The Effects of an Oxygen-Generating Dressing on Tissue Infection and Wound Healing

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ABSTRACT

Oxygen is a necessary component of normal wound healing and is required for multiple cell functions, including the killing of bacteria by leukocytes. A new oxygen-generating dressing has been developed that provides intermittent periods of hyperoxia interspersed with periods in which the wound oxygen tension is allowed to autoregulate.

Materials and Methods: The effects of an oxygen-generating dressing on healing of infected wounds were examined in 2 experiments using a rodent model of a chronically infected ($>10^5$ bacteria/g of tissue) granulating wound. Serial wound area measurements were compared among animals treated with the oxygen-generating dressing, the growth factor KGF-2, a vehicle control, a negative dressing control, and a positive dressing control. Serial biopsies of the wound for quantitative bacteriology were performed throughout both experiments.

Results In both experiments, infected wounds healed fastest when topically treated with an oxygen-generating dressing ($P< 0.05$). Although KGF-2 treated wounds healed faster than the vehicle control wounds in experiment 1, the oxygen-generating dressing was statistically better than KGF-2 ($P<0.05$). The oxygen-generating dressing decreased the bacterial burden in the granulating wounds 100-fold greater than did all other treatments.

Conclusions: The oxygen-generating dressing used in these experiments decreased the number of tissue bacteria in these infected wounds to less than 10^5 colony forming units (CFU) per gram of tissue. This, plus the stimulatory effect of oxygen on numer-

ous healing processes, facilitated more rapid healing of the infected granulating wounds.

INTRODUCTION

Oxygen is a necessary component of normal wound healing and is required for multiple cell functions, including the killing of bacteria by leukocytes.[1] Both hypoxia and infection adversely affect wound healing.[2] Factors that increase oxygen delivery to the wound can speed healing.[3] Low oxygen tensions in a wound can impair neutrophil, macrophage, and fibroblast functions.[2] This can result in abnormalities in each of the phases of wound healing.

Both oxygen-dependent and oxygen-independent systems are used by neutrophils and macrophages to kill bacteria.[2] Oxygen radicals derived from molecular oxygen are generated during the inflammatory phase of repair and effectively kill microbes. If cells are hypoxic, the oxygen-dependent pathway is severely incapacitated, leading to increased rates of infection.[4]

Collagen synthesis by fibroblasts also requires oxygen. Oxygen is an important co-factor required during the hydroxylation of proline and lysine during formation of procollagen.[2] Mature collagen synthesis requires prolyl-hydroxylase and lysyl-hydroxylase, both enzymes dependent on oxygen for function. If wounds are hypoxic, procollagen hydroxylation suffers, and mature collagen cannot be formed.[5] Hypoxia can cause acidosis in wounds with an accumulation of lactate. Although a relative hypoxia creating a lactate gradient can be stimulatory for some processes of wound repair such as angiogenesis, collagen secretion, and growth factor release, profound hypoxia inhibits all wound healing processes.[2,6] Higher rates of epithelial proliferation are observed under hyperoxic as opposed to hypoxic conditions.[7]

It would be useful to have a mechanism to increase wound oxygenation, when the patient's own delivery system has been maximized, yet is still deficient. In this setting, the technique of hyperbaric oxygenation has been successfully used to augment tissue oxygenation.[8] There have been clear benefits with this therapy in conditions in which the oxygen delivery to the wound is known to be compromised, such as osteoradionecrosis and in synergistic soft tissue infections.[9] In such conditions, the delivered oxygen is effective as a metabolic boost to the wound healing processes and as an antimicrobial, allowing leukocytes to kill ingested bacteria by increasing production of toxic oxygen free radicals during the respiratory burst.[10] The advantages of hyperbaric oxygenation are tempered by its local and systemic side effects due to prolonged hyperoxia. In addition, the hyperbaric chambers are costly and cumbersome. Due to the expense of and attendant risk engendered by systemic oxygen toxicity in large whole-body hyperbaric oxygen chambers, topical hyperbaric oxygen chambers have been developed. It is difficult to achieve more than a modest elevation in pressure using such devices, though some clinical benefit has been reported.[11,12]

