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BRIEF COMMUNICATION

Topical Oxygen Therapy Shifts Microbiome Dynamics in Chronic Diabetic Foot Ulcers

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Topical Oxygen Therapy Shifts Microbiome Dynamics in Chronic Diabetic Foot Ulcers

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ABSTRACT

Introduction. Bacterial biofilm in wounds prevents healing by acting as a physical barrier to wound closure and hyperactivating local inflammatory processes, thus making its removal a high priority. The authors previously have shown that adding topical oxygen to standard wound care increased healing of Texas Grade II and III diabetic foot ulcers (DFUs), which they hypothesized was a result of alterations of the wound microbiome/biofilm. **Objective.** This study aims to determine the mechanism of action of topical oxygen in DFUs by examining the diversity of bacterial genera present in DFUs treated with topical oxygen. **Materials and Methods.** Six patients with chronic DFUs had their wounds swabbed weekly over an 8-week period of continuous topical oxygen treatment, and microbiome diversity was assessed by metagenomic 16S rDNA sequencing using a next-generation sequencing platform. **Results.** The wound microbiome shifted toward a diverse flora dominated by aerobes and facultative anaerobes with oxygen therapy in 5 healed wounds. In contrast, anaerobic flora persisted in a single nonhealing ulcer in the present study cohort. **Conclusions.** Although the sample size was small, this study suggests topical oxygen therapy may have the ability to encourage the growth of aerobic members of the wound microbiome and be an effective alternative to antibiotics in this area.

KEY WORDS

microbiome, biofilm, diabetic foot ulcer, topical oxygen, foot ulcer

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Human skin is normally colonized by a consortia of bacteria, fungi, and viruses (together referred to as the skin microbiome), which occupies this niche to prevent colonization of the skin by more pathogenic bacteria.[1] When a break in the skin occurs, members of the skin microbiome may colonize the wound as part of a developing wound biofilm. Although the clinical diagnosis of wound biofilm remains controversial, it is now recognized that biofilms are present in 60% to 100% of chronic wounds,[2-4] where they form a physical barrier to wound closure and maintain a state of chronic inflammation that can damage surrounding tissue.[4] Biofilm removal is therefore a therapeutic priority, but treatment options are limited due to the fact that biofilms are intrinsically tolerant to antibiotics.[5] The diabetic foot ulcer (DFU) microbiome is enriched in anaerobic bacteria when compared with other wounds[6] and harbors a greater number of opportunistic pathogens when compared with contralateral intact skin.[7] The abundance of anaerobes may be due to host factors (eg, poor tissue perfusion) but also may be inherent to the biofilm mode of growth.

Recognizing deep, 3-dimensional wounds are often anaerobic, the authors recently introduced a topical oxygen delivery device into their wound care practice with excellent clinical results.[8] The present study evaluated whether topical oxygen therapy (TOT) causes shifts in the wound microbiome from anaerobic species toward an aerobic community, thereby favoring healing. Given that the wound microbiome represents the building blocks of biofilm formation, understanding its composition and response to treatment is the first step toward improving treatment of biofilms in chronic wounds.

MATERIALS AND METHODS

This study was approved by the Research Ethics Board at St. Michael's Hospital in Toronto, Canada. Following informed consent, 6 patients with diabetes and DFUs present for more than 4 weeks in duration were recruited in accordance with the inclusion and exclusion criteria listed in **Table 1**.

Patients were assessed weekly and received best practice standard care tailored to their wound (ie, iodine-based dressings, sharp debridement, and offloading as needed) as well as continuous topical oxygen via the NATROX Oxygen Delivery System (ODS; Inotec AMD, Cambridge, UK). This small, battery-operated oxygen generation device delivers a continuous stream of humidified oxygen to the wound bed via a small tube and sterile adhesive pad worn under the dressing. Patients wore the ODS device 24 hours per day for the full duration

Table 1. Study inclusion and exclusion criteria

INCLUSION CRITERIA	EXCLUSION CRITERIA
DFU >4 wk but <52 wk duration	Presence of invasive infection requiring IV antibiotics
No planned treatment for arterial disease	Pure neuropathic ulcer with no arterial insufficiency unless they fail to heal within 12 wk of optimum management
No planned surgical intervention	Significant reduced immunity or high-dose corticosteroids (>10mg prednisolone) or other second-line immunosuppressant
Patients aged ≥18 y	Need TCC
Patients who understand the study, agree to adhere to the treatment, and are able to give consent	Patients with a known sensitivity to any of the components of the ODS device
Patients who can be followed by the same investigating team for the whole period of their participation in the study	Patients with known or suspected malignancy in the ulcer or surrounding tissue
	Patients who do not have the physical or mental capacity or a significant other with the ability to change the ODS battery pack on a daily basis
	Patients with >10% of the ulcer surface area covered in hard eschar
	Patients with ulcer surface area of >10cmx10cm
	Patients participating in another clinical study for ulcer management
	Patients with a known history of poor compliance with medical treatment
	Patients unable to understand the aims of the study and not provide informed consent
	Patients who are pregnant

