

FROM OXIDATIVE SIGNALING TO CLINICAL PRACTICE: THE ROLE OF OZONE THERAPY IN SKIN HEALTH

DA SINALIZAÇÃO OXIDATIVA À PRÁTICA CLÍNICA: O PAPEL DA OZONIOTERAPIA NA SAÚDE DA PELE

DE LA SEÑALIZACIÓN OXIDATIVA A LA PRÁCTICA CLÍNICA: EL PAPEL DE LA OZONOTERAPIA EN LA SALUD DE LA PIEL



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ABSTRACT

Ozone (O₃) is a highly reactive triatomic gas whose bent molecular structure and strong oxidizing capacity underpin its broad biological activity. In physiological systems, O₃ is produced by activated leukocytes and functions as part of antimicrobial defense. Clinically, its instability requires in situ generation, typically via corona discharge applied to medical-grade oxygen. Upon dissolution in aqueous media, O₃ reacts rapidly with polyunsaturated fatty acids, producing hydrogen peroxide (H₂O₂) and lipid oxidation products (LOPs), which act as early and late biological messengers. These compounds activate controlled oxidative pathways that modulate cellular redox status. Therapeutically, O₃ induces mild, transient oxidative stress that activates the Nrf2–ARE pathway, enhances endogenous antioxidant defenses, and downregulates NF-κB–mediated inflammation. Its potent antimicrobial activity arises from oxidation of microbial membrane components, demonstrating efficacy against bacteria, fungi, viruses, and antibiotic-resistant strains. O₃ also promotes wound repair by improving oxygenation, stimulating growth factors (VEGF, TGF-β, FGF2, PDGF), and enhancing fibroblast activation and epithelial–mesenchymal transition. Ozonated oils further support tissue regeneration through sustained release of reactive intermediates. In aesthetic and metabolic applications, O₃ exhibits lipolytic effects through cleavage of fatty acid double bonds, triggering lipid peroxidation and adipocyte breakdown. Clinical studies report reductions in body mass index, waist circumference, submental fat, and lipoma size. Additional benefits include improvements in acne, pigmentary disorders, and photoaging, with increased collagen type I deposition. Despite promising therapeutic potential, standardized protocols remain lacking, and optimal concentrations, dosing frequency, and long-term safety require clarification. Methodological limitations, heterogeneous study designs, and insufficient follow-up hinder definitive conclusions. Precise dosing is critical, as

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excessive concentrations are toxic while insufficient doses may be ineffective. Overall, O₃ therapy functions as a biomodulatory intervention with antimicrobial, anti-inflammatory, regenerative, and metabolic effects, representing a minimally invasive complementary approach across diverse clinical contexts.

Keywords: Adiposity Reduction. Antimicrobial Activity. Lipid Peroxidation. Oxidative Stress. Wound Healing.