Given this background, it would appear desirable to create a device that is occlusive, in order to allow moist wound healing, and does not have to be removed frequently. Ultimately, this device would produce wound oxygen levels similar to that produced by moderate hyperbaric treatment without the necessity of maintaining an external supply of pressurized oxygen. A wound dressing with these characteristics has been developed (Oxygen Generating Wound Dressing).[13] This dressing consists of a hydrophilic colloid occlusive alginate (Kaltostat,® Convatec, Skillman N J), and incorporates an inorganic catalyst (manganese dioxide powder 60 to 80 $\mu g/cm^2$). When a moist substrate (0.3% H_2O_2) is intermittently added to the dressing, the manganese dioxide decomposes the substrate to produce high levels of oxygen and water. This dressing has been shown to achieve topical oxygen partial pressures (pO_2) of 350 mmHg (approximately 5 times

atmospheric pressure) after addition of substrate, while simultaneously maintaining the moist wound healing environment between periods of substrate addition.[13] A study using oxygen tonometry (TOPS—Tissue Oxygen Probe System, Innerspace Inc., Irvine, Calif.) in a rabbit ear ulcer model indicated that tissue pO_2 in the rabbit ear perichondrium beneath the ulcer was elevated to 138 mmHg (> 2x baseline) only 8 minutes after addition of the substrate to the dressing.[13,14] This elevation of pO_2 was maintained until the substrate was consumed.[13] Multiple hyperoxia treatments can be carried out with the same dressing by adding more substrate, as the MnO_2 catalyst is not consumed during oxygen production.[13] The dressing requires changing as often as one would normally change a hydrogel alginate dressing and is covered with a gas permeable thin film such as Opsite® (Smith & Nephew, LTD., Hull, England).

The purpose of this study is to test the effect of an oxygen-generating dressing in a chronically infected granulating wound model. The presence of a tissue level of >10^5 bacteria per gram of tissue in this wound model is known to inhibit various wound-healing processes.[15,16] It is hypothesized that supplying an exogenous source of oxygen to the wound will control the bacterial burden and restore healing capacity to the injured tissues, as well as enhance oxygen-dependent metabolic processes.

## MATERIALS AND METHODS
Animal Model

Chronic infected granulating wounds were prepared as previously described.[15-18] All experiments were conducted in accordance with the Animal Care and Use Committee guidelines of the Department of Veterans Affairs Medical Center, Bay Pines, Florida. Male Sprague-Dawley rats weighing 300 to 350 g were acclimatized for a week in our facility prior to use. Under intraperitoneal Nembutal anesthesia (35 mg/kg), the rat dorsum was shaved and depilated. A full-thickness dorsal burn measuring 30 cm^2 was created by immersion in boiling water. The burns were inoculated with 5 x 10^8 *Escherichia coli* (ATCC #25922, American Type Culture Collection, Rockville, Md.) after the rats had been allowed to cool for 15 minutes. Bacteria were obtained from fresh 18-hour broth cultures and inoculum size was confirmed by backplating.

Animals were individually caged and given food and water ad libitum. Five days after burning, the eschar was excised from anesthetized animals, resulting in a chronic granulating wound. Histological characterization of the wound has previously been shown to be comparable to a human chronic granulating wound.[19]

Any dried exudate was atraumatically removed from all wounds prior to application of test treatments, prior to wound biopsies, or prior to wound measurements. Wounds were biopsied for quantitative bacteriology at initial eschar removal and 3, 6, and 9 days after escharectomy to exclude superinfection and to confirm bacterial levels in the infected wounds.[20] Biopsies were obtained after cleaning the wound surface with 70% isopropyl alcohol to exclude surface contamination. The biopsies were weighed, flamed, and homogenized after being diluted 1:10 weight to volume with thioglycollate. Serial tube dilutions were prepared and then backplated onto selective media. Bacterial counts were completed after 48 hours incubation (37°C) and expressed as colony forming units (CFU) per gram of tissue.[20]

