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Review Article

Dynamic Role of Oxygen in Wound Healing: A Microbial, Immunological, and Biochemical Perspective

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Abstract

A wound is a temporary break in the continuity of the protective skin barrier. Wound healing is central in maintaining the body's normal homeostatic mechanism, and open wounds raise the risk of microbial infection and amputation. A successful wound healing event is achieved through a series of evolutionarily conserved biochemical pathways orchestrated by various cytokines, growth factors, and immune cells. Chronic wounds are generally oxygen-deficient, and wound hypoxia impairs the wound healing process. Therefore, the use of external oxygen may improve wound health by reducing wound hypoxia, promoting tissue regeneration and granulation tissue formation, reducing anaerobic bacteria colonization, and promoting the growth of beneficial aerobic bacteria. Relevant data were searched and gathered from scientific databases, including PubMed, ScienceDirect, and Google Scholar using relevant keywords, such as "Chronic Wounds", "Topical Oxygen Therapy", "Inflammatory Markers/ Lactate/ Matrix Metalloproteinase", "Collagen", and "Wound Healing". Relevant articles were shortlisted and used in the present study. Chronic wounds show higher expression of pro-inflammatory mediators, such as C-reactive protein, and higher levels of tissue-degrading matrix metalloproteinases. In addition, chronic wounds are generally oxygen-deficient, and wound hypoxia is directly associated with wound deterioration. Several microbial, immunological, and biochemical markers show a direct association with the oxygen availability in the wound. Therefore, a detailed understanding of these microbial, immunological, and biochemical markers will certainly help clinicians understand the interplay between various factors and topical oxygen therapy and may improve patient outcomes.

Keywords: Topical oxygen therapy, Chronic wounds, Wound hypoxia, Wound healing markers, Skin microflora

1. Context

Chronic wounds are difficult to heal, and the complete healing process takes three months or longer. The prevalence of chronic wounds is growing, similar to a silent epidemic, and places an enormous burden on the healthcare system. In addition, chronic wound management is associated with far-reaching financial and social implications since chronic wound patients are burdened with huge out-of-pocket expenses and have a suboptimal quality of life (1, 2). Importantly, the need for wound care and wound management will only increase with time due to the aging of the population and rising cases of obesity, type 2 diabetes mellitus, and cardiovascular disorders (3). Worldwide, approximately 40 million people develop chronic wounds, and 1-2% of the population in developed countries suffer from chronic wounds at any given point in time (4). Chronic wounds present inadequate oxygen, blood, and nutrient supply, have a higher risk of developing a microbial infection and suffer from

repeated trauma (5). Wound healing is a complex and multi-step process and is characterized by four overlapping stages, namely, hemostasis, inflammation, proliferation, and remodeling. Therefore, complex crosstalk among several molecular and cell signaling pathways is essential for the normal wound healing process (6, 7). In addition, such parameters as oxygen, chemokines, and nutritional requirements play a significant role in the overall wound healing process. Adequate intake of nutrients, such as protein, vitamins (vitamin A, C, folate, vitamin B12), and minerals (zinc, iron, and copper) is essential for normal wound healing (8). Similarly, adequate oxygen supply facilitates wound healing by promoting cell proliferation and angiogenesis, reducing the risk for microbial infections, and increasing collagen synthesis (9). Local delivery of oxygen to the wound has been used for wound care, and the idea is supported by *in vivo* and *in vitro* studies (10, 11). Moreover, *in vitro* models have shown an increase in Vascular Endothelial Growth Factor (VEGF) transcripts in macrophages and endothelial cells after hyperoxia (12), whereas an increased protein expression of VEGF has been observed in *in vivo* models (13). Woo, Coutts (14) reported reduced wound surface area and lower risk of bacterial infection after four weeks of transdermal continuous topical oxygen therapy. Overall, oxygen therapy may accelerate wound healing by directly inducing certain biochemical and immunological signaling pathways that aid wound healing. Therefore, the present review attempts to clarify the role of oxygen therapy in the wound healing process with a special focus on topical oxygen therapy, by presenting some of the recent findings in which the application of topical oxygen has been associated with changes in biochemical, microbiological, and immunological parameters. In addition, this review also discusses some of the shortcomings of topical oxygen therapy that limit its application to wound care and wound management.