RESUMO

O ozônio (O₃) é um gás triatômico altamente reativo cuja estrutura molecular angular e forte capacidade oxidante fundamentam sua ampla atividade biológica. Em sistemas fisiológicos, o O₃ é produzido por leucócitos ativados e atua como parte da defesa antimicrobiana. Clinicamente, sua instabilidade exige geração *in situ*, geralmente por descarga corona aplicada a oxigênio de grau médico. Ao se dissolver em meios aquosos, o O₃ reage rapidamente com ácidos graxos poli-insaturados, produzindo peróxido de hidrogênio (H₂O₂) e produtos de oxidação lipídica (LOPs), que atuam como mensageiros biológicos precoces e tardios, ativando vias oxidativas controladas que modulam o estado redox celular. Do ponto de vista terapêutico, o O₃ induz um estresse oxidativo leve e transitório que ativa a via Nrf2–ARE, potencializa as defesas antioxidantes endógenas e reduz a inflamação mediada por NF-κB. Sua potente atividade antimicrobiana decorre da oxidação de componentes da membrana microbiana, demonstrando eficácia contra bactérias, fungos, vírus e cepas resistentes a antibióticos. O O₃ também promove a reparação tecidual ao melhorar a oxigenação, estimular fatores de crescimento como VEGF, TGF-β, FGF2 e PDGF, além de intensificar a ativação de fibroblastos e a transição epitélio-mesenquimal. Óleos ozonizados contribuem para a regeneração tecidual por meio da liberação sustentada de intermediários reativos. Em aplicações estéticas e metabólicas, o O₃ apresenta efeitos lipolíticos por meio da clivagem das duplas ligações dos ácidos graxos, desencadeando peroxidação lipídica e degradação dos adipócitos, com estudos clínicos relatando reduções no índice de massa corporal, circunferência da cintura, gordura submentoniana e tamanho de lipomas, além de melhorias na acne, em distúrbios pigmentares e no fotoenvelhecimento, associadas ao aumento da deposição de colágeno tipo I. Apesar do potencial terapêutico promissor, ainda faltam protocolos padronizados, e as concentrações ideais, a frequência de aplicação e a segurança em longo prazo necessitam de esclarecimento, uma vez que limitações metodológicas, desenhos de estudo heterogêneos e seguimento insuficiente dificultam conclusões definitivas, sendo a dosagem precisa essencial, pois concentrações excessivas são tóxicas, enquanto doses insuficientes podem ser ineficazes. De modo geral, a ozonioterapia atua como uma intervenção biomoduladora com efeitos antimicrobianos, anti-inflamatórios, regenerativos e metabólicos, configurando-se como uma abordagem complementar minimamente invasiva em diferentes contextos clínicos.

Palavras-chave: Redução de Adiposidade. Atividade Antimicrobiana. Peroxidação Lipídica. Estresse Oxidativo. Cicatrização de Feridas.

RESUMEN

El ozono (O₃) es un gas triatómico altamente reactivo cuya estructura molecular angular y fuerte capacidad oxidante sustentan su amplia actividad biológica. En los sistemas fisiológicos, el O₃ es producido por leucocitos activados y actúa como parte de la defensa antimicrobiana. Clínicamente, su inestabilidad requiere generación *in situ*, generalmente mediante descarga corona aplicada a oxígeno de grado médico. Al disolverse en medios acuosos, el O₃ reacciona rápidamente con ácidos grasos poliinsaturados, produciendo peróxido de hidrógeno (H₂O₂) y productos de oxidación lipídica (LOPs), que actúan como mensajeros biológicos tempranos y tardíos, activando vías oxidativas controladas que

modulan el estado redox celular. Desde el punto de vista terapéutico, el O₃ induce un estrés oxidativo leve y transitorio que activa la vía Nrf2–ARE, potencia las defensas antioxidantes endógenas y atenúa la inflamación mediada por NF-κB. Su potente actividad antimicrobiana deriva de la oxidación de componentes de la membrana microbiana, mostrando eficacia frente a bacterias, hongos, virus y cepas resistentes a antibióticos. El O₃ también favorece la reparación tisular al mejorar la oxigenación, estimular factores de crecimiento como VEGF, TGF-β, FGF2 y PDGF, y potenciar la activación de fibroblastos y la transición epitelio-mesenquimal. Los aceites ozonizados contribuyen a la regeneración tisular mediante la liberación sostenida de intermediarios reactivos. En aplicaciones estéticas y metabólicas, el O₃ presenta efectos lipolíticos a través de la escisión de los dobles enlaces de los ácidos grasos, desencadenando peroxidación lipídica y degradación de adipocitos, con estudios clínicos que informan reducciones del índice de masa corporal, la circunferencia de la cintura, la grasa submentoniana y el tamaño de lipomas, además de mejoras en el acné, los trastornos pigmentarios y el fotoenvejecimiento, asociadas a un aumento de la deposición de colágeno tipo I. A pesar de su prometedor potencial terapéutico, aún faltan protocolos estandarizados y es necesario esclarecer las concentraciones óptimas, la frecuencia de dosificación y la seguridad a largo plazo, ya que las limitaciones metodológicas, los diseños de estudio heterogéneos y el seguimiento insuficiente dificultan conclusiones definitivas, siendo la dosificación precisa un aspecto crítico, dado que concentraciones excesivas resultan tóxicas, mientras que dosis insuficientes pueden ser ineficaces. En conjunto, la ozonoterapia actúa como una intervención biomoduladora con efectos antimicrobianos, antiinflamatorios, regenerativos y metabólicos, configurándose como un enfoque complementario mínimamente invasivo en diversos contextos clínicos.