DFU: diabetic foot ulcer; wk: week; IV: intravenous; y: year; TCC: total contact cast; ODS: oxygen delivery system

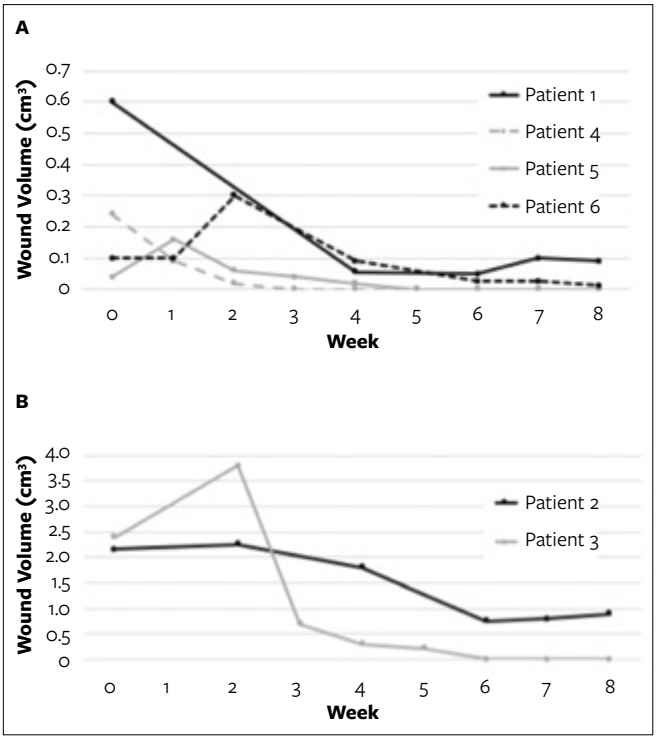

Figure 1. Clinical results of topical oxygen therapy on wound healing. Wound volume in cm^3 was plotted for (A) 4 smaller wounds and (B) 2 larger wounds.

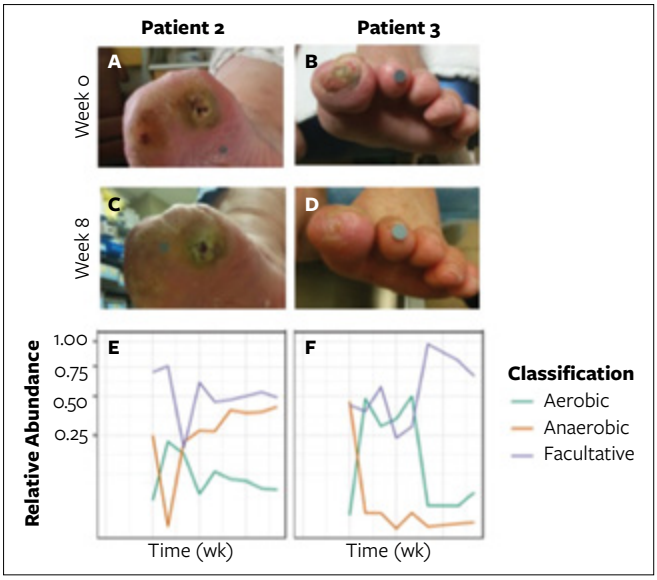

Figure 2. (A) Patient 2: nonhealed DFU compared with (B) a similarly sized healed wound (patient 3); (C, D) 16S rDNA sequencing was used to probe the microbiome over time while wounds were treated with continuous (24 h/d) topical oxygen, and the bacteria identified by sequencing were classified into aerobic, anaerobic, or facultatively anaerobic categories; (E) anaerobes were the most abundant organisms at the outset in both wounds, and persisted in the nonhealed wound; and (F) in contrast, topical oxygen induced a rapid transition to a flora dominated by aerobes in healed wounds.

