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Impact of continuous topical oxygen therapy on biofilm gene expression in a porcine tissue model

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Impact of continuous topical oxygen therapy on biofilm gene expression in a porcine tissue model

Objective: The effect of continuous topical oxygen therapy (cTOT) on *Pseudomonas aeruginosa* biofilm gene transcription profiles following inoculation onto porcine skin, using a customised molecular assay was determined.

Method: Sterilised porcine skin explants were inoculated with *Pseudomonas aeruginosa* in triplicate: 0 hours as negative control; 24 hours cTOT device on; 24 hours cTOT device off. The oxygen delivery system of the cTOT device was applied to the inoculated tissue and covered with a semi-occlusive dressing. All samples were incubated at 37±2°C for 24 hours, with the 0 hours negative control inoculated porcine skin samples recovered immediately. Planktonic suspensions and porcine skin biopsy samples were taken at 0 hours and 24 hours. Samples were processed and quantifiably assessed using gene specific reverse transcription-quantitative polymerase chain reaction assays for a panel of eight *Pseudomonas aeruginosa* genes (16S, pelA, pslA, rsaL, pcrV, pscQ, acpP, cbrA) associated with biofilm formation, quorum sensing, protein secretion/translocation and metabolism.

Results: Transcriptional upregulation of pelA, pcrV and acpP, responsible for intracellular adhesion, needletip protein production for type-3 secretion systems and fatty acid synthesis during proliferation, respectively, was observed when the cTOT device was switched on compared to when the device was switched off. Data suggest increased metabolic activity within bacterial cells following cTOT treatment.

Conclusion: cTOT is an adjunctive therapy that supports faster healing and pain reduction in non-healing hypoxic wounds. Oxygen has previously been shown to increase susceptibility of biofilms to antibiotics through enhancing metabolism. Observed gene expression changes highlighted the impact of cTOT on biofilms, potentially influencing antimicrobial treatment success in wounds. Further in vitro and clinical investigations are warranted.

Declaration of interest: CB, HT and HJ are employees of NAMSA, UK. EW and WC are employees of Inotec AMD Ltd., UK. GS was a consultant and chief scientific officer for Inotec AMD Ltd. This work was funded by Inotec AMD Ltd.

antibiotic treatment efficacy • biofilm • chronic wound • hard-to-heal wound • porcine model • wound • wound care • wound dressing • wound healing

Recurrent infections are commonly associated with biofilms. They are found in many chronic conditions, including sinusitis, urinary tract infections, prosthetic infections and hard-to-heal (chronic) wounds.[1] An estimated 80% of hard-to-heal wounds contain a biofilm.[2] There is growing recognition of their role as a barrier to wound healing in hard-to-heal wounds.[3–6] Another major clinical challenge of wound biofilms is their ability to evade the immune system[7] as well as their tolerance to antimicrobials,[8–10] both of which lead to persistence of biofilms and recurrent infections.

It is for this reason that scientists and clinicians recommend that biofilm infections are effectively managed, specifically to tackle these traits.[4] Biofilm-based wound care (BBWC) is a term coined by Wolcott et al.[11] whereby an aggressive regimen of debridement and cleansing is used to fragment biofilms, creating a window of opportunity to expose bacteria to antimicrobial action. This enhanced susceptibility of biofilms following physical disruption was shown by Alhede et al.,[12] with the disaggregation leading to enhanced antimicrobial and oxygen penetration, increased bacterial metabolism, and susceptibility to antimicrobials.

Oxygen gradients exist across biofilms, with levels particularly low towards the centre of the biofilm.[13] As a result, bacterial growth rates in the centre of a biofilm are low. This is postulated as one of the reasons for antimicrobial tolerance of these biofilms, resulting in reduced uptake and effect of antimicrobials on cellular processes.[14] It follows, therefore, that another complementary route to the physical BBWC would be to enhance oxygen penetration into a biofilm via supplementation.