Every 48 hours the outlines of the wounds were traced onto acetate sheets, and area calculations were performed by using computerized digital planimetry (Sigma Scan, Jandel Scientific, Corte Madeira, Calif.). All animals were weighed on a weekly basis. The animals were sacrificed by Nembutal overdose and bilateral thoracotomies when the wound was completely healed, or healed to less than 10% of its original area. Hayward et al. demonstrated that measurement of very small wounds by manual tracing introduced significant sys-
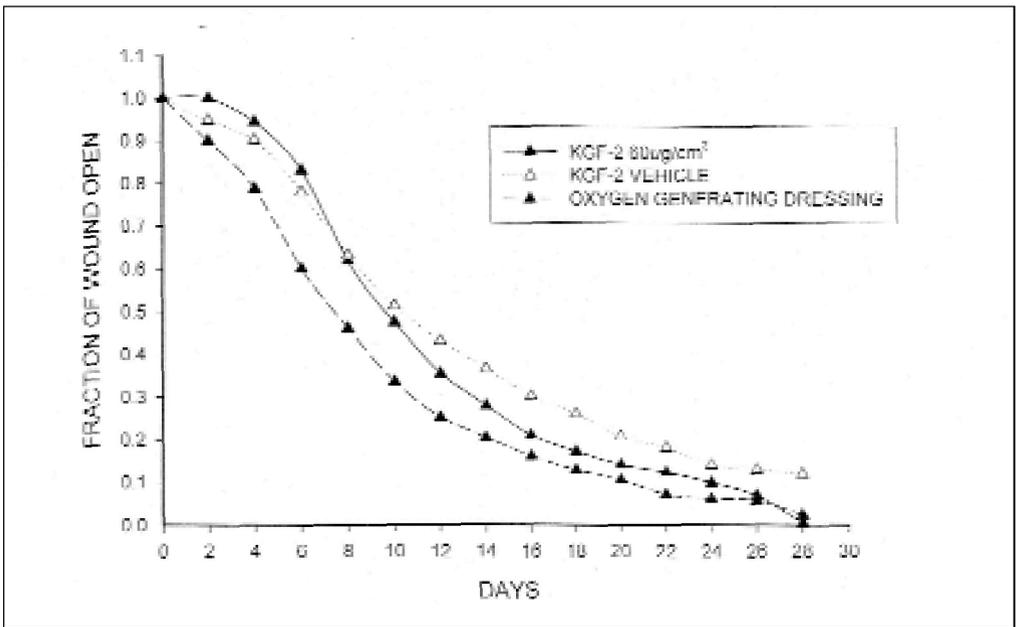
Figure 1. Oxygen-generating dressing-treated wounds healed statistically better than KGF-2 treated wounds or the vehicle control-treated wounds. Statistically significant improvement occurred at 25%, 50%, 75%, and 90% closure points (P < 0.05).

tematic error and found that wounds followed past this point remained static for prolonged periods of time.[16]

Two separate experiments were performed. In the first experiment, the oxygen-generating dressing was compared to the cytokine growth factor, Keratinocyte growth factor–2 (KGF-2), for its ability to overcome the known inhibition to wound healing due to large quantities of tissue bacteria. This direct comparison was chosen because KGF-2 was recently reported by our laboratory to accelerate wound contraction in this model when compared to vehicle controls.[21] Fifteen rats were divided into 3 equal groups. Group 1 (n = 5) animals had their infected wounds treated with KGF-2 (Human Genome Sciences, Rockville, Md.) 60 μg/cm² daily for 10 days following escharectomy. Group 2 (n = 5) animals were treated with the same topical vehicle as that used for the KGF-2 dilutions (infected controls) daily for 10 days. Group 3 (n = 5) animals had their wounds dressed with the oxygen-generating dressing covered by Op-site® daily for 10 days. These

animals had 0.3% H_2O_2 injected through the Op-site® at the time of dressing changes and 12 hours later.

