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## PERSPECTIVE ARTICLE

## The use of hyperbaric oxygen therapy to treat chronic wounds: A review

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### ABSTRACT

Chronic wounds, defined as those wounds which fail to proceed through an orderly process to produce anatomic and functional integrity, are a significant socioeconomic problem. A wound may fail to heal for a variety of reasons including the use of corticosteroids, formation of squamous cell carcinoma, persistent infection, unrelieved pressure, and underlying hypoxia within the wound bed. Hypoxia appears to inhibit the wound healing process by blocking fibroblast proliferation, collagen production, and capillary angiogenesis and to increase the risk of infection. Hyperbaric oxygen therapy (HBOT) has been shown to aid the healing of ulcerated wounds and demonstrated to reduce the risk of amputation in diabetic patients. However, the causal reasons for the response of the underlying biological processes of wound repair to HBOT, such as the up-regulation of angiogenesis and collagen synthesis are unclear and, consequently, current protocols remain empirical. Here we review chronic wound healing and the use of hyperbaric oxygen as an adjunctive treatment for nonhealing wounds. Databases including PubMed, ScienceDirect, Blackwell Synergy, and The Cochrane Library were searched for relevant phrases including HBOT, HBO/HBOT, wound healing, and chronic/nonhealing wounds/ulcers.

Leg ulceration is a significant socioeconomic problem.<sup>1</sup> Those who suffer from leg ulcer, whatever the etiology, experience a considerable amount of pain, immobility, and a decreased quality of life. It is estimated that 1% of the Western adult population will suffer from a leg ulcer at some stage of their life.<sup>2</sup>

In their 2001 publication, McGuckin et al.<sup>3</sup> estimated that it costs US\$3 billion each year solely to treat the symptoms of leg ulceration, not including the associated cost of approximately 2 million lost work days per annum. Leg ulcers become more common with age with the occurrence most common in the over 60s age demographic.<sup>1</sup>

Diegelmann et al.<sup>4</sup> claim that in the 15 years following their 2004 publication, the US population over age 85 will increase from 4 million to over 17 million. Hence, the treatment costs associated with ulcers are forecast to rise in the coming decades. Leg ulcers tend to persist for an extended period of time with 45% of patients in a Scottish study conducted by Cullum et al.<sup>5</sup> reporting venous ulceration for longer than 10 years. Diabetic, arterial, pressure, and mixed etiology leg ulcers that do not heal often leave medical personnel with no option but to amputate the lower limb.<sup>6</sup>

The term “leg ulcer” is widely used to indicate venous hypertension induced ulceration; however, an ulcer may develop on the lower limbs for a number of reasons. There are four main classifications of chronic leg wounds: venous, arterial, pressure, and diabetic.<sup>7</sup> Less commonly, nonhealing wounds may be the result of conditions such as vasculitis, cancer, and pyoderma gangrenosum. Table 1 summarizes the causes and treatments of the four main wound etiologies. It is important to note that a single wound may have a combination of underlying causes and

it is crucial to identify the etiology because standard treatments are recognized for each type of ulcer and inappropriate treatment based on a misdiagnosis may inadvertently impede healing and lead to wound chronicity. Recently, Chen et al.<sup>7</sup> concluded that unless the underlying causes of chronic wound formation are effectively managed, chronic wounds are not going to heal.

This paper will be presented as follows. In “Biology of Wound Healing” we review the biology of wound healing including the four phases of hemostasis, inflammation, proliferation, and remodeling. We also review the role that oxygen plays in tissue repair. Following this, in “Chronic Wounds,” the chronic wound process will be discussed. We summarize the reasons presented in the literature as to why chronic wounds heal slowly or not at all. Finally, we describe hyperbaric oxygen therapy (HBOT) and the current protocols and the rationale behind the use of this treatment.

### BIOLOGY OF WOUND HEALING

A successfully healing wound is typically thought to progress through four stages: hemostasis, inflammation, proliferation, and remodeling,<sup>16</sup> although these processes are interconnected and overlapping (see Figure 1). In the sections that follow the four phases of acute wound healing will be discussed.