The beneficial role of oxygen is well documented across multiple wound healing processes, including oxidative killing of bacteria, cellular signalling and proliferation, collagen deposition and angiogenesis.[15] Thus, reversal of hypoxic conditions in any non-healing wound can aid faster healing by supporting increased demand for oxygen tissue repair and the immune response. Supplementation with topical oxygen therapy has been demonstrated to improve wound healing rates in various randomised controlled trials and

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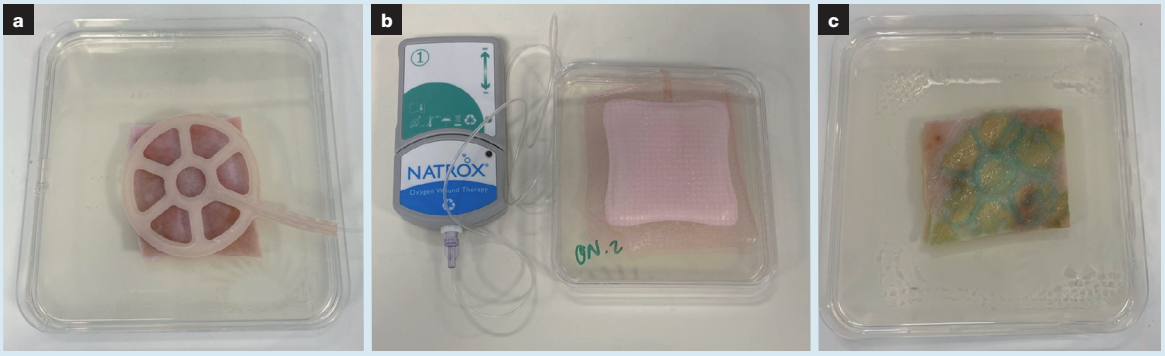
Fig 1. Continuous topical oxygen therapy (cTOT) device placed onto inoculated porcine skin **(a)**. Skin and cTOT device covered with 10×10cm semi-permeable dressing **(b)**. Final assay result following 24-hour humidified incubation at 37±2°C **(c)**



meta-analyses,[16–19] earning recommendations for use in non-healing wounds in various expert guidelines.[15,20–23]

Nevertheless, the exploration of the potential role of supplemental oxygen to tackle wound biofilms is in its infancy. Promising in vitro data using hyperbaric oxygen therapy (HBOT) on biofilm aggregates highlighted the possible role in enhancing antimicrobial susceptibility of biofilm bacteria, and reports that HBOT significantly increased the killing by ciprofloxacin in a *Pseudomonas aeruginosa* strain PAO1 biofilm.[24] Moreover, the study reported that the enhanced killing, in turn, lowered the oxygen consumption in the outer layers of the biofilm, and led to even deeper penetration of oxygen into the biofilm, accentuating its effect.[24]

Laboratory models that provide wound-like environments are challenging to create. Porcine tissue explant models have been used previously to provide a more realistic substrate for biofilm growth, where they are more likely to bind to biotic surfaces, thus creating a mature biofilm more reflective of that observed in real-life wounds.[25,26]

The objective of this ex vivo study was to further explore this mode of action by determining the effect of continuous topical oxygen therapy (cTOT) on *Pseudomonas aeruginosa* biofilm gene transcription profiles following inoculation onto porcine skin, using a customised molecular assay.

Methods

Preparation of ex vivo skin model

Gamma sterilised porcine skin samples (locally sourced) were aseptically dissected into 5×5cm sections and the subcutaneous fat layer removed. Porcine skin samples were then placed onto 1.5% agar in square Petri dishes. A $1\times10^6\pm5\times10^5$ colony forming unit (CFU)ml^{-1} *Pseudomonas aeruginosa* National Collections of Industrial, Food and Marine Bacteria (NCIMB) 10434 inoculum was prepared in tryptone soya broth. The inoculum was enumerated by performing tenfold dilutions in phosphate buffered saline (PBS) and plating out the resulting suspensions onto tryptone soya agar (TSA). Then, 100µl of bacterial inoculum was pipetted onto the centre of each porcine skin sample and spread over a 2.5×2.5cm area using a sterile loop. Triplicate inoculated porcine skin samples were prepared for contact times of: 0 hours as negative control; 24 hours device on and 24 hours device off.