2. Evidence Acquisition

Chronic wounds are a serious public health issue and annually affect millions of individuals across the globe. Relevant information was searched in various scientific databases including PubMed, ScienceDirect, and Google Scholar using such keywords as “Chronic Wounds”, “Chronic Wounds and Oxygen Therapy”, “Chronic Wounds and Topical Oxygen Therapy”, “Chronic Wounds and Microbial Infections”, “Microbial Infections in Wounds”, “Lactate and Wounds”, “Lactate and Wound Healing”, “Chronic Wounds and Inflammatory Markers”, “Chronic Wounds and Inflammatory Cytokines”, “Collagen and Wound Healing”, and “Matrix Metalloproteinase and Chronic Wounds”. Along with the search results obtained using the aforementioned keywords, several cross-references were also read and studied while writing the manuscript. The research team evaluated the obtained articles for their relevance before compiling the manuscript. The included articles in the final review consisted of the articles that discussed the association between “chronic wound and microbial infections or wound microbial profile”, “chronic wound and immunological parameters (e.g., cytokines, Matrix Metalloproteinase)”, and “chronic wound and biochemical parameters (lactate and collagen)”. All relevant articles in the PubMed database were included in the review paper, regardless of publication year.

3. Results

3.1 Role of oxygen in wound healing

The wound healing process is highly dynamic and complex and involves various cellular and molecular players, such as fibroblasts, blood cells, cytokines, and extracellular matrix components. Under normal circumstances, the closely coordinated processes ultimately restore skin integrity. Oxygen is a key player in the overall healing process due to its direct involvement in various essential physiological and cell signaling processes necessary for wound healing. An adequate oxygen supply is necessary during wound

healing for energy generation, production of reactive oxygen species (ROS), infection control, normal cell signaling, production of extracellular matrix components, remodeling of collagen, and angiogenesis (Figure 1) (11, 15). The increased energy requirements of damaged tissues are primarily met by glucose oxidation. At this crucial stage, inadequate oxygen supply can prevent energy generation, since, under hypoxic conditions, one glucose molecule undergoes anaerobic oxidation and produces only two molecules of ATP, whereas the aerobic oxidation of one glucose molecule produces 36 molecules of ATP (15). Insufficient oxygen supply at wound bed may have an adverse impact on wound healing by reducing the availability of energy. In addition, hypoxic conditions facilitate the generation of ROS, which further exacerbates tissue damage and adversely affects wound healing. Reduced energy generation also reduces angiogenesis in wounds, resulting in impaired wound healing (11). Given the crucial role of oxygen in the overall wound healing process, a wide range of adjunct oxygen therapies, such as Topical Oxygen Therapy (TOT), have been developed to deliver oxygen to the wound bed. Topical oxygen is delivered to wounds through several strategies, including delivery of pure oxygen to the wound site under pressure or in the ambient condition, use of chemicals and enzymes to facilitate oxygen release at wound bed, and the use of oxygen-binding molecules to release oxygen through the facilitated diffusion process (11). Fries, Wallace (16) tested the efficacy of topical oxygen in wound healing in a pig model. Results of tissue oximetry demonstrated that TOT increased tissue oxygenation, and partial pressure of oxygen (pO_2) in wound tissue increased from 5-7 mm Hg to 40-80 mm Hg. In addition, histological observations confirmed that pigs that underwent TOT had better angiogenesis, improved epidermis structure, and increased granulation tissue formation (16). A 15-month clinical trial showed that the application of TOT for 25 days reduced wound area by 83%, with a 47% wound closure rate in leg ulcers (17). Another large-scale review of 3,462 patients was conducted from 2007 to 2016, presenting the wound care of 4,127 wounds. All the wounds were treated with TOT and 46% of the wounds were on foot and toe, many of which were related to diabetes. All the patients were covered with Medicare and Medicaid (42.3 and 35.6, respectively). Size reduction occurred in a majority of the wounds (59.4%), of which 27.5 % healed completely, while 40.6% showed no signs of healing. The rate of cases with wound size larger than 16 cm^2 reduced from 24.4% to 16.2%, following TOT therapy (18). A multicentric, open, randomized controlled clinical trial also found that hard-to-heal diabetic foot ulcers (DFUs) were healed better when TOT was administered with standard care (19). However, some experimental findings in animal models showed little effect of topical oxygen in wound healing. A study in an experimental horse model found no significant difference in the mean healing time between the topical oxygen-treated horses and the control group, suggesting that application of topical oxygen may not have a therapeutic advantage in wound healing (20). Although the application of topical oxygen is well known in wound healing, its use has some limitations. One limitation is that topical oxygen supplement leads to diffusion of oxygen at a maximum distance of 50–100 microns; therefore, the amount of oxygen absorbed through open wounds would be extremely small in this therapy method (21). This may cause ischemia when the pressure within the closed Topical oxygen may cause cell toxicity. One of the biggest drawbacks of TOT which reduces its effectiveness for Wagner Grade 3 wounds is that it does not penetrate through the bone. This therapy is restricted to wounds that do not heal within 2-4 weeks of wound care treatment (22medium exceeds systolic pressure.).