#### Hemostasis

When a tissue injury is sustained, blood from the severed capillaries streams into the wound.<sup>4</sup> The immediate response of the body is aimed at impeding the blood loss.<sup>18</sup>

**Table 1.** Summary of the causes and treatments of different wound etiologies

Etiology	Causes	Treatments	References
Diabetic	Peripheral neuropathy Alterations in blood perfusion	Offloading by use of protective footwear, wheelchair, etc., debridement, moist wound care	Lerman and colleagues <sup>6,8,9</sup>
Venous	Venous hypertension Chronic venous insufficiency	Improve venous return/edema with compression therapy Moist wound care	Chen and colleagues <sup>7,10-12</sup>
Arterial	Arterial insufficiency	Restoration of arterial inflow with angioplasty, stents, bypasses Moist wound care (excluding unrevascularized arterial ulcers)	Enoch and colleagues <sup>12-14</sup>
Pressure (decubitus)	Unrelieved pressure leading to tissue death	Relieve pressure with dressings and pressure relieving surfaces Debridement, moist wound care	Byrne and Owen <sup>15</sup>

The blood flow into the wound carries platelets and fibrinogen with it, both of which are important in the wound healing process.<sup>19</sup>

Fibrinogen is activated in response to the exposed epithelium to form a fibrin mesh that traps platelets. These platelets also adhere to the ruptured blood vessels, preventing further blood loss.<sup>19</sup> Moreover, as platelets come into contact with damaged extracellular matrix (ECM) components, they release clotting factors, leading to the formation of a blood clot within the wound site.<sup>4</sup> Platelets within the wound release chemical stimuli such as platelet-derived growth factor (PDGF), transforming growth factor  $\beta$  (TGF- $\beta$ ), and vascular endothelial growth factor (VEGF).<sup>4,20</sup> This phase is referred to as hemostasis and, in the normal wound healing process, should last a matter of hours.

### Inflammation

The onset of the next phase, inflammation, is characterized by the arrival of phagocytic neutrophils at the wound site.<sup>17</sup> Typically, within 24 hours of injury, neutrophils have been attracted into the wound by the chemoattractants already

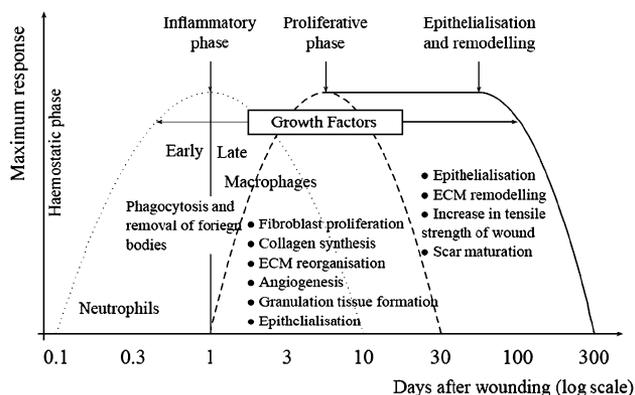
released and are in the process of phagocytosing foreign particles, bacteria, and the blood clot.<sup>21</sup> The chemical stimulants, provided by the growth factors, also stimulate large white blood cells, monocytes, to leave neighboring blood vessels and enter the wound tissue (see Figure 2).<sup>4</sup> At this stage, the cell is no longer referred to as a monocyte but rather as a macrophage.<sup>22</sup> Macrophages are vital for the healing of a wound, because they are involved in the promotion of angiogenesis, matrix deposition, and epithelialization.<sup>23</sup>

In the wound, as the macrophages actively migrate in response to a chemoattractant gradient, they consume the necrotic material in their path including dead neutrophils<sup>22</sup> while releasing a variety of growth factors such as macrophage derived growth factor (MDGF), VEGF, endothelial growth factor (EGF), PDGF, and TGF- $\beta$ .<sup>24</sup> The cocktail of chemicals present in the wound site attracts fibroblasts from the surrounding undamaged ECM into the wound site.<sup>4</sup> The inflammation phase typically lasts for a matter of days.