cTOT treatment and sample recovery

cTOT (NATROX O_2, Inotec AMD Ltd., UK) treatment was initiated as follows: the oxygen delivery system of the cTOT device was applied to the inoculated tissue and covered with a semi-occlusive dressing (ALLEVYN Gentle Border, Smith+Nephew, UK) as shown in Fig 1. All samples were incubated at 37±2°C for 24 hours with the 0 hours negative control inoculated porcine skin samples recovered immediately.

Following the incubation period, two 3mm biopsies were removed (one for culture, the other for molecular analysis) from directly under the area where the oxygen delivery system had been in contact with the skin. Each biopsy was then placed in separate bijou containers containing 1ml of PBS, sonicated for five minutes and vortexed for 10 seconds. A biopsy from each porcine skin sample was then used for bacterial quantification and the second biopsy was used for molecular assessment of eight genes. Samples of planktonic bacteria were also taken for molecular assessment at 0 hours and following 24 hours incubation.

Viable bacteria from the samples were quantified as follows: after vortexing, resultant suspensions were enumerated by performing tenfold dilutions in PBS and plating out the resulting suspensions onto TSA. Inoculated plates were incubated at 37±2°C for 24 hours followed by enumeration.

Molecular quantification of biofilm-related gene transcription profiles

Planktonic suspensions and porcine skin biopsy samples were taken at 0 hours and 24 hours.

Ribonucleic acid (RNA) was extracted from 140µl of each sample using the QIAamp Viral RNA kit (Qiagen, UK), according to manufacturer's instructions. Extracted RNA was converted to complementary (c)DNA using the QuantiTect Reverse Transcription kit (Qiagen) in a two-stage incubation process. Converted cDNA was

Table 1. Targeted genes and their role in *Pseudomonas aeruginosa* replication and biofilm production

Gene	Group	Role
16S	Housekeeping gene	Ribosomal RNA
pelA	Biofilm and quorum sensing	Intercellular adhesin
pslA		Biofilm structure formation and antibiotic resistance
rsaL		Transcriptional regulator to modify transition from planktonic to biofilm state
pcrV	Type-3 secretion systems (T3SS)	Needletip protein for T3SS protein secretion
pscQ		Translocation protein
acpP	Metabolic pathways	Fatty acid synthesis during proliferation
cbrA		Regulation of carbon and nitrogen utilisation

then diluted 1:50 with RNase-free water before being added to individual gene-specific quantitative polymerase chain reactions (qPCRs) to quantify the following *Pseudomonas aeruginosa* genes: 16S, pelA, pslA, rsaL, pcrV, pscQ, acpP and cbrA (Table 1).

Samples were processed and quantifiably assessed using gene specific reverse transcription (RT)-qPCR assays for the panel of eight *Pseudomonas aeruginosa* genes associated with biofilm formation, quorum sensing, protein secretion/translocation and metabolism.

Statistical analysis

For viable bacteria quantification, an F-test was applied to assess variance of $\log_{10}$CFUml^{-1} recovery data. A student's unpaired t-test (two-tailed) was applied to assess statistical differences between the $\log_{10}$CFUml^{-1} recovery data from the negative control and test samples. The minimum limit of detection was 1 log for $\log_{10}$CFUml^{-1} data. Data were considered statistically significant when p-values were <0.05.

To determine the transcription regulation for each gene, the obtained target gene quantification cycle data for each sample were analysed by normalisation against the 16S gene, followed by comparison with the planktonic bacteria sample at the same timepoint (0 hours or 24 hours). Fold change in gene transcription was calculated using the double-delta method (Fig 2).

