve healing. It exerts effect in the activation of aerobic processes (glycolysis, Krebs cycle, beta-oxidation of fatty acids), secretion of vasodilators (like nitric oxide), activation of the protein synthesis mechanism, and increases in the number of ribosomes and mitochondria in the cells.[36] The increase of functional activity potentiates the tissue regeneration.[37] Ozone enters into the cell membrane of formed ROS, which activate the NF-κB pathways increasing their translocation to the nuclei, causing the activation of intracellular inflammation pathways, such as interleukin-1β, interleukin-6, tumor necrosis factor-α and cyclooxygenase-2, which awaken the apoptotic cascade.[38]

## Ozonated oil: production and stability

The purpose of ozonated oil is to obtain formulations containing ozone with better stability to facilitate handling, to improve its storage, to avoid its rapid degradation, to allow extra-hospital treatment and to reduce the risk of using it in gaseous form, in high and inadequate doses. The scientist Nicola Tesla bubble the ozone continuously for three weeks through the oil, creating a natural gel with ozone in suspension. This product was called "ozo-oil."[3]

Ozone reacts with the double bonds of fatty acids present in vegetable oils, forming specially ozonides (1,2,4-trioxolanes) and peroxides such as hydroperoxides, $H_2O_2$, polymer peroxides and other organic peroxides.[39,40]

In order to characterize the ozonated oils it is essential to understand the physicochemical properties. Analytical techniques have been used to determine the quality of vegetable oils and ozonated products.[40]

The characterization of the ozonated and pure oils by nuclear magnetic resonance, by peroxide and acid index, by determination of viscosity and molar mass showed a gradual decrease of the unsaturation with increase of ozonation time and formation of ozonides. Ozonation increased the peroxide and acid values. After long ozonation periods, upper and lower molar mass species were observed, as oligomeric ozonides and cross-ozonides.[3]

The first vegetable ozonated oil, known as OLEOZON®, was registered as a medicinal product for oral and topical therapeutic purposes by the National Center for Scientific Research in Cuba. In a toxicological study, OLEOZON® not showed deaths or toxicity signals. The animals showed a normal behavior, without macroscopic alterations in any of the parenchymal organs in the both groups (treatment and control). OLEOZON® is not classified as an acute toxicity substance and its safety is greater than 2000 mg/kg body weight.[41]

The production of the ozonated oils can be directly related to the method for generating foams by means of gas diffusion. These methods explain the liquid phase oxidation process when an oxygen-based gas is injected into a vessel of a bubble column reactor containing an oxidizable organic liquid. This is a high energy system that produces small diameter uniform bubbles, strongly driven in jet form resulting in foaming. This technique is known as Compressed Air Foam and has been used for several decades in oil, food, cosmetics and pharmaceutical industry, among others.[42]

To solidify ozonated vegetable oil is necessary two days of ozone gas continuously bubbled in vegetable oil, enabling that one gram of the oil contains about 160 mg of ozone. However, it can also be manufactured in less time becoming more viscous and less durable. When refrigerated it is valid for 2 years.[10] Nonetheless these informations are imprecise because it does not explain what the ozone concentration produced by the generator, as well as, there is no ozone meter in oil available in the market.

The stability of the resulting product is strongly influenced by the size of the bubbles, by the fraction of the liquid gas and by the circularity of the bubbles, which is the determinant factor of their persistence. As the bubbles became smaller and circular, their distribution is more uniform and higher, having

good quality and stability.[43]

## Ozonated oil in inflammations

Ozone is recognized as one of the best bactericides, antivirals and antifungals, and has been used empirically as a clinical therapeutic agent for post-surgical fistulas and wounds, pressure ulcers, as well as chronic wounds such as trophic ulcers, ischemic ulcers, diabetic wounds, psoriasis, and athlete's foot. The beneficial effects of ozone on wound healing are related to the reduction of microbial infection, debridement effect, modulation of the inflammatory phase, stimulation of angiogenesis as well as biological and enzymatic reactions that favor oxygen metabolism improving wound healing.[13]

Tissue repair and restoration is a complex and dynamic process. The improvement of this process characterized by the closure of the wound is crucial for several pathologies, as the case of diabetics.[33]

Ozonated oil presents an early response and has a larger number of cells involved in the repair process when used on wounds, as well as superior angiogenesis with increased vascular endothelial growth factors and cyclin D1 expression.[12]

There are millions of patients with cutaneous lesions that degenerate into infected ulcers and with little expectation of cure, especially in diseases such as diabetes mellitus, atherosclerosis and in the aging process, which implies a high socioeconomic cost. Daily use of ozonated oil in these lesions eliminates infection and promotes rapid healing.[34]

The first evidence that evaluates the benefits of ozone in skin disease was provided by Shpektorova[44] in 1964.

Diabetic complications can be also attributed to oxidative stress. In 2005, a clinical trial was published to investigate the ozone therapeutic effectiveness in the treatment of patients with type 2 diabetes who had diabetic feet and to compare ozone with antibiotic therapy.[45] 101 patients were selected and divided into two groups (52 patients treated with ozone (local and rectal gas insufflation) and 49 patients treated with topical and systemic antibiotics for 20 days). Ozone improved glycemic control, prevented oxidative stress, normalized organic peroxide levels and activated superoxide dismutase. It was also seen an improvement in the healing of the lesions, resulting in less amputations than in the control group. Therefore, medical treatment with ozone activates the antioxidant system and may be an alternative therapy in the treatment of diabetes and its complications. This work was performed with high methodological rigor.