The second experiment also consisted of 3 groups of 5 animals each. Group 4 (n = 5) animals were treated with a plain hydrophilic colloid alginate dressing, covered by Op-site® and changed daily for 10 days beginning at the time of escharectomy. This group was labeled as a negative dressing control. Group 5 (n = 5) animals were treated with a hydrophilic colloid alginate dressing containing manganese dioxide (60 to 80 μg/cm²) co-factor and covered by Op-site®. As with Group 4, the dressings were changed daily for 10 days beginning on the day of escharectomy. This dressing served as a positive dressing control. Group 6 (n = 5) was treated with the oxygen-generating dressing moistened with the 0.3% H_2O_2 substrate every 12 hours and was identical to Group 3 in the first experiment.

Statistical Analysis

Serial area measurements of the rat infected
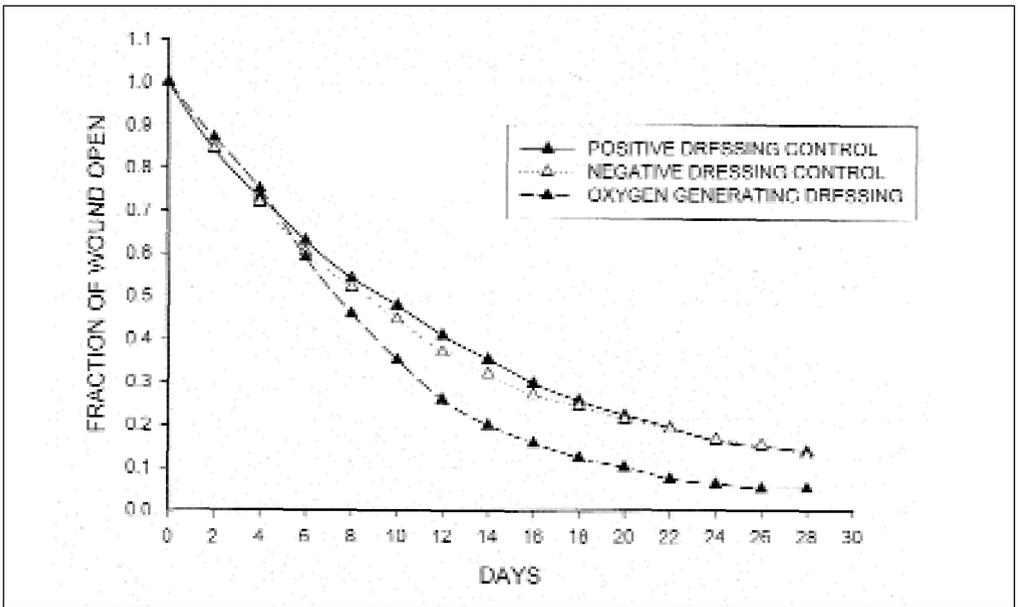
Figure 2. Oxygen-generating dressing-treated wounds showed a statistically shorter time to reach 75% and 90% wound closure when compared with both the negative dressing control and the positive dressing control (P < 0.05).

granulating wounds were plotted against time. For each animal's data a Gompertz equation was fitted (typical r2 = 0.85).[15,22] Using this curve, the wound half-life was estimated as well as points of 25%, 75%, and 90% wound closure. Comparisons between groups were performed using life table analysis and Wilcoxon rank test.[22] These statistical analyses were performed by using the SAS (SAS/STAT Guide for Personal Computers, Version 6 Edition, Cary, North Carolina, 1987, p. 1028) and BMDP (BMDP Statistical Software Manual, Los Angeles, BMDP Statistical Software, Inc. 1998) packages on a personal computer.