Significant upregulation was regarded as a value ≥2.0, and significant downregulation was regarded as a value ≤0.50. A value of 1 indicated no change in gene transcription when compared with the planktonic organisms. Through comparison with the 0 hours planktonic bacteria sample, 0 hour negative control values were determined. The 24-hour cTOT device on and off values were determined by comparison with the 24-hour planktonic bacteria sample.

Fig 2. Double-delta (Δ) formula for calculating fold-change difference between biofilm (treated) and planktonic (untreated) samples. Δ—change; Ct—cycle threshold

$$\textit{Fold change} = 2^{-(\Delta Ct\ (\text{treated sample}) - \Delta Ct\ (\text{untreated sample}))}$$

Results

Quantification of viable *Pseudomonas aeruginosa*

At 0 hours, a mean viable *Pseudomonas aeruginosa* recovery of 4.58 $\log_{10}$CFUml^{-1} was observed for the negative control. Following 24 hours of incubation, an average viable *Pseudomonas aeruginosa* recovery of 8.16±0.36 $\log_{10}$CFUml^{-1} and 7.93±0.10 $\log_{10}$CFUml^{-1} was observed when the cTOT device was switched off and on, respectively. This resulted in a non-significant reduction of 0.24±0.10 $\log_{10}$CFUml^{-1} when the device was switched on compared to when the device was switched off (Table 2).

Molecular gene transcription profiles of selected biofilm related genes

Overall, a greater impact was observed on the *Pseudomonas aeruginosa* transcription profile when the cTOT device was on, when compared to when the device was off, as summarised in the heat map (Fig 3) and detailed in Fig 4a–g.

Genes responsible for metabolism-related functions—pelA (intracellular adhesion); pcrV (needle-tip protein production for type-3 secretion systems); and acpP (fatty acid synthesis during cell proliferation)—were observed to be upregulated when the cTOT device was switched on compared to when the device was switched off (Fig 4). The pcrV gene was significantly upregulated to the greatest extent (6.46-fold change increase), with pelA significantly upregulated (2.30-fold change increase, and acpP was also upregulated (1.89-fold change increase; $p<0.05$).

In contrast, genes linked to biofilm formation—cbrA (regulation of carbon and nitrogen use); pscQ (translocation protein); and pslA (biofilm structure formation and antibiotic resistance)—were observed to be down-regulated following cTOT application. The cbrA gene was significantly down-regulated to the greatest extent (0.07-fold change increase), with pslA significantly down-regulated (0.30-fold change) and pscQ was also down-regulated (0.52-fold change).

Discussion

Biofilms pose major challenges to hard-to-heal wounds, leading to a cycle of low-level inflammation, tissue damage and recurrent infections.[1,4,6] In contrast to acute

Table 2. Mean log recoveries and log reductions of viable *Pseudomonas aeruginosa* at 0 hours and following 24 hours on porcine skin when the cTOT device was switched off and on

Sample	Mean recovery±SD ($log_{10}CFUml^{-1}$)	Mean reduction±SD ($log_{10}CFUml^{-1}$)
0h negative control	4.58*	n/a
24h cTOT device off	8.16±0.36	n/a
24h cTOT device on	7.93±0.10	0.24±0.10†

*No standard deviation; †not statistically significant; CFU—colony-forming units; cTOT—continuous topical oxygen therapy; h—hour; n/a—not applicable; SD—standard deviation

Fig 3. Heat map representation of the gene transcription profiles for the seven genes investigated following 24 hours of incubation with continuous topical oxygen therapy (cTOT) device on or off

Gene	Function	Sample: 0h negative control	Sample: 24h cTOT device off	Sample: 24h cTOT device on
pelA	Intracellular adhesin			
pslA	AMR and biofilm structure (exopolysaccharide synthesis)			
rsaL	Transcription regulator for transition to biofilm			
pcrV	Needle-tip protein, secretion			
pscQ	Quorum sensing system translocation protein			
acpP	Fatty acid synthesis			
cbrA	Regular biofilm genes, carbon and nitrogen utilisation			