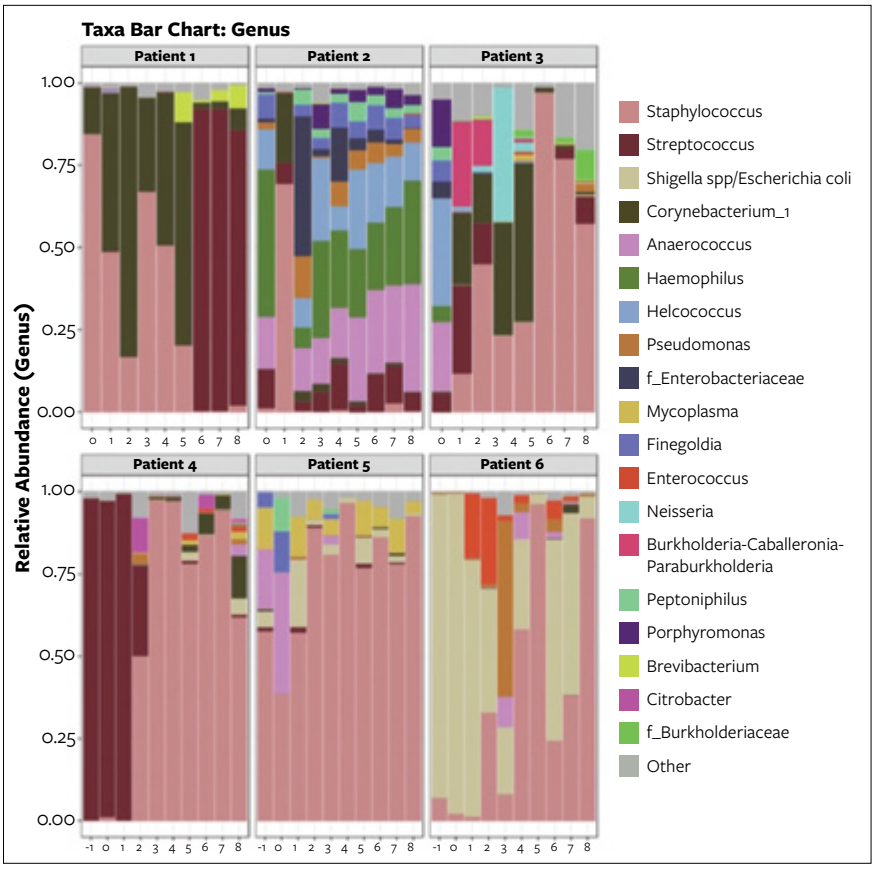

Figure 3. Sequencing of the diabetic foot ulcer microbiome over 8 weeks of topical oxygen therapy. Results are shown for the most abundant 99% of bacterial genera in each sample and expressed as a relative abundance measure.

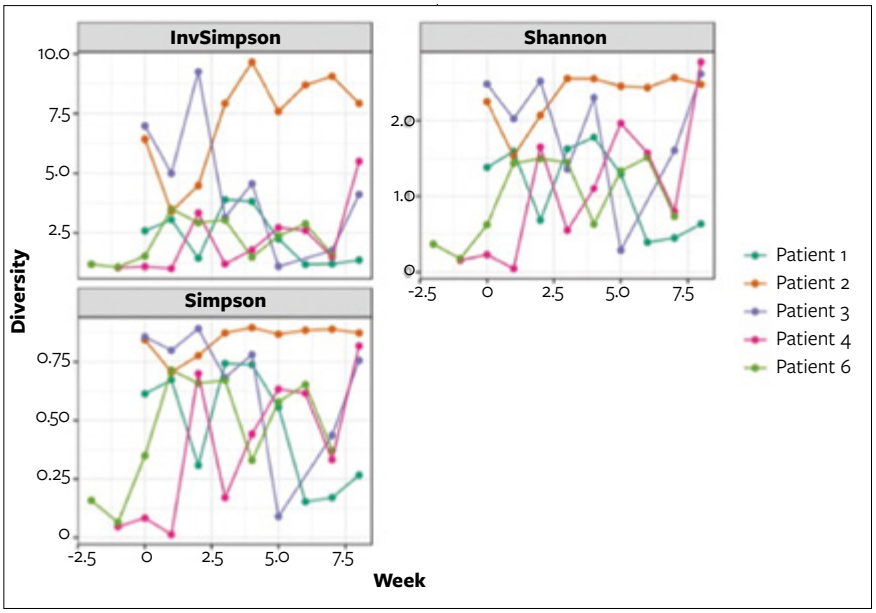

Figure 4. Diversity indices of wound microbiome samples. The diversity of each sample was plotted over time, where higher numbers indicated less diversity. In all diversity measures used, the nonhealing ulcer of patient 2 was stable and had little change over time. Healing wounds showed large fluctuations in diversity over time.

of the 8-week study so that their DFUs were continuously exposed to higher than ambient concentrations of oxygen. Ulcers were photographed and swabbed using Levine's technique at each weekly visit.

