How Oxygen Works in Wound Healing

The following is a summary overview of the current scientific literature regarding the efficacy of oxygen in wound healing. The primary modes of action listed below are detailed in the following pages. Literature references are included at the end.

1. **Increases Cell Metabolism and Energy Production**
   Oxygen is required for intracellular processes like biosynthesis, movement, and transport need energy to be functional, as well as for cell survival

2. **Increases Rate of Cell Proliferation and Reepithelialization**
   Epithelial cells "march in" from the sides to close the wound and form a barrier between the wound and the environment – this is the foundation for forming new skin

3. **Increases Collagen Synthesis and Tensile Strength**
   Oxygen is essential to make and properly organize collagen, which is the primary component of skin, accounting for 70-80% (dry weight – without water) and acts as the structural scaffold of skin. Organized collagen is bundled into fibers (like strands in rope), which are interwoven and can be stretched in multiple directions without tearing (the collagen fibers are woven similar to fabric)

4. **Increases Anti-bacterial Activities**
   Oxygen is essential for respiratory burst, the production of reactive oxygen species (ROS), used by phagocytes such as neutrophils and macrophages in bactericidal activity and the removal of necrotic cellular debris

5. **Increases Angiogenesis and Promotes Revascularization**
   The creation of new blood vessels, angiogenesis, is essential to the growth and survival of repair tissue. Oxygen levels directly affect the rate and quality of new blood vessel growth

6. **Promotes Growth Factor Signaling Transduction**
   Reactive oxygen species (ROS) are essential for the signaling processes of growth factors and processes such as leukocyte recruitment, cell motility, angiogenesis and extracellular matrix formation

"First and foremost it needs to be borne in mind that the overarching goal of oxygen therapy should be to correct wound hypoxia. … Second, approaches to keep a wound oxygenated over a longer period of time, as opposed to a few hours usually targeted in HBO therapy, should prove to be beneficial. In response to HBO, there is no sustained change in tissue oxygen tension much beyond the period of treatment."[1,2]

How Oxygen Works in Wound Healing

1. Increases Cell Metabolism and Energy Production
Oxygen is required for intracellular processes like biosynthesis, movement, and transport need energy to be functional, as well as for cell survival
- Oxygen dependent enzymes include:
  - Adenosine triphosphate (ATP) for chemical energy, which fuels most active cellular processes such as during wound healing.[3] Increased energy demand of the healing tissue leads to a hypermetabolic state wherein additional energy is generated from oxidative metabolism increasing the oxygen demand of the healing tissue.[4,5,6,7] ATP thus generated powers tissue repair
  - NADPH oxygenase for respiratory burst (reactive oxygen species release), the activity of which is critically required to produce the redox signals required for wound healing[8,9,10]
- Aerobic glycolysis, β-oxidation of fatty acids, and the citric acid cycle are tightly attached to the energy acquisition by oxidative phosphorylation and are therefore oxygen dependent[11]
- If oxygen levels are too low (<20 mmHg pO_2), cells convert to anaerobic metabolism and go into survival mode in which wound healing activities such as mitosis (cell division, and therefore reepithelialization) and collagen production are impaired[12,13,14]
- Prolonged exposure to extremely low oxygen levels, if not alleviated by oxygen, can result in cell death and tissue necrosis due to the inability of the cells to repair the spontaneous decay of cell components (DNA, RNA, proteins) and inability to maintain calcium pumps which require ATP to function[15,16]

2. Increases Rate of Cell Proliferation and Reepithelialization
Epithelial cells "march in" from the sides to close the wound and form a barrier between the wound and the environment – this is the foundation for forming new skin
- The addition of pure oxygen over a diabetic wound has been shown to increase the rate of wound closure, as measured by endothelial gap closure, by as much as 69%, indicating more rapid reepithelialization[17]
- Fibroblast proliferation and protein production have been reported to be optimal at 160 mmHg, i.e. at pO_2 levels 2-fold to 3-fold higher than those found in healthy tissues[18], indicating that supplemental oxygen increases the rate of wound repair
- Endothelial progenitor cells (EPCs) are essential in wound healing, but their circulating and wound level numbers are decreased in diabetes. Elevated oxygen levels (hyperoxia) reverse the diabetic defect in EPC mobilization[19]
- EPC mobilization into circulation is triggered by hyperoxia through induction of nitric oxide (NO) with resulting enhancement in ischemic limb perfusion and wound healing[20,21,22]