Palabras clave: Reducción de Adiposidad. Actividad Antimicrobiana. Peroxidación Lipídica. Estrés Oxidativo. Cicatrización de Heridas.

1 INTRODUCTION

Ozone (O₃) is a triatomic gas formed spontaneously in the atmosphere by the interaction between oxygen (O₂) and ultraviolet (UV) radiation in the 220–290 nm range. The three oxygen atoms of the O₃ molecule are arranged in a structure described by valence shell electron pair repulsion (VSEPR) theory: the electrons of the central oxygen atom repel the electron clouds of the two terminal atoms, resulting in a bent (V-shaped) molecular geometry with an obtuse angle of approximately 116°49' and a bond length of 1.278 Å.

The central atom of O₃ has one double bond and one single bond with its neighboring oxygen atoms, which continuously interchange positions. This electronic configuration confers high reactivity to the gas, whose oxidizing power is second only to that of fluorine.

The term “ozone”, from the Greek *ozein* (“to smell”), refers to the characteristic pungent odor of the gas. O₃ is colorless at low concentrations and appears blue at high concentrations in air. At 0 °C, it is slightly denser than air (2.14 vs. 1.28 g/L), and its solubility in water depends on temperature and atmospheric pressure.

Physiologically, O₃ acts as a biomolecule produced by activated leukocytes as part of immune defense mechanisms. Catalysis performed by human neutrophils coated with antibodies generates O₃ via a water oxidation pathway, leading to efficient bacterial killing. Consequently, O₃ has been used as a powerful oxidant for medical purposes for more than 150 years, initially as an antiseptic for operating rooms and for disinfecting surgical instruments in 1856.

Because O₃ is unstable, with a half-life of approximately 40 minutes at 20 °C, it spontaneously degrades within minutes of production. Its stability is directly influenced by its concentration. Therefore, O₃ must be generated *in situ* immediately before application, using specific medical devices. Typically, the gas is produced by the corona discharge method applied to high-purity medical O₂, yielding final mixtures with O₃ concentrations of up to 16%.

1.1 REACTION PATHWAYS WITH WATER AND VEGETABLE OILS

In clinical practice, O₃ can be administered as a gas mixture, dissolved in aqueous media, or incorporated into ozonated oils. The instability of O₃ results in a short half-life, of approximately 40 minutes in the gas phase and about 20 minutes in water.

When introduced into aqueous media such as blood plasma or saline solution, O₃ rapidly dissolves and reacts with biological components. In plasma and serum, it preferentially reacts with polyunsaturated fatty acids (PUFAs), generating two key biological messengers: hydrogen peroxide (H₂O₂) and lipid oxidation products (LOPs). H₂O₂ is a reactive oxygen species (ROS) that acts as an early, rapid-onset messenger, whereas LOPs, such as 4-

hydroxynonenal (4-HNE), are late and long-lasting messengers that diffuse into cells and signal a state of minimal oxidative stress.

In the liquid phase, O_3 decomposition also yields hydroxyl radicals as major reactive species. O_3 reacts with compounds in aqueous solution either directly or indirectly through free OH radicals. These radicals are highly reactive and can attack organic compounds approximately 100 times faster than molecular O_3 . Disinfection generally occurs via molecular ozone, whereas oxidation processes involve both direct and indirect mechanisms.