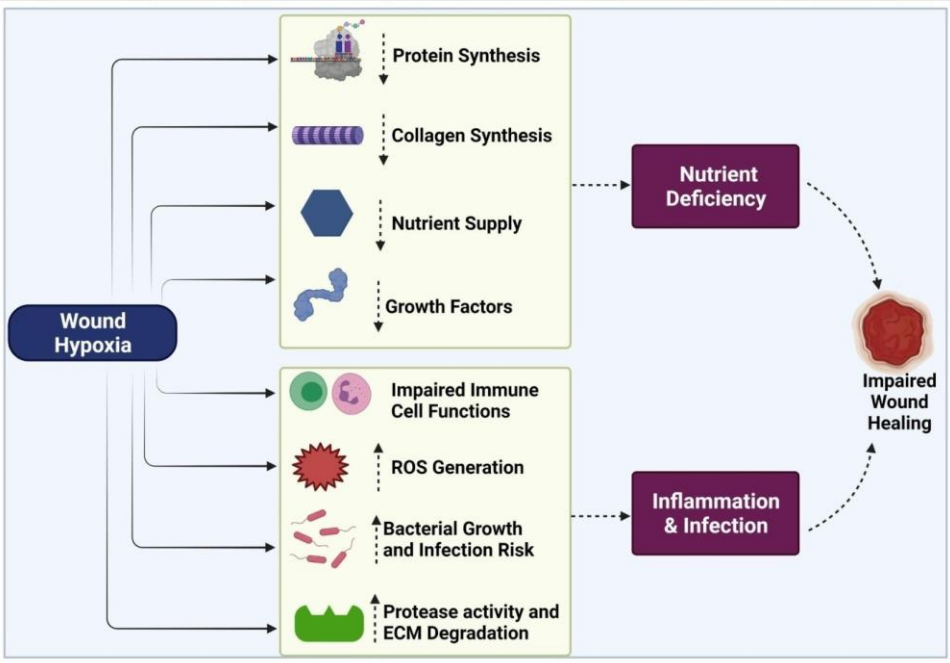

Figure 1. Lower oxygen pressure in wounds leads to wound hypoxia. A hypoxic wound shows an overall reduction in protein synthesis, including collagen, as the major protein in the extracellular matrix. Wound hypoxia also reduces nutrient supply and secretion of growth factors in the wound. Overall, wound hypoxia generates nutritional deficiency in the wound region. Further, hypoxia impairs the functioning of primary immune cells and causes the excessive generation of reactive oxygen species. It also increases the activity of tissue degrading enzymes. Eventually, hypoxic wounds are more prone to develop life-threatening infections by anaerobic bacteria. Therefore, nutritional deficiency increased inflammation, and the growth of anaerobic bacteria leads to impaired wound healing (11, 15, 23, 24).

