Battling the Biofilm: Topical Oxygen Shifts the Bacterial Biofilm in Chronic Wounds

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**BACKGROUND**

Biofilms in wounds prevent healing by both acting as a physical barrier to wound closure, and by hyper-activating local inflammatory processes. Eradicating wound biofilms is therefore a therapeutic priority. However, bacteria living as a biofilm are phylogenetically diverse, semi-dormant and matrix-embedded, and are therefore highly resistant to antibiotic therapy. To underscore the problem, clinical diagnostic microbiology uses traditional culture-based systems that miss up to 99% of bacteria present in a wound biofilm. Given the empirical nature of treatment and the rise of antibiotic resistance, new therapeutic strategies are needed to combat biofilm infections.

**OBJECTIVE & STUDY DESIGN**

Using culture-independent whole-metagenome DNA sequencing captures a more complete picture of what bacteria are present in a wound, and how they are changing over time. It has been previously reported that dynamic communities are associated with healing, and temporal stability is associated with delayed healing.

**Primary objective:** To better understand the mechanism of action of the Natrox™ Topical Oxygen device in patients with non-healing diabetic foot/leg ulceration in conjunction with standard best practice.

**Inclusion Criteria**

1. Diabetic foot ulcer, which has had optimum multi-disciplinary team management for >4 week but <52 weeks.
2. No Planned treatment for arterial disease.
3. No planned surgical intervention
4. Patient aged >18 years.
5. Patients who understand the study, agree to adhere to the treatment and are able to give consent.
6. Patients who can be followed by the same investigating team for the whole period of their participation in the study.

**Exclusion Criteria**

1. Presence of invasive infection requiring intravenous antibiotics.
2. Pure neuropathic ulcer with no arterial insufficiency unless they fail to heal within 12 weeks of optimum management.
3. Significant reduced immunity or high dose corticosteroid (10mg Prednisolone) or other second line immune suppressant.
4. Need for total contact cast.
5. Patients who present with more than 10% of the ulcer surface covered in hard eschar.
6. Patients with ulcer surface area of more than 10x10cm.

**RESULTS**

**Figure 2:** Wound healing results from two patients treated with topical oxygen. Five of the six patients had results similar to DWA006, such that their wounds healed over the course of 8 weeks of topical oxygen therapy. Interestingly, one patient out of our cohort did not respond to topical oxygen, and did not heal despite regular acetic acid washes for clinically suspected Pseudomonas.

Results of microbiome analysis for these two patients are presented in Figure 3.

**Figure 3:** Bacterial genera present in a wound that did not heal (A) and a wound that healed (B) over 8 weeks of topical oxygen therapy. Genera are expressed as percentage of the whole microbiome. Classifying taxa according to respiration shows a non-healing wound dominated by anaerobes (C) and a healing wound that switches from anaerobic phyla to aerobic phyla upon application of topical oxygen (D). This is also the trend in pooled analysis from 5 healed wounds (E).

**Study Protocol**

6 Patients with diabetic foot ulcers for >4 weeks but <52 weeks are screened and informed consent obtained.

DFU is swabbed and photographed at baseline (Week 0) and weekly clinic visits for 8 weeks (Week 1-8). Topical oxygen is added to standard wound care.

Swabs have metagenomic DNA extracted. 16S rRNA amplicons are sequenced on the Illumina platform. Bacterial relative abundance is determined for the most abundant 1% genera and classified.

**CONCLUSION**

The wound microbiome in healing DFUs shifts towards a diverse flora dominated by aerobes when topical oxygen is added to standard wound care. In contrast, anaerobic flora persisted in a non-healing wound. These results suggest that treating the wound biofilm with topical oxygen has a positive impact on healing through diversification and enrichment of aerobes. Further studies are needed.

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