Ozonated oils are produced by bubbling ozone into cold-pressed vegetable oils rich in unsaturated fatty acids, such as olive or sunflower oil. This formulation is advantageous for topical use, as it has a long shelf life (up to 2 years if refrigerated). The ozonation process leads to formation of the 1,2,4-trioxolane moiety (ring structure) within the oil, which represents the active form of ozone in the material. When applied to moist wounds or ulcers, the trioxolane ring rapidly decomposes into compounds that accelerate healing and provide antimicrobial and antimycotic effects. In the stratum corneum, the reaction of O_3 with PUFAs generates O_3 -derived reactive species and lipo-oligopeptides, which can be partially absorbed through the skin.

2 MAIN THERAPEUTIC APPLICATIONS OF OZONE THERAPY

O_3 therapy is indicated as an adjuvant treatment for conditions characterized by chronic hypoxia, inflammation, and redox imbalance. Its primary therapeutic efficacy derives from the induction of controlled “micro-oxidation” (hormesis), allowing O_3 to function as a prodrug that generates a cascade of beneficial biochemical signals, including redox modulation, anti-inflammatory effects, and antimicrobial activity.

2.1 REDOX MODULATION AND ANTI-INFLAMMATORY EFFECTS

The controlled oxidative stress induced by O_3 , via H_2O_2 and LOPs, is central to its biological effects. Low or moderate doses of O_3 /LOPs activate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, a key regulator of cytoprotective responses. Upon activation, Nrf2 binds to antioxidant response elements (AREs), inducing synthesis of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and heme oxygenase-1 (HO-1), thereby protecting against chronic oxidative stress.

Activation of the Nrf2 pathway is also associated with reduced activity of the pro-inflammatory nuclear factor kappa B (NF- κ B) pathway, resulting in decreased expression of pro-inflammatory cytokines. In parallel, O_3 oxidizes compounds containing carbon–carbon

double bonds, such as prostaglandins and leukotrienes, contributing to the attenuation of inflammation.

2.2 ANTIMICROBIAL ACTIVITY

O₃ is a potent germicidal agent, effective against bacteria, fungi, viruses, and parasites. It acts rapidly by damaging bacterial cell walls through oxidation of phospholipids and lipoproteins, compromising cellular function and often leading to lysis. The germicidal effect of O₃ is relatively selective, because microorganisms generally have poor antioxidant defenses (e.g., lack of CAT and GSH-Px), rendering them more susceptible to transient oxidative stress than host cells.

2.3 HEALING AND CIRCULATION

O₃ therapy enhances tissue wound repair by promoting epithelialization, stimulating cell proliferation, increasing matrix deposition, and inducing production and release of growth factors crucial for healing, such as vascular endothelial growth factor (VEGF), transforming growth factor β (TGF- β), fibroblast growth factor 2 (FGF2), and platelet-derived growth factor (PDGF). Clinically, O₃ is used in chronic wounds, diabetic foot ulcers, infected wounds, gangrene, and burns.

O₃ also increases local microcirculation by enhancing nitric oxide (NO) production and improves oxygen delivery to hypoxic tissues by increasing 2,3-diphosphoglycerate (DPG) levels, thereby reducing hemoglobin affinity for O₂.

2.4 PAIN AND MUSCULOSKELETAL DISORDERS

Administered by local injection, O₃ has been extensively studied in painful conditions. Analgesia is achieved by oxidizing algopeptides (products of albuminolysis) that stimulate nerve endings, thus reducing pain intensity.

Intradiscal or paravertebral injections of the O₂-O₃ gas mixture are widely used for lumbar and cervical disc herniation. The gas reacts with proteoglycans and collagen in the nucleus pulposus matrix, promoting a “mummification” process that shrinks the disc and reduces nerve root compression.

Injections of O₃ have also demonstrated efficacy in knee osteoarthritis, supported by multiple systematic reviews. Systemic O₃ therapy (e.g., rectal insufflation and autohemotherapy) is used as an adjuvant in disorders such as fibromyalgia and rheumatoid arthritis, in which chronic oxidative stress and redox imbalance are prominent.