3.2 Topical Oxygen Therapy and Microbial Infections

Normal oxygen pressure at the skin surface is between 45 and 65 mm Hg and decreases to 0-5 mm Hg in the non-vascularized central portion of the wound. These extremely low oxygen levels promote an anaerobic metabolic state and significantly reduce the wound healing process (25, 26). Bacterial infection of wounds is a major reason for delayed wound healing and the chronicity of wounds. It has been reported that chronic wounds harbor multiple bacterial species, and open chronic wounds are frequently exposed to polymicrobial infections. The genera of *Corynebacterium, E. coli, Enterobacter, Enterococcus, Finegoldia, Peptoniphilus, Providencia, Proteus, Pseudomonas, Serratia, Staphylococcus,* and *Stenotrophomonas* are commonly found in open and chronic wounds (27-30). In addition, the formation of bacterial biofilm further contributes to the chronicity of wounds by depleting the oxygen needed for the normal wound healing process (31). Chronic wounds harbor a distinct microflora rich in anaerobic bacteria species, such as *Anaerococcus, Peptoniphilus,* and *Finegoldia,* and other harmful bacteria genera, such as *Staphylococcus* and *Corynebacterium*. In addition, there is lower bacterial diversity in the wound microflora at species and genus levels (32). Culture-based and 16s rRNA sequencing data showed that the wound microbiome was rich in Gram-negative bacteria including *Alcaligenes, Pseudomonas, Burkholderia,* and *Corynebacterium* in descending order of abundance in the wound microbiome. In addition, Wagner grade 5 wounds (26.5%) showed a higher presence of facultative anaerobes, while Wagner grade 1 wounds were rich in strict anaerobes (26%) (33). The presence of certain facultative anaerobic bacteria in chronic wounds significantly delay wound healing, with genus *Enterobacter* completely preventing wound healing. Since facultative anaerobes show robust survival under different metabolic environments, their

presence in a wound site is negatively associated with wound healing (34). The pO_2 between 5 and 20 mm Hg is common in non-healing wounds, while pO_2 of 30 mm Hg is common in infected and traumatized wounds. A pO_2 of 30 mm Hg is essential for normal cell division. In the absence of normal oxygen saturation, hypoxia worsens wounds by promoting cell death and tissue necrosis and creating a suitable growth environment for anaerobic microbes (5). Interestingly, *Pseudomonas aeruginosa* growing under oxygen-depleted wounds show higher resistance to antibiotics, demonstrating that anaerobic conditions reduce the antibiotic susceptibility of *Pseudomonas aeruginosa* to tobramycin, ciprofloxacin, carbenicillin, ceftazidime, chloramphenicol, or tetracycline in the wound (35). These scientific observations suggest that the presence of bacterial species, especially anaerobes, impairs the normal wound healing process, and reduction of bacterial load can accelerate the wound healing process (36). The use of topical oxygen has also been found to modulate the bacterial flora, the property which effectively improves wound healing. The application of topical oxygen in DFUs of grades II and III increased microbial diversity with a higher proportion of aerobes and facultative anaerobes in five patients with DFU, suggesting that topical oxygen application facilitates wound healing by increasing aerobic bacteria at the wound site (37). It has been shown that the application of topical oxygen not only prompted a shift of the wound genera from anaerobic to aerobic but also forced the facultative anaerobe *Staphylococcus* to switch to aerobic respiration (38). It has been reported that the culture-positive rate was significantly lower in patients who were given a combination of TOT and negative pressure wound therapy (n=56), compared to patients who received only negative pressure wound therapy (n=56). The group that received combined therapy showed a significantly lower abundance of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, and other bacterial species than the control group (only NPWT). A lower culture-positive rate in the intervention group is possibly due to better availability of oxygen which might have inhibited bacterial growth and proliferation (39). In addition, the use of Topical Oxygen Jet Therapy is associated with reduced pain, accelerated healing, and better clinical outcomes, compared to the control group (40).

