Impact of continuous Topical Oxygen Therapy on biofilm gene expression in a porcine tissue model

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Introduction

Up to 80% of chronic non-healing wounds contain a biofilm.[1]

Oxygen and nutritional limitations in bacterial biofilms, particularly pronounced towards the centre,[2] can result in slow bacterial growth & metabolism.[3,4] This reduced metabolism can lead to reduced efficacy of antibiotics against these communities.[5]

Supplemental oxygen has been shown to increase oxygen penetration into a biofilm with increased metabolism linked to subsequent increased susceptibility of biofilm bacteria to antibiotics demonstrated *in vitro*.[6,7]

The aim of this *ex vivo* study was to determine 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

Sterilized porcine skin explants were inoculated with *P. aeruginosa* in triplicate (0h negative control, 24h cTOT on, 24h cTOT off). The oxygen delivery system (ODS) of the cTOT device was applied to the inoculated tissue and covered with a semi-occlusive dressing. All samples were incubated at 37°C ± 2°C for 24h with the 0h negative control inoculated porcine skin samples recovered immediately.

Planktonic suspensions and porcine skin biopsy samples were taken at 0 and 24h. Samples were processed and quantifiably assessed using gene specific RT-qPCR assays for a panel of eight *P. aeruginosa* genes (16S, pelA pslA, rsaL, pcrV, pscQ, acpP, cbrA) associated with biofilm formation, quorum sensing, protein secretion/translocation and metabolism.

*cTOT device tested was NATROX® O_2 Wound Therapy

NAMSA® NATROX® Wound Care

Results

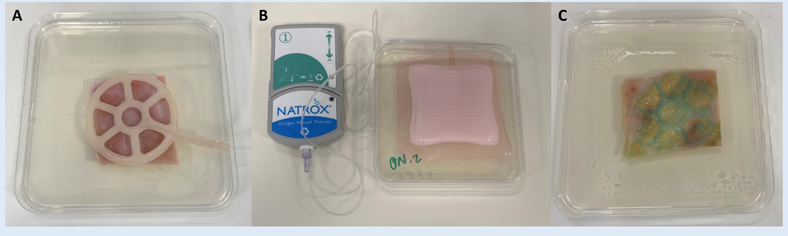
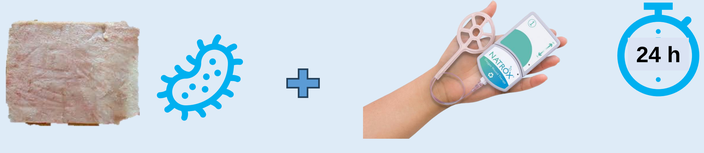


Figure 1. A) cTOT device placed onto inoculated porcine skin, B) skin & cTOT device covered with 10 cm x 10 cm semi-permeable dressing, C) Final assay result following 24 hour humidified incubation at 37 °C ± 2 °C.



Transcriptional **up-regulation** of genes linked to increased metabolism (*pelA, pcrV* and *acpP)*

Transcriptional **down-regulation** of genes linked to biofilm formation (*cbrA, pscQ, pslA)*

Suggests **increased** metabolic activity within bacterial cells and **less** requirement to form biofilm following cTOT treatment

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

AMR = Antimicrobial Resistance

Figure 2. Heat map representation of the gene transcription profiles for the seven genes investigated following 24 hours incubation with cTOT device on or off.
Red = significant up-regulation, Blue = significant down-regulation, white = no significant change in gene expression

Discussion

cTOT is an adjunctive therapy that supports faster healing[8-10] and pain reduction[11] in non-healing hypoxic wounds. Data in this study suggests increased metabolic activity within bacterial cells following cTOT treatment. Oxygen has previously been shown to increase susceptibility of biofilms to antibiotics[7] through enhancing metabolism.

Observed gene expression changes here highlight the impact of cTOT on biofilms potentially influencing antimicrobial treatment success in wounds warranting further *in vitro* and clinical investigations.

References

1.Malone, M. *et al.* J Wound Care 26, 20–25 (2017), **2.** de Beer, D., *et al.* Biotechnol Bioeng 43, 1131–1138 (1994), **3.** Hall-Stoodley, L. & Stoodley, P. Trends Microbiol 13, 7–10 (2005), **4.** Schultz, G. *et al.* Wound Repair and Regeneration 25, 744–757 (2017), **5.** Stewart, P. S. & Costerton, J. W. Lancet 358, 135–8 (2001), **6.** Jensen, P. Ø. *et al.* Biofilm 1, 100008 (2019), **7.** Gade, P. A. V. *et al.* PLoS One 13, e0198909 (2018), **8.**Carter, M. J. *et al. Adv Wound Care (New Rochelle)* **12**, 177–186 (2023), **9.** Serena, T. E. *et al. J Wound Care* **30**, S7–S14 (2021), **10.** Kaufman, H. *et al. Wounds International* **12**, 63–68 (2021), **11.** Jebril, W. *et al. J Wound Care* **31**, 4–11 (2022)

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