2.5 NEURODEGENERATIVE AND DERMATOLOGICAL CONDITIONS

The use of O₃ and its derivatives is being investigated in neurodegenerative and systemic diseases, including multiple sclerosis and systemic sclerosis. In these conditions, its potential benefits are linked to modulation of the Nrf2 pathway, attenuation of chronic inflammation, and reduction of cellular oxidative stress.

2.6 AESTHETIC CONDITIONS

Localized fat and cellulite can be treated with O₃ therapy. The gas induces lipid peroxidation by cleaving carbon–carbon double bonds in fatty acids, damaging adipocyte membranes and potentially promoting cell lysis and lipolysis. Additionally, its antibacterial action is relevant in acne, and its stimulatory effect on fibroblasts contributes to collagen biostimulation and anti-aging effects, partly through Nrf2 activation. O₃ therapy has also been proposed to reduce oxidative stress associated with pigment disorders such as vitiligo and hyperchromia.

3 OZONE THERAPY AND WEIGHT REDUCTION

Adiposity is intrinsically linked to overweight and obesity, which are major risk factors for diabetes, cancer, and cardiovascular diseases. Localized fat deposits in regions such as the abdomen, hips, and submental area negatively affect self-esteem and mental health. In this context, and considering the demand for minimally invasive therapeutic and aesthetic strategies, O₃ therapy has emerged as a complementary method for treating localized adiposity and related conditions, including lipomas.

3.1 LIPOLYTIC EFFICACY OF OZONE

O₃ is considered a lipolytic agent capable of significantly reducing adiposity, often with relatively few applications and limited reported side effects. Its efficacy in localized adiposity is mainly attributed to well-defined biochemical mechanisms that promote lipolysis.

After application, O₃ dissolves in interstitial water and preferentially targets lipids. It reacts with carbon–carbon double bonds of unsaturated fatty acids, promoting their scission and forming lipoperoxides, hydroperoxides, and low molecular weight, water-soluble lipid oxidation products. This controlled oxidative stress initiates lipid peroxidation and contributes to adipocyte breakdown.

O₃ also induces metabolic and circulatory changes that favor adiposity reduction. It increases metabolic activity, including glycolysis, enhancing intracellular adenosine

triphosphate (ATP) availability, and participates in protein metabolism by reacting with sulfhydryl (–SH) groups in essential amino acids.

The O₂–O₃ mixture improves microcirculation and tissue oxygenation. By reacting with unsaturated fatty acids in erythrocyte membranes, it increases red blood cell deformability and reduces blood viscosity, facilitating microvascular flow and increasing DPG production, thereby improving O₂ supply and local metabolism.\

Because localized adiposity is associated with inflammation and oxidative stress, the anti-inflammatory and anti-edematous properties of O₃ assist in removing stagnant interstitial fluids and counteracting oxidative damage. Activation of Nrf2 by O₃ contributes to the restoration of redox homeostasis.

3.2 CLINICAL EVIDENCE IN WEIGHT MANAGEMENT AND ADIPOSITY

Clinical studies report promising results for O₃ therapy as an adjuvant in weight reduction and localized adiposity. Effective weight management protocols usually combine a low-calorie diet, regular physical exercise, and, when indicated, aesthetic treatments.

In a randomized study of adults with Grade II obesity, patients receiving O₃ therapy (rectal insufflation, from 20 to 40 µg/mL) combined with a low-calorie diet showed greater reductions in body mass index (from 39.53 to 30.67 kg/m²) and waist circumference (from 119.47 to 100.20 cm) compared with patients on diet alone.

For localized concerns such as submental fat (double chin), O₃ therapy has been associated with significant reductions in adipometry and pachymetry after only three weekly applications at 5 µg/mL. O₃ therapy has also been used in painful lipomas, yielding reductions in lesion size (e.g., approximately 4 cm over 10 sessions) and immediate pain relief.

3.3 PROTOCOL STANDARDIZATION AND FUTURE PERSPECTIVES

Despite favorable results in localized fat reduction and in combination with dietary restriction, the literature still lacks standardized protocols. Optimal O₃ concentrations, application frequency, and treatment duration to ensure long-term efficacy remain to be defined.