3.3 Topical Oxygen Therapy and Changes in Immunological Markers

The normal wound healing process involves a cross-talk between various immune cell types and immune mediators. The role of the immune system is cardinal in the overall wound healing process, with macrophages, neutrophils, growth factors, cytokines, chemokines, and interferons playing crucial roles at various stages of wound healing (41). Wound healing is an evolutionarily conserved and complex phenomenon aimed at restoring the integrity of the epithelium. The highly complex network of signals controls growth, differentiation, and cellular metabolism at the wound site. Unhealed wounds often exhibit dysregulated signaling of pathways controlled by various growth factors, such as VEGF, platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF), suggesting that growth factors can be used to improve clinical outcomes of complex non-healing wounds (42). It has been demonstrated that inflamed wounds have higher activity of Metalloproteinase (MMPs) and higher levels of pro-inflammatory mediators, such as IL-1ß. In addition, highly inflamed wounds show a higher bacterial load, compared to wounds with a low degree of inflammation (43). A prospective pilot study by Liu, Yang (44) showed that levels of inflammatory mediators C-reactive protein (CRP) and IL-6 significantly reduced during the healing process. In this study, the CRP level was reduced from 66.4 mg/L to 10.4 mg/L, while the level of IL-6 was reduced from 44.1 pg/mL to 8.6 pg/mL (44). Therefore, reduced concentration of inflammatory mediators at the wound bed may facilitate the wound healing process by reducing tissue damage and bacterial load. In this regard, the use of topical oxygen can be explored to

ameliorate wound inflammation and accelerate the wound healing process. Studies have demonstrated that topical oxygen application promotes the production of reactive oxygen species (ROS), and ROS generation is directly associated with pO_2. H_2O_2 is one of the most prevalent and stable ROS and acts as an intracellular messenger in the micromolar range due to its ability to efficiently cross cellular membranes. This ability of H_2O_2 facilitates neutrophil movement, regulates TGF-β signaling, induces expression of VEGF and EGF, and promotes collagen deposition (24). Another study revealed that TOT promoted complete wound closure at the molecular level by increasing the expression of growth factors, such as VEGF (45). Gordillo, Roy (13) also reported a significantly higher VEGF expression in wound edges of diabetic patients who were given portable topical oxygen therapy, compared to patients who received hyperbaric oxygen therapy. Increased VEGF expression was associated with improved closure of chronic wounds observed in diabetics (13). It should be noted that TOT is FDA approved for diabetic ulcers, venous insufficiency, postsurgical infections, gangrenous lesions, pressure ulcers, skin grafts, burns, frostbite, and amputations (46). A study performed by He, Liang (47) on a cohort of DFU patients demonstrated that a combination of moist wound dressing (MWD) and continuous diffusion of oxygen (CDO) was more effective in wound healing than the application of MWD and CDO alone. The group receiving combined therapy showed a higher wound healing rate, lower levels of high CRP, and lower count of white blood cells than the MWD and CDO groups, suggesting a promising strategy to deal with complex clinical wounds (47).