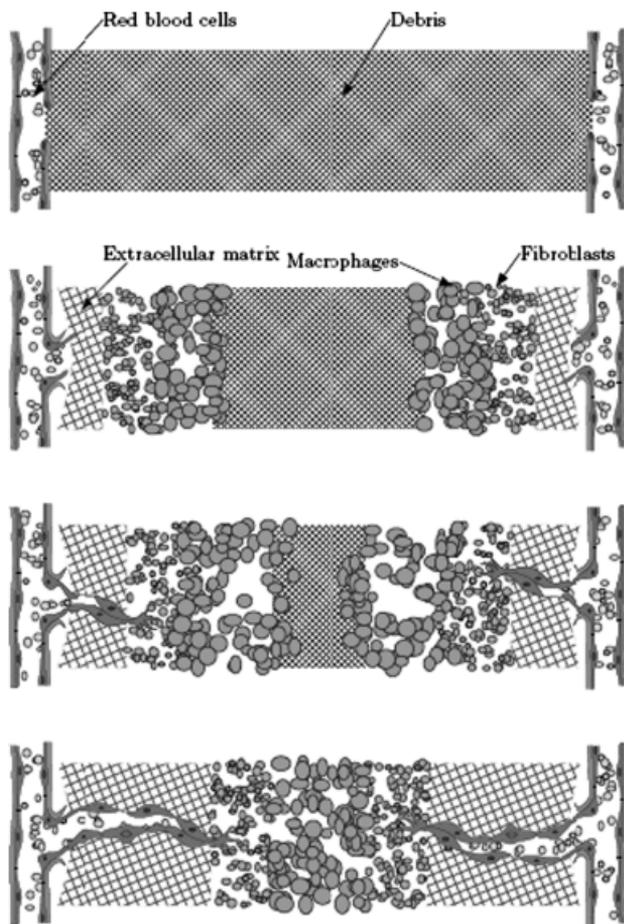
### Proliferation

The fibroblast is the dominant cell within the wound during the proliferative phase of healing.<sup>20</sup> Fibroblast proliferation is oxygen-dependent and the cells can only survive and perform their necessary functions in the wound where sufficient oxygen is available.<sup>25</sup> The moving band of phagocytosing macrophages typically leads the fibroblast front into the wound.<sup>26</sup>

Fibroblasts, stimulated by chemoattractants such as PDGF, produce collagen, which is a major component of the ECM.<sup>17</sup> This granulation tissue is the formation of collagenous scaffolding upon which the vascular network can extend (see Figure 2).<sup>19</sup> Fibroblasts differentiate into myofibroblasts that align themselves along the newly formed ECM and generate tensile strength across the wound, leading to contraction.<sup>27</sup> The matrix acts as a "bed," across which endothelial cells, the cells that make up blood vessels, can migrate.<sup>28</sup> Fibroblasts continue to secrete growth factors such as VEGF, TGF- $\beta$ , and PDGF.<sup>6</sup> Activated to do so by the growth factors present in the wound, keratinocytes migrate and proliferate,



**Figure 1.** The four stages of the wound healing process, adapted from Enoch et al. with permission from BMJ Publishing Group.<sup>17</sup>



**Figure 2.** Schematic diagram of wound healing.

creating an epithelial layer that covers the top of the wound.<sup>19</sup> The wound then enters the remodeling phase of healing.<sup>19</sup>

The cocktail of growth factors present in the wound stimulates the endothelial cells of vessels in the nearby healthy tissue to release proteases such as matrix metalloproteinases (MMPs).<sup>29</sup> Keratinocytes, fibroblasts, and macrophages also release MMPs.<sup>30</sup> MMPs digest the basement membrane, allowing endothelial cells to escape the confines of their parent vessel.<sup>20</sup> Growth factors such as VEGF, EGF, and TGF- $\beta$  stimulate the systematic rearrangement of endothelial cells from neighboring blood vessels.<sup>4</sup> The cells elongate and align to form a capillary sprout,<sup>29</sup> extending away from the original vessel (see Figure 2).<sup>31</sup> This signals the start of angiogenesis, a critical process in the healing of a wound.<sup>25</sup> Angiogenesis, the formation of new blood vessels from preexisting ones, is oxygen dependent in that oxygen is needed for collagen deposition which, in turn, provides the scaffold on which blood vessels can form.<sup>25</sup> Hypoxia-inducible factor (HIF) is known to up-regulate the production of VEGF, which stimulates endothelial cell proliferation and thus aids angiogenesis during the proliferation phase.<sup>32</sup>

Vasculogenesis, the formation of blood vessels from the differentiation of progenitor cells, also plays a part in

the formation of new blood vessels during this stage of wound healing.<sup>33</sup> Progenitor cells differentiate into early endothelial progenitor cells in the bone marrow and further differentiate into late endothelial progenitor cells in the vasculature system before arriving at the site of vessel formation.<sup>20</sup> Some authors have argued that a significant contribution to new blood vessel growth is made by the process of vasculogenesis (see e.g.,<sup>34</sup>).