RESULTS

In experiment 1, both KGF-2 and the oxygen-generating dressings improved the rate of healing by contraction of the infected wound compared to the vehicle control (Figure 1). However, KGF-2 did not reach statistical significance versus the vehicle control until 75% wound closure ($P = 0.033$). This difference persisted at 90% closure ($P = 0.006$). The oxygen-generating

dressing was statistically better at improving wound closure. At 25% closure the dressing statistically improved healing compared to the vehicle control ($P = 0.016$) and to KGF-2 treatment ($P = 0.003$). At 50% closure the oxygen-generating dressing was again better than the vehicle control ($P = <0.001$) and KGF-2 ($P = 0.005$). This significant improvement remained at 75% wound closure (vehicle $P = 0.004$, KGF-2 $P = 0.003$) and at 90% wound closure (vehicle $P = <0.001$, KGF-2 $P = 0.027$).

In the second experiment, the oxygen-generating dressing was statistically more effective at facilitating wound healing than the hydrophilic colloid alginate negative dressing control or the identical dressing containing manganese dioxide (positive dressing control) (Figure 2). This statistical difference became apparent at 75% wound closure for the oxygen-generating dressing versus the negative dressing control ($P = 0.019$) and versus the positive dressing control ($P =<0.001$). At 90% closure, the difference remained with the oxygen-generating dressing versus the negative control ($P=0.005$) and

Table 1. Quantitative bacteriology results for wound biopsies collected on the day of eschar removal (day 0) and on day 3, 6, and 9 after eschar removal.*

Group	Escharectomy Day 0	Day 3	Day 6	Day 9
1-Exp 1 (KGF-2 60 ug/cm^2)	8.2 x 10^7	5.0 x 10^7	9.9 x 10^6	2.9 x 10^5
2-Exp 1 (Infected vehicle control)	8.0 x 10^7	6.0 x 10^7	2.0 x 10^7	5.6 x 10^6
3-Exp 1 (O$_2$ generating dressing)	8.0 x 10^7	6.0 x 10^7	5.0 x 10^6	6.3 x 10^3
4-Exp 2 (Negative dressing control)	5.2 x 10^7	2.5 x 10^7	6.0 x 10^6	6.0 x 10^5
5-Exp 2 (Positive dressing control)	3.1 x 10^7	6.0 x 10^6	8.0 x 10^6	6.0 x 10^5
6-Exp 2 (O$_2$ generating dressing)	4.8 x 10^7	5.0 x 10^6	5.0 x 10^5	8.0 x 10^3

* Results are mean counts for treatment groups expressed in colony forming units per gram of tissue.

vs. positive dressing control ($P<0.001$).

Biopsies of the wounds for quantitative bacteriology demonstrated that all animals had 10^7 or greater *E coli* CFU/g of tissue on the day of escharectomy (granulating wound day 0). This remained the case for the infected control animals, treated with vehicle only, for the 9 days following escharectomy when bacterial counts were monitored (Table 1). Only the oxygen-generating dressing decreased the bacterial burden in the granulating wounds to < 10^5 CFU /g of tissue. In the first experiment the mean bacterial count 9 days after escharectomy was 6.3 x 10^3 CFU/g of tissue in the Group 3 oxygen-generating dressing animals. In the second experiment the mean bacterial count in the Group 6 oxygen-generating dressing treated animals was a similar 8.0 x 10^3 CFU/g of tissue.

There was an equivalent gain in body weight among all groups during the various periods of study in both experiments with no statistically significant variation among the groups.