3.4 Topical Oxygen Therapy and cHanges in Biochemical Markers

3.4.1 Lactate

Lactate is a by-product of the leukocytic (bactericidal) ‘‘oxidative burst’’ that is essential for wound immunity. Lactate accumulation occurs in wounds due to anaerobic and aerobic glycolysis, and studies have shown that lactate facilitates wound healing by activating numerous molecular signaling pathways and promoting angiogenesis. Increased levels of wound lactate by implanting purified, solid, and hydrolyzable polyglycolide increased the short-term levels of interleukin-1 beta and caused a long-term elevation in the levels of VEGF and TGF-β. It also increased the level of collagen by 50% and reduced insulin-like growth factor-1 by 90%. According to Trabold, Wagner (48), increased lactate concentration at the wound bed is a “perception” for hypoxia, and the cellular machinery responds to this signal by increasing the synthesis of various cytokines and mediators involved in the wound healing process. In addition, the continuous presence of oxygen facilitated the synthesis and deposition of collagen by endothelial and fibroblast cells (48). It has now been recognized that there are lactate “sensitive genes” that code important signaling molecules, such as hypoxia-inducible factor, VEGF, and TGF-band, pertinently, MMPs. Lactate can also be considered a surrogate marker for hypoxia in initiating the healing cycle (49). Studies in mice models have also demonstrated that sustained release of lactate at the wound site by implanting poly-D, L-lactide-co-glycolide (PLGA) promoted wound closure through increased angiogenesis. In this study, lactate increased the vascular density by 3.4 times, compared to the control group (50). Since the majority of chronic wounds are managed within the community setting, measurement of wound lactate may help understand the overall progress of wound healing. In this regard, automated lactate meters and the dipstick method that distinguishes between “physiological” and “pathological” lactate concentrations can help identify patients at higher risk (51).

3.4.2 Matrix Metalloproteinase (MMPs)

It should be mentioned that MMPs are proteases responsible for the degradation of extracellular matrix components and play an important role during the wound healing process (52). The MMPs are vital during all phases of wound healing and remove temporary Extracellular Matrix (ECM) and damaged proteins, help in angiogenesis and cell migration by

breaking down the capillary basement membrane, and contract and remodel the tissue during the remodeling phase. Therefore, a certain activity of MMPs is essential for the normal progression of wound healing; however, excessive activity of MMPs extensively leads to prolonged inflammation and damages (ECM) which causes impaired wound healing (52, 53). Higher activity of MMPs is commonly observed in chronic wounds, and strategies, such as the use of the protease-absorbing dressing, photodynamic therapy, and certain peptides have been explored to control MMP activity (54). Interestingly, there is some evidence from both human trials and preclinical studies showing that externally supplied oxygen can improve wound healing by reducing the expression of MMPs. For instance, a prospective, randomized clinical trial by Driver, Yao (55) reported lower expression of tissue-degrading proteases (MMP-1,-2,-9,) in DFU patients that received transdermal continuous oxygen therapy (TCOT) for four weeks as an adjunct therapy to the standard care (debridement, offloading, and moisture). In addition, the group receiving TCOT also showed a lower expression of inflammatory mediators IL-6 and IL-8 which accelerated healing in DFU patients, compared to the control group that received standard care (55).

3.4.3. Collagen

Collagen is the most abundant protein present in the human body (56). The structure of collagen in the human skin is proportional to the amount and quality of collagen present in the human tissues. Therefore, the deposition of collagen is considered to be a fundamental step in wound healing (57). Importantly, several post-translational steps in the collagen synthesis pathway are oxygen dependent, since oxygen acts as a cofactor for key enzymes prolyl hydroxylase, lysyl hydroxylase, and lysyl oxidase. In addition, the Km and Vmax values for collagen synthesis are pO_2 20-25 mm Hg and 25 mm Hg, respectively. These pO_2 values are higher than those usually found in wounds, indicating that supplying oxygen to the wound bed will increase collagen production (58, 59). Therefore, accelerated wound healing after TOT may be attributed to improved reaction kinetics of collagen synthesis. In DFU patients, Lavery, Killeen (60) reported that topical oxygen significantly increased the expression of genes associated with collagen production (i.e., TGF-β, VEGF, and IL-6) (60). Scientific evidence showed that supplying adequate oxygen at the wound bed increased collagen deposition, which in turn led to the acceleration of the overall wound healing process. Therefore, collagen measurement at the wound bed may be used as an excellent biomarker to understand the overall progression of wound healing.