Sprout extension is facilitated by endothelial cell proliferation and further migration toward the chemical attractant.<sup>4</sup> The joining of two capillary sprouts within a healing wound forms a loop through which blood can flow and new sprouts develop from this vessel thus propagating angiogenesis.<sup>28</sup> As described above, wound healing is observed to consist of the migration of a structural “wound healing unit” of macrophages, fibroblasts, ECM, and capillary sprouts through the wound site.<sup>35</sup>

The extension of a blood vessel into a wound allows oxygen and other critical nutrients to be transported further into the injured region.<sup>19</sup> This, in turn, allows the macrophage and fibroblast fronts to migrate further into the wound, and thus the healing unit progresses into the injured tissue.<sup>28</sup> When the unit has completely swept through the wound site, blood vessels are networked over the entire space, oxygen levels are returned to normal and the healing process stops.<sup>4</sup>

### Remodeling

The remodeling phase can last for several months or even years.<sup>19</sup> During the remodeling stage, the absence of hypoxia results in a marked decline in the vascular density and increased cellular apoptosis.<sup>36</sup> Also during this phase, the collagen matrix is remodeled, complete wound contraction occurs, and wound strength is increased from about 20% normal tensile strength 3 weeks post injury to 80% within a timeframe of 2 years.<sup>37</sup>

### The importance of oxygen in wound healing

As already discussed, oxygen is a critical nutrient required for successful healing of a wound and this notion is well documented in the literature.<sup>24,28,35,38–48</sup> Oxygen plays an important role in each stage of the wound healing process. According to Tompach et al.,<sup>35</sup> in the inflammatory stage, oxygen controls the migration and proliferation of fibroblasts. Bacteria are kept under control in acute wounds by intracellular oxidative mechanisms of leukocytes and molecular oxygen must be present for the production of the required oxidants.<sup>49</sup> For successful healing to occur, infection must be prevented and resistance to infection depends on oxygen content within the wound as neutrophils become ineffective under hypoxic conditions.<sup>50</sup>

In the proliferative phase, angiogenesis requires oxygen.<sup>25</sup> In the remodeling stage, the production of collagen by fibroblasts is well-documented as being dependent on oxygen.<sup>21</sup> There are several steps in collagen synthesis that require oxygen. For example, the enzymes responsible for converting proline to hydroxyproline, allowing procollagen peptides to achieve their triple helix form, require molecular oxygen.<sup>25</sup>

Conversely, it is widely thought that the absence of oxygen from the central space of the wound initially

stimulates the healing process.<sup>13,25,35,46,51</sup> This wound hypoxia (a deficiency of oxygen) must be corrected in order to provide sufficient oxygen to support tissue repair.<sup>25</sup> It is thought that correction of the oxygen level throughout the entire wound may arrest the healing process due to the failure of macrophages to produce growth factors such as VEGF.<sup>38,52</sup> Lack of oxygen within a wound stimulates the healing mechanism but will frustrate the overall process if hypoxia persists.<sup>53</sup> Furthermore, the macrophage production of the chemical stimulus, VEGF, has been shown to be up-regulated by both hypoxic and hyperoxic conditions.<sup>19,54</sup> It is clear that a delicate relationship exists between the healing of a wound and the oxygen level within the wound site.<sup>13</sup>

### Other important factors in wound healing

There are many other intrinsic factors, besides molecular oxygen, which are thought to affect the healing of wounds including oxidants species, lactate, and HIF. During the phagocytosis of debris in the inflammation stage of wound healing, phagocytic cells such as macrophages and neutrophils consume increased amounts of oxygen.<sup>55</sup> This is commonly referred to as the “oxidative burst” and the consumption leads to the conversion of oxygen to oxidation species such as superoxide and hydrogen peroxide.<sup>56</sup> These oxidants may assist wound healing by up regulating fibroblast migration and proliferation.<sup>57</sup> Lactate is produced by fibroblasts and is a by-product of the oxidative burst. VEGF and collagen production are elicited by lactate.<sup>58</sup> Interestingly, lactate levels are similar in both low and high levels of oxygen.<sup>59</sup> In fact, Trabold et al. propose that “lactate induces a biochemical perception of hypoxia and instigates several signals that activate growth factor signals while the continued presence of oxygen allows endothelial cells and fibroblasts to reproduce and deposit collagen.”<sup>55</sup> Finally, expression of HIF by endothelial cells is simulated during times of hypoxia. This HIF then binds to DNA that regulates the expression of VEGF.<sup>4</sup>