In 2003, 23 patients were treated with ozone in septic chronic complications and broad-spectrum antibiotic-resistant following trauma, surgical procedures, and secondary skin infections.[46] Ozone therapy was administered superficially, by an authorial technique. Septic processes were inhibited and wound healing was much faster than normal in all patients receiving ozone therapy, confirming the advantages of surface ozone therapy in the treatment of septic wounds.

The effect of extra virgin olive ozonated oil was *in vitro* evaluated to validate antifungal activity and *in vivo* evaluated on oral levels of *Candida* spp. in patients with prosthetic stomatitis. The antifungal activity was performed against *Candida albicans* and five non-albicans species (*Candida tropicalis*, *Candida dubliniensis*, *Candida krusei*, *Candida guilliermondii* and *Candida parapsilosis*). The effects on planktonic *C. albicans* and biofilm were also evaluated. *In vivo*, ozonated oil was used by 30 patients, and another 20 used sodium bicarbonate for 14 days. *In vitro* ozonated oil showed antifungal activity against all *Candida* species, as well as anti-biofilm activity of *C. albicans*. Oral candidiasis levels were lower than baseline after 14 days of intervention with both treatments. Remission of prosthetic stomatitis was observed in all patients after 7 days of treatment in both groups. Therefore, the study concluded that ozonated oil may be an alternative for biofilm control in patients with prosthetic stomatitis.[47]

The effect of different ozonated oils (olive, sesame and linseed) on wound healing was compared in mice.[48] Eight microliters of ozonated or non-ozonated oil were applied in 36 mice divided into 4 groups of 9 mice each, twice daily in wounds for 14 days. Wounds were performed on the back of the mice preventing them from licking. The skin was compressed and folded to obtain a circular wound of equal diameter. The wounds were photographed at 0, 3, 7 and 14 days. Olive oil, sesame oil and linseed without ozonization (control group) and the same ozonated oils (treatment group) were applied to each wound. The wound closure was greater than 90% in all cases, however the treatment group showed a significant increase in the closure rate in relation to the control group on the first day and was significantly higher on days 3 to 7. Sesame oil showed a significant increase of the wound closure speed relative to the non-ozonated sesame oil in the first 7 days, demonstrating that the oil composition is important in the wound healing process.

The therapeutic effects of topical ozonated olive oil were evaluated in the healing of surgical sites of gingival graft.49 A randomized, triple-blind clinical trial with 20 patients was divided into 2 groups (treatment: ozonated oil or control: olive oil without ozonization). They were evaluated postoperatively by cytological analysis at the beginning of the study, after 24 hours, 3, 7, 14 and 21 days, and 2, 3, 8 and 18 months. There was a significant improvement in epithelial healing at 7, 14 and 21 days, as well as at 2, 3 and 8 months in the ozonated oil group. The efficacy of topical application of ozonated vegetable oil at gingival surgical sites has been proven. Ozonated oil promoted a significant improvement in the epithelial healing of gingival wounds compared to the control group. The methodology of this study is well defined, but its limitation is not to have a sample calculation.

Ozonated oil and chlorhexidine gel were evaluated in the treatment of gingivitis. At the beginning of the treatment (up to the third week) the ozone presented advantages; however, at the end of the study there was no significantly statistical difference between the ozonated oil and the chlorhexidine gel. Gingival massage with ozonated oils shows an effective alternative against plaque-induced gingivitis.[50]

Ozonated oil and α-bisabolol were compared to control creams (vitamin A, vitamin E, talc and zinc oxide) in the vascular treatment of leg ulcer. Complete ulcer healing was greater with ozonated oil and α-bisabolol. In addition, changes in the surface area of the ulcer were significant only for ozonated oil and α-bisabolol, with a significant and progressive

reduction of the wound surface by 34%, 59% and 73% after 7, 14 and 30 days, respectively.[51] This study was performed without double-blindness and sample calculation, and these facts may represent a limitation.

The effects of ozonated camellia oil were evaluated in atopic dermatitis.[52] Eighteen mice were injured in the back and were treated with ozonated oil. Ozonated oil inhibited inflammation significantly and healed lesions within 7 days. Therefore, ozonated oil can suppress inflammation in atopic dermatitis demonstrating may be a potential treatment for the disease.

In acute skin wounds of guinea pigs, ozonated oil significantly improved the healing process.[53]

Among the papers studies, several of them showed that ozonated oil is effective in the healing of cutaneous wounds. However, some studies evaluating the ozonated oil in humans[45-47,49-51] indicate limitations in the methodologies used, without clear patients randomization, double-blinding or sample calculation.

## Conclusion

Ozone via topical in gaseous form or into oils stands out as a treatment for tissue repair, since it promotes wound healing and has antimicrobial, immunological, antioxidant and oxygenating properties. The efficacy of ozonated oil may represent an integrative therapy in the treatment of tissue lesions, especially in patients presenting pathologies such as diabetes mellitus, atherosclerosis and in the aging process. For diseases such as ulcers or aphthous stomatitis, gingivitis, and dermatitis, ozonated oil aids on the pain relief and healing process acceleration. However, some studies showed limitations in the methodology, without randomization, or double-blind or sample calculation, demonstrating the need for further clinical studies to confirm the topical efficacy of ozone.

**Author contributions**
All authors participated in the research of articles, construction and revision of the text.
**Conflicts of interest**
The authors have no conflicts of interest to declare.
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