In clinical practice for localized adiposity, subcutaneous applications typically use ozone gas at concentrations between 1 and 40 µg/mL, according to technique and patient characteristics. Experimental data indicate that low concentrations (from 5 to 10 µg/mL) may induce adipogenesis *in vitro* in human adipose-derived stem cells, whereas higher concentrations (≥20 µg/mL) lead to cell destruction by oxidative stress. These findings reinforce the need for technical competence and precise dosing.

Overall, O₃ therapy may represent a minimally invasive therapeutic strategy for managing localized adiposity, contributing to weight reduction mainly through lipolytic and microcirculatory effects. However, its efficacy and safety require confirmation by robust randomized controlled trials with larger samples and long-term follow-up before its role in clinical practice can be definitively established.

4 OZONE THERAPY IN SKIN CARE

O₃ therapy has been applied to a broad range of conditions, including ocular, orthopedic, pulmonary, renal, and hematological diseases. In skin care, its main reported effects are improved cell metabolism, enhanced peripheral tissue oxygenation, and germicidal, analgesic, and reparative actions. Studies suggest its use as an adjuvant treatment in infectious skin diseases, eczema, psoriasis, atopic dermatitis, systemic sclerosis, herpes, skin aging, ulcers, and scarring.

In dermatology, the O₂–O₃ gas mixture is typically administered via topical applications using bags or through superficial application of ozonated water or oils. Rectal insufflation, ozonated autohemotherapy, and subcutaneous applications are secondary options aimed at systemic effects.

4.1 TREATMENT OF CUTANEOUS INFECTIONS

O₃ is a strong oxidant and effective disinfectant, which underlies its broad-spectrum antimicrobial activity against bacteria, viruses, and fungi. This property led to its early use in treating infected wounds, such as gaseous gangrene.

The antimicrobial mechanism of O₃ rests on its oxidative action on membrane phospholipids and lipoproteins in microbial cell walls. This disruption compromises membrane integrity, allowing O₃ to diffuse into the cell and oxidize amino acids and nucleic acids, ultimately inhibiting replication, promoting denaturation, and causing cell death. In viral infections, such as herpes, O₃ damages the viral capsid via lipid peroxidation, thereby inhibiting viral proliferation.

Experimental and clinical data confirm the potency of O₃ and its derivatives against common skin pathogens, including Gram-positive strains like *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA). In *in vitro* tests, ozonated water (1 mg/L) eliminated 100% of both *S. aureus* and MRSA within one minute. Ozonated oil also showed a high killing rate, eliminating nearly 100% of *S. aureus* in five minutes and MRSA in fifteen minutes. Clinically, topical O₃ therapy has been associated with complete healing of MRSA skin

infections. Ozonated oils have additionally shown efficacy in fungal conditions such as tinea pedis and onychomycosis.

O₃ can also act as an adjunct therapy against drug-resistant Gram-negative bacteria. By inducing oxidative damage and forming pores in the outer membrane, gaseous O₃ greatly increases susceptibility of Gram-negative bacteria, such as *Escherichia coli* and *Pseudomonas aeruginosa*, to antibiotics like linezolid and vancomycin, which are usually ineffective due to intrinsic resistance mechanisms.

4.2 BENEFITS FOR REGENERATION AND WOUND HEALING

O₃ therapy exerts biomodulatory effects on tissue repair and regeneration, especially in wound healing. Topical and systemic O₃ therapies have been associated with attenuation of acute inflammatory features.

In animal models of induced skin wounds, O₃-treated groups showed mixed inflammation at seven days, evolving to absence of inflammation by fifteen days, whereas control groups progressed from intense acute inflammation to chronic inflammation. O₃ also accelerates wound contraction in the initial healing phase, shortening overall healing time. By the second week, both control and O₃ groups reach complete re-epithelialization, but O₃-treated wounds exhibit better muscle regeneration and more intense hair follicle formation, suggesting higher tissue quality.