Blue—significant down-regulation; Red—significant upregulation; White—no significant change in gene expression; AMR—antimicrobial resistance; h—hour

infections caused by planktonic bacteria, biofilm-related infections are particularly recalcitrant to clearance by the immune system, and antibiotic or antimicrobial effects.[7–10] Antimicrobial interventions may kill the faster growing bacteria on the outer edge of a biofilm, resolving acute clinical signs and symptoms of infection. However, reduced impact is observed on the bacteria towards the centre of the biofilm, which then acts as a reservoir for reinfection, leading to the multiple cycles of recurrent infections characteristic of biofilms.[4]

A major factor in biofilm tolerance to antimicrobials, including antibiotics and many antiseptics, is thought to be related to the extremely slow metabolism of bacteria towards the centre of a biofilm which, in turn, limits uptake of antimicrobials into the cell as well as the cell functions these antimicrobials may act against, such as replication, protein synthesis and respiration.[14]

Oxygen concentration gradients also exist in biofilms, with higher levels at the outer edges and decreasing towards the centre.[13] They are thought to be a key influencing factor on metabolism and antimicrobial effectiveness.[14]

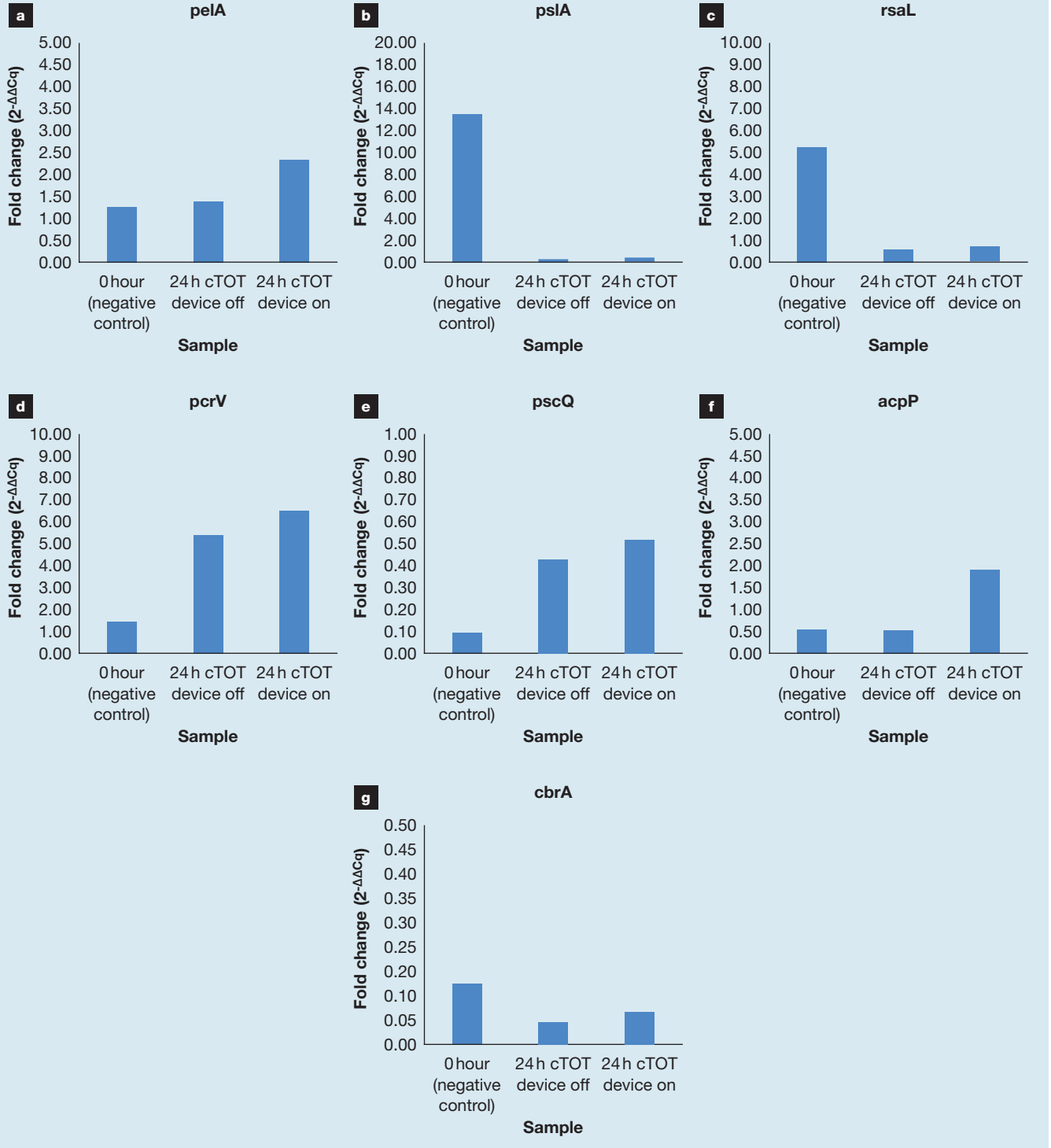
Previously published data highlight the impact of increasing oxygen levels using HBOT on biofilm bacteria, demonstrating an increased susceptibility of these biofilms following oxygen treatment.[24] The findings of this paper, and that of a subsequent review of the potential of this treatment option for biofilm tolerant infections,[27] indicate that increased metabolism of the bacterial cells following increased oxygen penetration deeper into the biofilm structure, may be responsible for this enhanced antimicrobial effect.