Extrinsic factors that affect wound healing are numerous and include medication, stress, depression, nutrition, and age. Many medications are known to affect wound repair. For example, steroids are thought to down-regulate growth factors and collagen deposition,<sup>60</sup> aspirin suppresses inflammation, and chemotherapeutic drugs arrest cell replication.<sup>61</sup> Stress has been shown to delay the healing of punch biopsies and may be caused by changes in the secretion of proinflammatory cytokines during times of stress.<sup>62</sup> Similarly, depression has been linked to a decrease in cellular immunity and may delay wound repair.<sup>63</sup> Wounding can alter nutritional requirements and it is therefore crucial that patients at risk of malnutrition change their diet accordingly as failure to do so can adversely affect the healing process.<sup>64</sup> The wound healing process is often delayed in aged individuals due to a decrease in cell proliferation, collagen synthesis, and angiogenesis.<sup>65</sup> As Smith et al.<sup>66</sup> concluded, it is likely that supplementing oxygen to wounds that fail to heal due to reasons besides hypoxia, such as those mentioned above, will have little benefit.

### Mathematical models of wound healing

Mathematical modeling can provide independent insight into a biological process and has the potential to generate theoretical predictions that could not have been anticipated in advance, thereby stimulating further biomedical research and reducing the need for time-consuming, technically difficult, and often costly experiments.<sup>23</sup> As such, many would argue that mathematical models must form a crucial part of research into any biological process, including the healing of wounds.<sup>67</sup> Mathematical modeling gives the wound healing researcher an insight into the mechanisms and relative importance of processes, information that is also useful in the treatment methodologies to be implemented by medical staff.<sup>68</sup> The development of mathematical models that describe the numerous components of the wound healing process, together with their synergistic or antagonistic interactions, will provide the means to identify which elements of the wound healing process may be manipulated in a rational, mechanism-based strategy for improved clinical management of wound repair.

## CHRONIC WOUNDS

A chronic wound is one in which healing fails to proceed through an orderly and timely process to produce anatomic and functional integrity, or proceed through the repair process without establishing a sustained anatomic and functional result.<sup>69</sup> These wounds are often a surface manifestation of an underlying disease such as venous insufficiency, arterial disease, or diabetes.<sup>21</sup> Treatment tends to focus on treating the surface ulcer and commonly ends in nothing more than added expense without successful healing.<sup>70</sup>

### The chronic wound healing process

The factors involved in the development of a chronic wound remain unclear;<sup>6,71</sup> however, the most common cause, according to Mathieu et al.<sup>21</sup> is thought to be related to the detrimental effects of prolonged wound hypoxia (oxygen deficiency). It has been suggested that while oxidant species, produced by neutrophils and macrophages within the wound, may serve as messengers to promote healing, overproduction may in fact overwhelm the immune system and delay healing.<sup>56</sup> Chronic wounds can be arrested in any one of the stages of wound healing,<sup>4</sup> but disruption commonly occurs in the inflammatory or proliferative phases.<sup>17</sup> Braiman-Wiksman argue that within the proliferative phase, reepithelialization is crucial for successful healing.<sup>72</sup>

There is growing consensus that a chronic wound remains “stuck” in the inflammatory stage of healing with an overabundant production of neutrophils.<sup>73,74</sup> Diegelmann et al.<sup>4</sup> state that this excessive production can have a detrimental effect on healing by destroying growth factors (and thus reducing the chemotactic and proliferative stimulus that these chemicals provide) through neutrophil production of proteolytic enzymes and degrading ECM components.<sup>73</sup> In chronic wounds, insufficient ECM is deposited (which is likely due to fibroblast production and remodeling of collagen being dependent on a sufficient

supply of oxygen), leading to a weakened tissue that can easily rupture.<sup>4</sup> Others have reported high levels of MMPs, which can lead to excessive ECM degradation.<sup>8,75</sup> Furthermore, blocked or defective cell proliferation may prevent healing.<sup>20,76</sup>

Numerous mathematical models of chronic wound healing have been developed. For example, Pettet et al.<sup>77</sup> were able to simulate wound chronicity by assuming that capillary budding is down-regulated in chronic wounds. Byrne et al.<sup>28</sup> simulated a nonhealing wound by assuming that endothelial cell proliferation and chemoattractant production rates are reduced in chronic wounds.