3. Increases Collagen Synthesis and Tensile Strength
Oxygen is essential to make and properly organize collagen, which is the primary component of skin, accounting for 70-80% (dry weight – without water) and acts as the structural scaffold of skin. Organized collagen is bundled into fibers (like strands in rope), which are interwoven and can be stretched in multiple directions without

tearing (the collagen fibers are woven similar to fabric)
- Oxygen is required for the hydroxylation of proline and lysine in procollagen[23]
- Several posttranslational steps in collagen synthesis are oxygen dependent. The enzymes prolyl hydroxylase, lysyl hydroxylase and lysyl oxidase all require oxygen[24,25,26]
    - o Formation of cross-linked triple-helices via the oxygen-dependent enzyme prolyl hydroxylase and excreted as collagen fibers
    - o Collagen fibers are arranged into linear fibrils via cross-linking by lysyl hydroxylase
    - o Linear fibrils are cross-linked by lysyl oxidase - a necessary step to achieve the necessary tensile strength for healed wounds
- Higher oxygen concentrations increase the amount of collagen deposition[27] and tensile strength[28,29,30]
- The rate limiting step is the rate of prolyl hydroxylation[25,26]
- The oxygen level required for optimal prolyl hydryoxlase activity is at oxygen levels approaching 250 mmHg, exceeding those present in normal wounds[31,32]
- It has been shown that increasing oxygen above normal physiologic levels enhances collagen synthesis and tensile strength in both animal and human subjects[28,29,30] and can increase the level of collagen organization[17]
- Correction of vasoconstriction and hypoxia can result in a 10-fold increase in collagen deposition in wound repair[27,29,33,34]

4. Increases Anti-bacterial Activities
Oxygen is essential for respiratory burst, the production of reactive oxygen species (ROS), used by phagocytes such as neutrophils and macrophages in bactericidal activity and the removal of necrotic cellular debris
- NADPH (nicotinamide adenine dinucleotide phosphate) oxidase, also known as leukocyte oxidase, supports macrophage survival (delay of apoptosis)[35] and enables dead cell cleansing by phagocytosis[36]
- NADPH oxidase in wound phagocytes, such as neutrophils and macrophages, produces superoxides (O_2^- and H_2O_2) for bactericidal activities[37] – in fact, ~98% of oxygen consumed by these cells is used to produce reactive oxygen species (ROS) during phagocytosis[38]
- Leukocyte activity (production of ROS and hence oxidative killing) is directly proportional to local oxygen concentration[39]
- Optimal ROS production is seen at oxygen levels of greater than 300 mmHg[38], levels which can only be achieved with supplemental oxygen[40]
- At the wound site, ROS are generated by almost all wound-related cells[24]
- The efficacy of supplemental oxygen has been shown to be similar to antibiotic administration and has additive effects when used together[41,42]

5. Increases Angiogenesis and Promotes Revascularization
The creation of new blood vessels, angiogenesis, is essential to the growth and survival of repair tissue. Oxygen levels directly affect the rate and quality of new blood vessel growth
- Sufficient oxygen levels are required for correct collagen synthesis

(posttranslational hydroxylation)[43], without which the new capillary tubes assemble poorly and remain fragile[44,45,46]

- Supplemental oxygen accelerates blood vessel growth[47]
- Moderate hyperoxia increases the appearance of new blood vessels in wounds[48]
- The rate of angiogenesis is directly proportional to oxygen levels in injured tissues and rates of collagen deposition increase proportionally with oxygen levels to more than 250 mmHg[45]
- Conversely, hypoxic wounds deposit collagen poorly and become infected easily[23,27]

6. Promotes Growth Factor Signaling Transduction

Reactive oxygen species (ROS) are essential for the signaling processes of growth factors and processes such as leukocyte recruitment, cell motility, angiogenesis and extracellular matrix formation

- Signal transduction of growth factors happens through ROS[49]
- ROS such as hydrogen peroxide (H_2O_2) increase vascular endothelial growth factor (VEGF) production in macrophages and keratinocytes[50,51]
- VEGF is a major long-term angiogenic stimulus at the wound site
  - o oxygen treatment induces VEGF mRNA levels in endothelial cells and macrophages[52,53,54]
  - o oxygen treatment increases VEGF121/165 protein expression in wounds[55] and facilitates the release of VEGF165 from cell-associated stores[56]
- Platelet-derived growth factor (PDGF) requires ROS in its role to regulate cell growth and division[57], and PDGF plays a significant role in blood vessel formation (angiogenesis)[24]
- ROS has effects on other processes such as cytokine action, cell motility and extracellular matrix formation[8]
- Conversely, tissue hypoxia will limit redox signaling and disable the function of several growth factors (e.g., PDGF, VEGF, keratinocyte growth factor, insulin-like growth factor, transforming growth factor-a) and numerous molecular mechanisms (e.g., leukocyte recruitment, cell motility, integrin function), which rely on redox signaling[10,58,59]

NOTES:
- Some sections of the above text with citations are directly extracted from the referenced literature without quotations.
- Recommended summary articles on oxygen in wound care include articles by Sen[1], Tandara and Mustoe[11], and Gordillo and Sen[24].

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