Genomic DNA was extracted from wound swabs as described by Stearns et al,[9] and the variable region 3 of the 16S rRNA gene was amplified.[10] Positive amplicons were normalized using the SequalPrep Normalization Plate Kit (Thermo Fisher Scientific, Waltham, WA) and sequenced on a MiSeq System (Illumina, San Diego, CA). Reads were filtered and trimmed using Cutadapt[11] and processed using DADA2.[12] Taxonomy was assigned using the SILVA database version 1.3.2 (Max Planck Institute for Marine Microbiology and Jacobs University, Bremen, Germany).

RESULTS AND DISCUSSION

Outcomes of DFUs

Of the 6 DFUs, 5 healed over 8 weeks of TOT (**Figure 1**). The DFU that did not heal in the cohort was 1 of 2 ulcers that was significantly larger than the others at the outset (**Figure 2**). Clinically, the nonhealed ulcer (patient 2; ~2 cm^3) was on the plantar surface of the first metatarsal head and had been wrapped in a compression dressing with acetic acid washes for suspected *Pseudomonas aeruginosa* for the 8-week study period (**Figure 1B**). The largest healed ulcer (patient 3; ~2.4 cm^3) in the study cohort was on the first digit (hallux tip) (**Figure 1B**), which was offloaded using a DARCO shoe (DARCO International, Inc, Huntington, WV) and intermittently cleansed with povidone-iodine when deemed clinically necessary. Despite being larger than patient 2's DFU at the outset of treatment, patient 3 healed completely by week 7 of TOT. The clinical course of all 6 wounds in the current study is presented in **Figure 1**.

Microbiome results

Sequencing identified typical DFU pathogens (ie, *Staphylococcus, Streptococcus, Pseudomonas*) as well as some less commonly encountered genera (ie, *Anaerococcus, Helcococcus, Mycoplasma,*

Haemophilus) in all ulcers in the present study (**Figure 3**). There was no obvious association between the presence of a specific pathogen and the duration/severity of an ulcer. In keeping with the results of Loesche et al,[13] it was found that the microbiome of the 5 healing DFUs in the present study cohort diversified and transitioned over time (**Figures 3, 4**). In contrast, the microbiome of the 1 nonhealing ulcer in the present cohort (patient 2) was the least diverse and did not change over time despite standard care plus TOT (**Figures 3, 4**). The most abundant 1% of organisms identified according to oxygen utilization were classified (**Table 2**), and their abundance over the course of treatment was plotted (**Figures 2, 5**). Averaged results from all 6 ulcers demonstrated a transition from a flora dominated by anaerobes at the outset of monitoring to a flora rich in aerobic species after 8 weeks of continuous (24 hours/day) topical oxygen. In contrast, the wound microbiome of the nonhealed DFU remained anaerobic, with the exception of a timepoint in which the patient missed their scheduled acetic acid wash (**Figure 2**). Although it is recognized this is a single data point, it is speculated acetic acid washes may have sterilized the commensal aerobic flora that were able to proliferate in other patients' healed ulcers.

LIMITATIONS

This study, to the best of the authors' knowledge, is the first to document changes to a chronic wound microbiome with TOT. However, the sample size was limited to 6 patients due to the longitudinal nature of the study. Because of the small sample size, the authors were unable to determine keystone species common to healing versus nonhealing wounds. The authors are currently conducting a similar, larger trial to map changes in the microbiome on a finer scale and calculate the statistical significance of the findings.

CONCLUSIONS

Topical oxygen therapy can enrich aerobes to drive the microbiome toward a flora that more closely resembles that of intact skin. These changes may contribute to healing through suppression of dysregulated inflammatory cascades induced by chronic wound colonization. Understanding the mechanism-of-action of topical oxygen may help in determining which patients will benefit most from its use and guide treatment decisions by distinguishing responders from non-responders earlier.