### Treatment of chronic wounds

Chronic wounds are resistant to treatment and healing progresses very slowly or not at all.<sup>13</sup> Traditional approaches to treating leg ulcers have involved debridement, pressure management, antibiotic treatment, compression therapy, and the use of specialized wound dressings.<sup>78</sup> Some more recent approaches to treating foot ulcers include the application of cultured skin substitutes (Apligraf and Dermagraft), electrical stimulation, HBOT, and vacuum assisted closure.<sup>51,79–82</sup> The use of HBOT to treat certain chronic wounds will be discussed in the following section.

### HBOT

HBOT is the intermittent exposure of the body to 100% oxygen at a pressure > 1 atmosphere absolute (ATA).<sup>44</sup> HBOT has been demonstrated to reduce the rate of major lower limb amputations among diabetics.<sup>51,83–86</sup> The analysis by Fife et al.<sup>87</sup> showed that the economic and emotional cost of amputation undoubtedly exceeds the cost of successful treatment with HBOT. HBOT is administered either in a multiplace chamber where the air within the chamber is pressurized and each patient receives 100% oxygen through a gas mask or a monoplace chamber where the sole patient is entirely surrounded by pressurized oxygen.<sup>44</sup>

HBOT can only be delivered to a patient systemically.<sup>39</sup> Confusion sometimes exists with the use of the term, “topical HBO” used to describe the application of 100% oxygen to the surface of the wound at 1 ATA. This is typically done by injecting oxygen into a plastic bag encasing the lower limb. As it is physiologically impossible to apply pressure to specific body parts such as the lower leg, at pressures much above ambient without creating a tourniquet effect, the use of this modality should more accurately be referred to as “topical oxygen therapy” or TOT for short. Gordillo et al.<sup>25</sup> argue that this therapy is unlikely to result in deep perfusion of oxygen through the skin and wound surface, making the topical approach less than optimal.

Other research has shown a very limited depth of penetration for topical oxygen perhaps as shallow as 50–100  $\mu\text{m}$ .<sup>88</sup> Advocates of this technique for wound healing point out certain advantages, such as lower costs, greater portability, and reduced risks of oxygen toxicity when compared with conventional HBOT<sup>56</sup> and, although the technique seems to hold theoretical promise, the only randomized-controlled trial done to date showed no

difference in healing rates between topical oxygen therapy and conventional treatment.<sup>89</sup>

There are currently 13 indications for which the Undersea and Hyperbaric Medical Society (UHMS) recommend treatment with HBOT.<sup>90</sup> Wound related indications include delayed radiation injury (osteoradionecrosis), necrotizing soft tissue infections, enhancement of healing in selected problem wounds, thermal burns, compromised skin grafts and flaps, crush injury, clostridial myositis, myonecrosis (gas gangrene), and refractory osteomyelitis. Nonwound related indications include decompression sickness, air/gas embolism, carbon monoxide poisoning, exceptional blood loss anemia, and intracranial abscess. Babul et al.<sup>41</sup> state that HBOT may also serve to improve the short and long-term prognosis of sports-related injuries; however, this is currently not a UHMS-recognized condition for treatment with HBOT as evidence is lacking.

### Side effects of HBOT

Although HBOT is generally regarded as a safe treatment, there are several potential side effects. The most common side effect is ear and sinus barotrauma, which occur in approximately 52 of every 10,000 cases.<sup>19</sup> Another side effect of HBOT is myopia, which can last several weeks after treatment has stopped.<sup>91</sup> HBOT is associated with an oxygen toxicity-induced convulsion rate of about 1 in 10,000.<sup>19</sup> It should be noted that these convulsions are not life threatening. To prevent oxygen toxicity, it is recommended that HBOT protocols involving 2.4 ATA allow for 5 minutes of air breathing in the chamber every 30 minutes.<sup>45</sup> The incidence of decompression sickness in multiplace chamber attendants is also estimated to be 1 in 10,000.<sup>19</sup>

Fires within chambers occur at a rate of about one per year worldwide.<sup>92</sup> Fire hazard is the most common fatal complication, yet hospital fires, in general, are far more common.<sup>45,92</sup> Some patients with severe congestive heart failure experience a decline in cardiac function as a result of receiving HBOT.<sup>93</sup> To safeguard against this, patients with lower than 35% cardiac ejection are typically not treated with HBOT. To minimize the chance of side effects, patients should be thoroughly screened and monitored during their treatment by trained hyperbaric physicians and nurses.