The reparative effects of ozone are attributed to four main biological actions: improved oxygenation and circulation, stimulation of growth factors, fibroblast activation and migration, and induction of epithelial–mesenchymal transition (EMT).

O₃ increases O₂ delivery to ischemic tissues by enhancing peripheral tissue oxygenation, raising red blood cell and hemoglobin levels, and increasing erythrocyte metabolism, thereby reducing wound hypoxia, particularly in chronic wounds such as diabetic foot ulcers.

O₃ treatment promotes release of growth factors essential for tissue repair and extracellular matrix deposition, including PDGF, TGF-β, and VEGF. Increased VEGF expression supports neovascularization, consistent with higher vessel scores observed in O₃-treated wounds during early healing.

Ozonated oil further facilitates wound healing by stimulating fibroblast migration and activation, increasing expression of genes such as collagen I, alpha-smooth muscle actin (α-SMA), and TGF-β. It also promotes EMT in fibroblasts, characterized by decreased E-cadherin and increased mesenchymal markers (vimentin, fibronectin, N-cadherin), a process mediated by the PI3K/Akt/mTOR signaling pathway and crucial for tissue remodeling.

4.3 TREATMENT OF PIGMENTARY AND AGING-RELATED SKIN CHANGES

In aesthetic dermatology, O₃ therapy has been explored for facial rejuvenation, with reported improvements in dermal texture and appearance. In case reports, patients undergoing facial ozone therapy showed reduction or disappearance of age-related pigmentary spots and improved skin thickness, firmness, and homogeneity of color and surface. These anti-aging effects are associated with biostimulatory activity, including increased production of type I collagen.

4.4 UNEXPLORED KNOWLEDGE GAPS

Despite promising findings, the efficacy and safety of O₃ in skin care remain limited by methodological issues in the available literature. Many studies on acne, dermatitis, psoriasis, herpes, and systemic sclerosis are non-randomized or show only moderate quality scores. In addition, inconsistent reporting of dose, concentration, and route of application hinders protocol standardization.

Although major mechanisms—antioxidant, anti-inflammatory, and pro-regenerative—have been identified, the exact cellular and molecular pathways of O₃ therapy require further elucidation. Additional research is needed to clarify its potential biosynthetic, analgesic, and vasodilatory properties. The scarcity of long-term follow-up studies also makes it difficult to define the persistence of benefits and the risk of delayed adverse effects.

Furthermore, many aesthetic studies rely primarily on clinical or photographic assessments, without anatomopathological analyses to quantify dermal thickness or collagen content induced by biostimulation.

4.5 THEORETICAL AND PRACTICAL IMPLICATIONS

O₃ therapy functions as a biomodulator of tissue regeneration and inflammatory responses. Its therapeutic activity is based on controlled mild oxidative stress, which, unlike excessive oxidative damage, activates the Nrf2 pathway and represses the NF-κB pathway. This balance modulates the organism's oxidant/antioxidant systems, reducing pro-inflammatory cytokines and increasing antioxidant enzyme activity.

Fibroblast activation and EMT promotion are key molecular findings supporting the role of O₃ in accelerating wound closure and improving tissue quality, mainly via the PI3K/Akt/mTOR pathway. The ability of O₃ to neutralize infectious agents and modulate immune responses positions it as an intervention that simultaneously addresses infection control and tissue repair.

Bio-oxidative therapy with O₃ gas appears to provide additional benefits to tissue repair processes and has been proposed as an option for patients with impaired immunity, coagulation, tissue repair, or healing, particularly in ulcers and scarring.

However, clinical safety and efficacy depend critically on precise dose control. O₃ concentrations must be rigorously regulated by accurate generators. Very high concentrations are potentially toxic, especially when inhaled, and may cause malaise, fatigue, or severe pulmonary effects, whereas very low concentrations may be ineffective.

For topical use, ozonated oils offer advantages in stability and portability compared with ozonated water, which degrades rapidly. When used appropriately, O₃ therapy is considered a relatively economical and conservative option with minimal reported side effects and may serve as a complementary strategy to conventional pharmacological treatments.

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