In this study, the gene transcriptional analysis suggested an increased metabolic activity within bacterial cells following cTOT treatment which may concur with these previous studies. This study also highlights that topical application of oxygen may induce similar effects as HBOT on biofilms, thus providing a useful addition to the clinician toolbox in managing these hard-to-heal wounds. It also has the practical advantages of a portable, easy-to-use treatment outside of the specialist clinical setting. The potential of cTOT to ameliorate antimicrobial susceptibility in biofilm infections could improve antimicrobial effectiveness at first use and minimise recurrent infections, thus supporting more appropriate antimicrobial use as part of antimicrobial stewardship. These new data, combined with the growing evidence supporting improved healing rates,[15,16,18] pain reduction[28] and reduced ulcer recurrence,[17] emphasise cTOT as an essential adjunct to gold standard of care in hard-to-heal wounds.

Limitations

Extensions to this work should include analysis of the depth of penetration of oxygen into biofilms following cTOT treatment and subsequent direct analysis of the impact of the increased metabolism identified here, on antimicrobial effect, which was not covered by this

Fig 4. Fold change quantification of genes following 24 hours of incubation of *Pseudomonas aeruginosa* on porcine skin and the continuous topical oxygen therapy (cTOT) device switched off or on **(a–g)** represent the genes pelA, pslA, rsaL, pcrV, pscQ, acpP, cbrA, respectively. Zero hour (0 h) values produced from comparison to 0 h planktonic bacteria, 24 h values produced from comparison to 24 h planktonic bacteria. A fold change ≥2.00 indicates significant upregulation, and a fold change ≤0.50 indicates significant downregulation—both compared to the 24 h planktonic bacteria samples. Δ—delta; Cq—quantification cycle



study. Translation from laboratory studies to impact in clinical scenarios should also be assessed in parallel to confirm this effect.

Conclusion

This research adds further clarity to the multimodal action of topical oxygen therapy observed in hard-to-heal

wounds and adds support to its wider application as an adjunctive therapy in these types of wounds. JWC

Reflective questions

- How frequently do you see recurrent infections in hard-to-heal wounds?
- How would more effective treatment of biofilm help you support appropriate antimicrobial use in your daily practice?
- Are adjunctive therapies considered in your clinical pathways? Why?