### The HBOT protocol

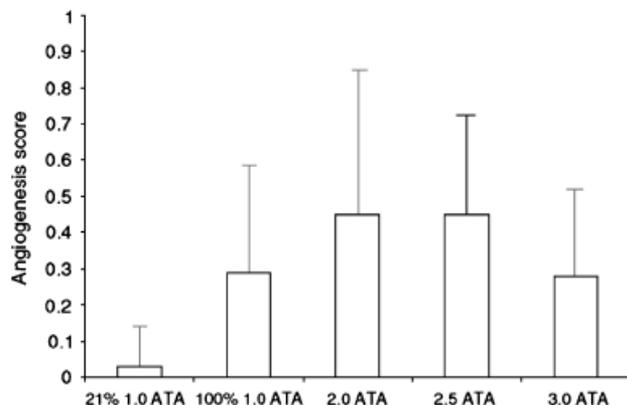
There is much debate about the optimal HBOT protocol in treating chronic wounds.<sup>35,94–96</sup> Quantity and quality of evidence varies by etiology, stage, and chronicity of the wound; evidence is best for efficacy in osteoradionecrosis and ischemic diabetic ulcers. Although HBOT is typically used as an adjunctive therapy for treating chronic wounds, many clinicians lack a full knowledge of the evidence-based data that support its use.<sup>97</sup> Mason et al.<sup>78</sup> concluded that the role of HBOT in wound care could neither be ruled in nor out based on the available information and evidence at the time. There are also several authors who conclude that there is sufficient evidence to support the use of HBOT as an adjunctive treatment for nonhealing wounds.<sup>39,51,83,98,99</sup> In 1999, the American Diabetes Association endorsed the use of HBOT in severe diabetic

wounds that fail to respond to more traditional approaches.<sup>100</sup> The Centers for Medicare and Medicaid Services stated in 2002 that there was adequate evidence to conclude that HBOT is clinically effective, reasonable and necessary in the treatment of certain (Wagner grade III or higher and recalcitrant to 30 days of appropriate wound treatment) diabetic lower limb-threatening wounds.<sup>101</sup> In 2003, the UHMS committee reported that the available evidence supports the use of adjunctive HBOT for diabetic foot ulcers.<sup>90</sup> More recently, in 2006, guidelines for the treatment of arterial insufficiency ulcers and diabetic ulcers, established by request of the Wound Healing Society, supported the use of adjunct HBOT for ischemic, diabetic wounds, and recommended further investigation for ischemic, nondiabetic ulcers.<sup>102,9</sup> It should be noted that the evidence supporting the use of HBOT for chronic wounds is limited mainly to diabetic wounds.<sup>51,71,84,85,91,92,100,103</sup>

Whether HBOT is effective or not, there is the question as to whether or not HBOT is cost effective. In their reports to the Australian Federal Government, the Medical Services Advisory Committee (MSAC) stated that although there is evidence of HBOT's effectiveness in treating diabetic wounds, there is inadequate proof to show that the use of HBOT is cost-effective.<sup>86</sup> Leach et al.<sup>45</sup> state that more work is needed to establish timing, indications and regimens to achieve the best clinical and cost-effective results.

Even among those that advocate the use of HBOT in wound healing, the HBOT protocol varies significantly. There is still much uncertainty surrounding the choice of a regimen that will obtain the optimal therapeutic affect of pressure, duration of treatment, and number of repeat sessions of HBOT for the healing of chronic wounds.<sup>35,40,104,105</sup> Moreover, there is insufficient evidence to ascertain at which stage of the healing process to start the treatment.<sup>99</sup>

Some authors think that the stimulation of angiogenesis is one of the most important features of HBOT in the treatment of chronic wounds. Figure 3 shows the calculated values of the mean angiogenesis score by Hopf et al.<sup>53</sup> with changes in the percentage oxygen inspired and the atmospheric pressure applied to wounded mice. The angiogenesis grading was stepwise with 0 indicating no vessels,



**Figure 3.** Bar graph of the mean angiogenesis score at various pressure and oxygen levels from Hopf et al.<sup>53</sup> Reprinted with permission from Blackwell Publishing.

0.5 scattered vessels, and 1 maximal vessels. The mean angiogenesis score is then calculated by averaging over the entire sample. We see that the mean angiogenesis score increases as the oxygen level changes from that in room air to pure oxygen. The score increases with increasing pressure until 2.5 ATA, when it begins to decline. Oxygen was delivered to the mice in an enclosed chamber for 90 minutes twice a day for 7 days. Hopf et al.<sup>53</sup> concluded that angiogenesis was significantly greater in all hyperoxic groups compared with room air-exposed controls.