Table 2. Classification of the most abundant 1% bacteria identified by 16S rDNA sequencing

AEROBES	FACULTATIVE ANAEROBES	ANAEROBES
Pseudomonas	*Streptococcus*	*Porphyromonas*
Corynebacterium	*Staphylococcus*	*Peptoniphilus*
Burkholderia-Caballeronia-Paraburkholderia	*Mycoplasma*	*Finegoldia*
Brevibacterium	*Helcococcus*	*Anaerococcus*
	Haemophilus	
	Shigella spp/*Escherichia coli*	

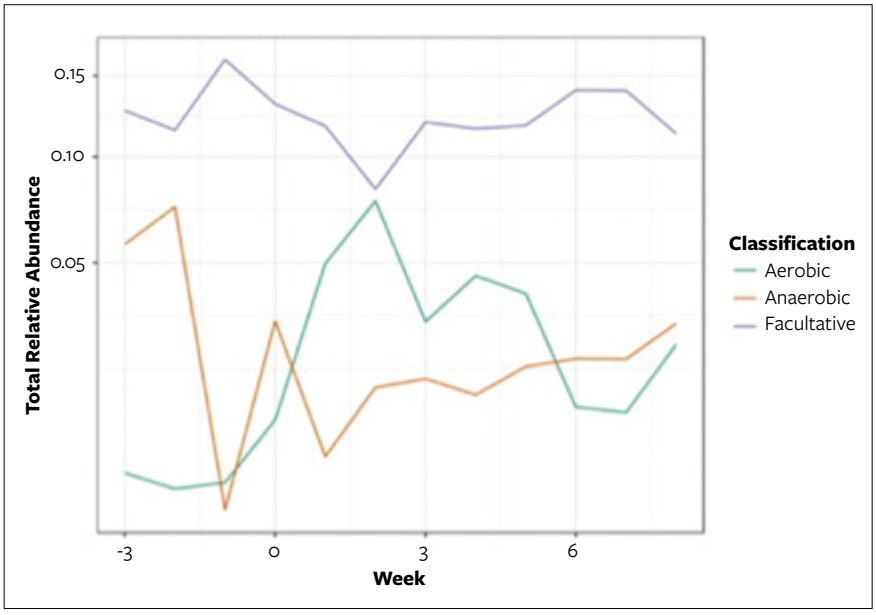

Figure 5. Mean abundance of each class of microbe across all patients. Wounds were sampled for sequencing beginning 3 weeks (-3) before topical oxygen therapy commenced (week 0) to ensure the changes in abundance of aerobes were in fact due to TOT and not random fluctuations in the microbiome. We found that indeed, there was a clear increase in abundance of aerobic species within 1–2 weeks of topical oxygen therapy with a corresponding decrease in anaerobes.

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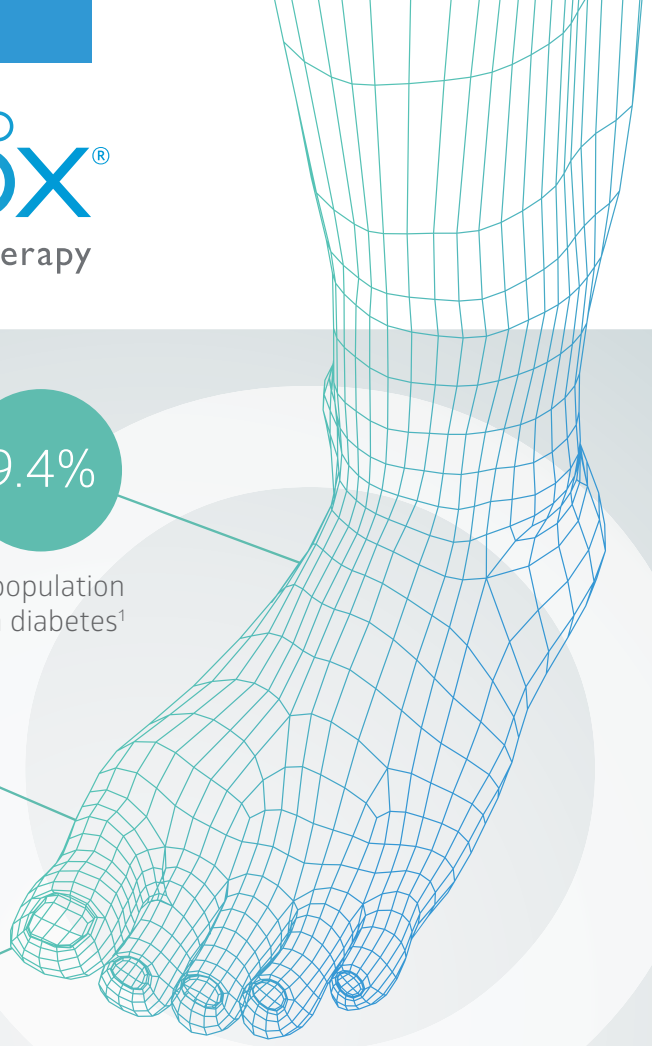

9.4%

US adult population with diabetes[1]

13%

US prevalence of diabetic foot ulceration[2]

33%

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100%

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