References

1 Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999; 284(5418):1318–1322. https://doi.org/10.1126/science.284.5418.1318
2 Malone M, Bjarnsholt T, McBain AJ et al. The prevalence of biofilms in chronic wounds: a systematic review and meta-analysis of published data. J Wound Care 2017; 26(1):20–25. https://doi.org/10.12968/jowc.2017.26.1.20
3 Schierle CF, De la Garza M, Mustoe TA, Galiano RD. Staphylococcal biofilms impair wound healing by delaying reepithelialization in a murine cutaneous wound model. Wound Repair Regen 2009; 17(3):354–359. https://doi.org/10.1111/j.1524-475X.2009.00489.x
4 Schultz G, Bjarnsholt T, James GA et al.; Global Wound Biofilm Expert Panel. Consensus guidelines for the identification and treatment of biofilms in chronic nonhealing wounds. Wound Repair Regen 2017; 25(5):744–757. https://doi.org/10.1111/wrr.12590
5 Roche ED, Renick PJ, Tetens SP et al. Increasing the presence of biofilm and healing delay in a porcine model of MRSA-infected wounds. Wound Repair Regen 2012; 20(4):537–543. https://doi.org/10.1111/j.1524-475X.2012.00808.x
6 Bjarnsholt T. The role of bacterial biofilms in chronic infections. Acta Pathol Microbiol Scand Suppl 2013; 121(s136):1–58. https://doi.org/10.1111/apm.12099
7 Alhede M, Lorenz M, Fritz BG et al. Bacterial aggregate size determines phagocytosis efficiency of polymorphonuclear leukocytes. Med Microbiol Immunol (Berl) 2020; 209(6):669–680. https://doi.org/10.1007/s00430-020-00691-1
8 Stewart PS, William Costerton J. Antibiotic resistance of bacteria in biofilms. Lancet 2001; 358(9276):135–138. https://doi.org/10.1016/S0140-6736(01)05321-1
9 Stewart PS, Rayner J, Roe F, Rees WM. Biofilm penetration and disinfection efficacy of alkaline hypochlorite and chlorosulfamates. J Appl Microbiol 2001; 91(3):525–532. https://doi.org/10.1046/j.1365-2672.2001.01413.x
10 Bjarnsholt T, Kirketerp-Møller K, Kristiansen S et al. Silver against Pseudomonas aeruginosa biofilms. Acta Pathol Microbiol Scand Suppl 2007; 115(8):921–928. https://doi.org/10.1111/j.1600-0463.2007.apm_646.x
11 Wolcott RD, Rumbaugh KP, James G et al. Biofilm maturity studies indicate sharp debridement opens a time-dependent therapeutic window. J Wound Care 2010; 19(8):320–328. https://doi.org/10.12968/jowc.2010.19.8.77709
12 Alhede M, Kragh KN, Qvortrup K et al. Phenotypes of non-attached Pseudomonas aeruginosa aggregates resemble surface attached biofilm. PLoS One 2011; 6(11):e27943. https://doi.org/10.1371/journal.pone.0027943
13 de Beer D, Stoodley P, Roe F, Lewandowski Z. Effects of biofilm structures on oxygen distribution and mass transport. Biotechnol Bioeng 1994; 43(11):1131–1138. https://doi.org/10.1002/bit.260431118
14 Walters MC 3rd, Roe F, Bugnicourt A et al. Contributions of antibiotic penetration, oxygen limitation, and low metabolic activity to tolerance of Pseudomonas aeruginosa biofilms to ciprofloxacin and tobramycin. Antimicrob Agents Chemother 2003; 47(1):317–323. https://doi.org/10.1128/AAC.47.1.317-323.2003
15 Frykberg R, Andersen C, Chadwick P et al. Use of topical oxygen therapy in wound healing. J Wound Care 2023; 32(Sup8b):S1–S32. https://doi.org/10.12968/jowc.2023.32.Sup8b.S1