Mathematical models allow us to investigate the optimal HBOT protocol. By simulating the healing of a chronic wound under treatment by various durations and frequencies of HBOT sessions, one may be able to determine, from a theoretical viewpoint, the optimal procedure for administering HBOT. This can be achieved without costly laboratory experiments or clinical trials.

### The rationale behind HBOT

The primary rationale behind the use of HBOT in the treatment of chronic wounds is to correct the amount of oxygen delivered to the wound site.<sup>105</sup> This is achieved by the HBOT-induced increase in the blood-oxygen level within the injury and the reallocation of blood flow to hypoxic areas due to hyperoxic vasoconstriction in the surrounding normal tissue.<sup>21</sup>

There are two methods by which oxygen reaches the wounded tissue. The first is attachment to hemoglobin molecules and the second as dissolved oxygen in the plasma. According to Kessler et al.,<sup>51</sup> HBOT cannot significantly increase the amount of oxygen bound to hemoglobin molecules but can increase the amount of oxygen dissolved in the plasma. A person breathing room air already can have at any one time up to 97% of their hemoglobin molecules bound to oxygen so that increases in the oxygen level and pressure will not significantly increase the oxygen content delivered via hemoglobin.<sup>19</sup> Furthermore, even if HBOT did significantly increase the amount of oxyhemoglobin present in the vasculature, the lack of blood vessels in the wound would prevent oxygen reaching the innermost parts of the injury, where hypoxia is greatest and the oxygen is needed the most.<sup>48</sup> On the other hand, even poorly perfused wounds can receive oxygen via hyperoxygenated plasma.<sup>19</sup>

Table 2 shows theoretical arterial oxygen tensions and the amount of oxygen dissolved in the blood for various HBOT protocols. These figures are calculated using ideal oxygen pressure laws (see e.g.,<sup>19,88</sup>) and an oxygen

**Table 2.** Theoretical arterial oxygen tension and oxygen blood content for different treatment protocols

Oxygen % level	Arterial oxygen tension (mmHg)	Milliliters of oxygen in plasma per dL of blood
21	100	0.31
100	660	2.0
100	1400	4.3
100	2200	6.8

ATA, atmosphere absolute.

solubility in blood of 0.0031 mL/dL of blood per mmHg of arterial oxygen tension. A healthy adult has been reported to require about 6 mL of oxygen/dL of blood and hence the data in Table 2 suggest that HBOT can provide sufficient oxygen in the plasma to support the body's requirements.

The positive effects of HBOT stem from the benefit of increasing the tissue oxygen tension and/or pressure within the wound site and include<sup>19</sup>:

1. Alteration of ischemic effect.
2. Reduction of edema.
3. Modulation of the production of nitric oxide.
4. Modification of the effect of growth factors and cytokines.
5. Promotion of cellular proliferation.
6. Acceleration of collagen deposition.
7. Stimulation of capillary budding.
8. Accelerated microbial oxidative killing.
9. Interference with bacterial proliferation.
10. Modulation of the immune system response.
11. Enhancement of oxygen radical scavengers, thereby reducing ischemia reperfusion injury.

Therapeutic effects such as increased collagen synthesis, improved bacterial killing, and antibiotic potentiation have been reported to occur during the periods of elevated oxygen tension, while other effects including suppression of bacterial propagation, down-regulation of inflammatory signals, and prevention of leukocyte activation and adhesion following ischemic reperfusion may continue even once the treatment has ceased.<sup>90</sup>

## DISCUSSION

It is clear that more reliable clinical data are needed in order for HBOT to be recognized as an appropriate adjunct treatment for certain nonhealing wounds. In order to fully understand the chronic wound and, more importantly, to identify exactly what prevents these wounds from healing, time-consuming, technically difficult and costly experiments, such as in vitro and animal studies, need to be performed. Alternatively, a validated mathematical model may provide independent insight into the wound healing process and has the potential to generate theoretical predictions which could not have been anticipated in advance.<sup>23</sup>

This paper has reviewed the chronic wound process, the possible reasons as to why chronic wounds heal slowly and the use of adjunct HBOT to accelerate the healing rate of chronic wounds. As theoreticians, experimentalists, and clinicians work together, perhaps we can establish the role for HBOT in the treatment of chronic wounds.

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