4. Conclusions

Chronic wounds are difficult to heal and have a negative impact on patients' quality of life. Globally, an estimated 40 million people develop chronic wounds each year, which places an enormous burden on the existing healthcare infrastructure. Non-healing of wounds is usually attributed to poor nutrition, inadequate oxygen supply, bacterial infections, improper wound management, and lack of understanding of wound healing dynamics. In addition, a detailed understanding of various microbial, immunological, and biochemical markers that either indicate wound deterioration or confirm wound healing can help physicians make appropriate treatment decisions which in turn reduces mortality and morbidity. This review study has summarized some of the recent evidence supporting the use of TOT for wound healing (Figure 2). Based on the results of available scientific studies, TOT is a promising therapeutic intervention for wound care and management. Application of topical oxygen eliminates hypoxic conditions in wounds and promotes wound healing by increasing the expression of growth factors (VEGF) and decreasing the secretion of tissue-degrading MMPs which promotes collagen deposition and granulation tissue formation.

Therefore, topical oxygen therapy may be a promising therapeutic option for accelerating wound healing and improving clinical outcomes in patients with chronic wounds. Further studies are recommended to explore the benefits of TOT, including the minimal use of antibiotics.

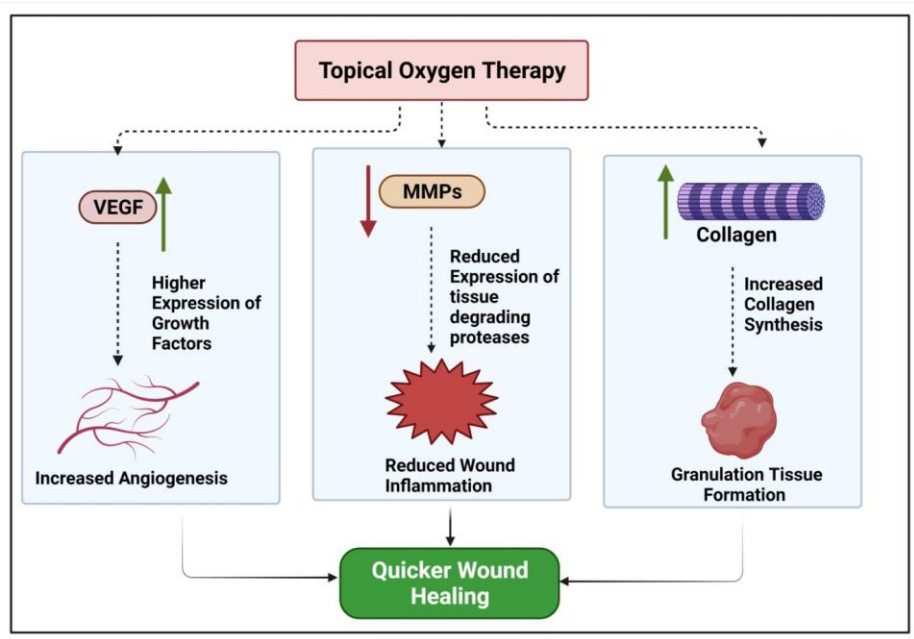

Figure 2. Topical oxygen accelerates wound healing by modulating several signaling pathways and biological reactions. Topical oxygen application increases the expression of growth factors such as VEGF, thereby promoting angiogenesis in wound tissue. It also reduces tissue degrading enzymes such as matrix metalloproteinases (MMPs) leading to lower tissue inflammation. Topical oxygen promotes the synthesis of collagen protein in wounds. Since collagen is a major constituent of the extracellular matrix, its increased level promotes granulation tissue formation and aids wound closure. A combined effect of increased angiogenesis, lower wound inflammation, and higher ECM synthesis accelerates wound healing. Prepared using Biorender.com

List of Abbreviations

ATP: Adenosine triphosphate
DFU: Diabetic foot ulcers
ECM: Extracellular matrix
GPAC: Gram-positive anaerobic Cocci
MMP: Matrix metalloproteinase
PLGA: Poly-D, L-lactide-co-glycolide
pO_2: Partial oxygen pressure
ROS: Reactive oxygen species
TO/TOT: Topical oxygen therapy
VEGF: Vascular endothelial growth factor

Authors' Contribution

The authors contributed equally to this study

Conflict of Interest

The authors declare that they have no conflict of interest.

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