16 Serena TE, Bullock NM, Cole W et al. Topical oxygen therapy in the treatment of diabetic foot ulcers: a multicentre, open, randomised controlled clinical trial. J Wound Care 2021; 30(Sup5):S7–S14. https://doi.org/10.12968/jowc.2021.30.Sup5.S7
17 Al-Jalodi O, Kupcella M, Breisinger K, Serena TE. A multicenter clinical trial evaluating the durability of diabetic foot ulcer healing in ulcers treated with topical oxygen and standard of care versus standard of care alone 1 year post healing. Int Wound J 2022; 19(7):1838–1842. https://doi.org/10.1111/iwj.13789
18 Carter MJ, Frykberg RG, Oropallo A et al. Efficacy of topical wound oxygen therapy in healing chronic diabetic foot ulcers: systematic review and meta-analysis. Adv Wound Care (New Rochelle) 2023; 12(4):177–186. https://doi.org/10.1089/wound.2022.0041
19 Niederauer MQ, Michalek JE, Liu Q et al. Continuous diffusion of oxygen improves diabetic foot ulcer healing when compared with a placebo control: a randomised, double-blind, multicentre study. J Wound Care 2018; 27(Sup9):S30–S45. https://doi.org/10.12968/jowc.2018.27.Sup9.S30
20 ElSayed NA, Aleppo G, Aroda VR et al.; on behalf of the American Diabetes Association. 12. Retinopathy, neuropathy, and foot care: standards of care in diabetes—2023. Diabetes Care 2023; 46(Supplement_1):S203–S215. https://doi.org/10.2337/dc23-S012
21 ElSayed NA, Aleppo G, Bannuru RR et al.; American Diabetes Association Professional Practice Committee. 12. Retinopathy, neuropathy, and foot care: standards of care in diabetes—2024. Diabetes Care 2024; 47(Supplement_1):S231–S243. https://doi.org/10.2337/dc24-S012
22 Chen P, Campillo Vilorio N, Dhatariya K et al. Guidelines on interventions to enhance healing of foot ulcers in people with diabetes. IWGDF 2023 update. 2023. https://tinyurl.com/rj9c8dym (accessed 6 August 2024)
23 Lavery LA, Suludere MA, Attinger CE et al. WHS (Wound Healing Society) guidelines update: diabetic foot ulcer treatment guidelines. Wound Repair Regen 2024; 32(1):34–46. https://doi.org/10.1111/wrr.13133
24 Gade PA, Olsen TB, Jensen PØ et al. Modelling of ciprofloxacin killing enhanced by hyperbaric oxygen treatment in Pseudomonas aeruginosa PAO1 biofilms. PLoS One 2018; 13(6):e0198909. https://doi.org/10.1371/journal.pone.0198909
25 Phillips PL, Yang Q, Davis S et al. Antimicrobial dressing efficacy against mature Pseudomonas aeruginosa biofilm on porcine skin explants. Int Wound J 2015; 12(4):469–483. https://doi.org/10.1111/iwj.12142
26 Roche ED, Woodmansey EJ, Yang Q et al. Cadexomer iodine effectively reduces bacterial biofilm in porcine wounds ex vivo and in vivo. Int Wound J 2019; 16(3):674–683. https://doi.org/10.1111/iwj.13080
27 Jensen PØ, Møller SA, Lerche CJ et al. Improving antibiotic treatment of bacterial biofilm by hyperbaric oxygen therapy: not just hot air. Biofilm 2019; 1:100008. https://doi.org/10.1016/j.bioflm.2019.100008
28 Jebril W, Nowak M, Palin L et al. Topical oxygen treatment relieves pain from hard-to-heal leg ulcers and improves healing: a case series. J Wound Care 2022; 31(1):4–11. https://doi.org/10.12968/jowc.2022.31.1.4

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