DISCUSSION

Bacteria have been demonstrated to affect all of the various processes of the wound-healing scheme.[23] The effects are like the yin-yang effects of certain pharmacological agents. Small amounts of bacteria seem to stimulate or accelerate the repair processes; whereas, bacterial burdens exceeding 10^5 CFU/g of tissue inhibit or delay the processes.[23,24] In the infected granulating wound model used in this study, a tissue level of bacteria significantly delays the healing of the wound by contraction and epithelialization.[15-18] Cytokine growth factors can overcome the inhibition to healing in this model but require very large doses because of the bacterial degradation of the growth factors.[25] Of the various factors tested, only GM-CSF appears to lower the bacterial burden and accelerate wound closure.[17,21]

Oxygen is a critical element in the healing of wounds.[1] Cellular proliferation during angiogenesis, fibroplasia, and epithelialization proceeds at a more rapid pace in response to higher oxygen levels.[2,26,27]

Bacterial killing by phagocytic cells is also an oxygen-dependent process.[10] In both of the experiments conducted, the oxygen-generating dressing significantly facilitated closure of the infected wounds. Since the growth factor KGF-2 and the oxygen-generating dressing both accelerated healing compared to the vehicle control, one wonders if a combination growth factor and oxygen-generating dressing might be more effective than either one alone. Recent animal data involving both acute and chronic wounds treated with hyperbaric oxygen and growth factors indicate that the combination of the two modalities is synergistically beneficial to healing.[28,29]

The superior effect on wound closure by the oxygen-generating dressing in the first experiment might have been attributed

to the fact that the KGF-2 treated animals (Group 1) and the vehicle-treated animals (Group 2) had their wounds left exposed while the Group 3 animals were dressed with the hydrophilic colloid alginate dressing, which retained moisture in the wounds. Certainly, moisture balance is an important consideration for healing.[30,31] However, Hayward, et al. demonstrated that exposure in this model did not significantly impede healing when the wounds were treated with growth factors in a liquid medium.[15]

In the second experiment, all animals (Groups 4,5,6) were treated with an occlusive moisture-retentive dressing. There was no difference in healing between animals treated with the plain hydrophilic colloid alginate dressing (Group 4) and the identical dressing with manganese dioxide added (Group 5). Significant improvement occurred when the H_2O_2 substrate was added to generate oxygen (Figure 2). Therefore, it is clear that the superiority of Groups 3 and 6 was due to the oxygen-generating capacity of the dressing.

The oxygen and oxygen radicals generated when the manganese dioxide catalyst decomposed the H_2O_2 substrate appeared sufficient to significantly lower the bacterial burden in the wounds (Table 1). Only in wounds treated with the oxygen-generating dressing (Groups 3 and 6) did the bacterial level fall to less than 10^5 CFU/g of tissue. Because the 0.3% H_2O_2 substrate was added intermittently, hyperoxia was produced on an intermittent basis. This was effective enough to lower the tissue bacterial level. These treatment periods were interspersed with "oxygen quiescent" periods when the oxygen tension of the wound approached normal or even hypoxic levels.[13]

A second positive control dressing using intermittent 0.3% H_2O_2 in a moist hydrocolloid alginate dressing could have been tested. Hydrogen peroxide has been shown to have an antimicrobial effect.[32] However, when H_2O_2 alone has been tested in ischemic guinea pig ulcers, it did not yield a faster rate of healing.[33] In the present experiments, H_2O_2 was used at one-tenth the usual concentration, and relied on only as a catalyst to create maximum oxygen generation from a minimal amount of substrate.

Dangers of systemic oxygen toxicity or wound complications seen with systemic hyperbaric oxygen are eliminated with the use of the intermittently hyperoxic, oxygen-generating dressings. Multiple daily treatment regimens increasing the application of the H_2O_2 substrate can be used if more wound hyperoxia is desired. Bacterial killing, collagen synthesis, and epithelialization can be improved with the hyperoxia. During the periods without substrate decomposition, the oxygen levels will be normal or hypoxic, which can contribute to oxygen and/or lactate gradients. The wound-healing processes such as angiogenesis and fibroblast migration that are stimulated by relative wound hypoxia can be facilitated during these periods. From the data presented, it is concluded that the oxygen-generating dressing is effective in facilitating the healing of chronic infected granulating wounds, and that its efficacy is due at least in part to the reduction in the wound bacterial burden.

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