The role of nitric oxide in the killing & prevention of surface-associated bacterial communities by a prototype nitric oxide-generating wound dressing

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Introduction

- Hard-to-heal wounds, such as diabetic foot ulcers (DFUs), are often compromised by microorganisms that contribute to chronicity and infection risk, particularly in high-risk patients such as those with diabetes[1]
- Nitric oxide (NO) is a potent antimicrobial[2] and antibiofilm[3] gas produced by the mammalian innate immune response to microorganisms, yet it has unrealised potential in wound care[4]
- A novel NO-generating dressing (NOGD) showed superiority over standard of care (SoC) in a randomised controlled trial (RCT) in DFUs[5], and has demonstrated antibiofilm activity *in vitro*[6]
- NO is generated in NOGD when two component parts are placed together: a Carrier Layer (CL) containing aqueous nitrite and a low pH Absorbent Protective Layer (APL; which acidifies nitrite to NO)

Objective

The aim of these *in vitro* studies was to examine the antibiofilm activity of the NO-generating technology within NOGD

Methods

- Wound-associated pathogens were used in biofilm kill and prevention assays, while varying nitrite concentration and treatment times
- Initial prototype test dressings comprised APLs plus CLs containing varying concentrations of nitrite
- Nitrite (NO precursor) concentration:
  - Biofilm kill: biofilms of methicillin-resistant *Staphylococcus aureus* (NCTC 12493; MRSA) were grown on 25 mm dia. nitrocellulose filters on agar for 24 hours, as described elsewhere[6], before prototype and controls (no dressing, and dressings without nitrite) were applied
  - Biofilm prevention: 1x10^5 CFU/mL planktonic MRSA-inoculated filters were immediately treated with prototypes and controls for 24 hours
    - In both assays, surviving bacterial cells were enumerated in quadruplicate on agar
- Treatment duration:
  - The final NOGD design containing a 1 M nitrite CL was used to regularly measure biofilm survival or formation over a 24-hour period, as described above and elsewhere[6]

Results

Nitrite concentration:

- MRSA biofilm was reduced by 3 $\log_{10}$ in 24 hours by prototypes with CLs containing 0.2 M nitrite, and was eradicated by prototypes with CLs containing 0.5 M nitrite (Fig 1)
- MRSA biofilm formation was not prevented by prototypes with CLs containing 0.1 M nitrite, but was completely prevented by prototypes with CLs containing 0.2 M nitrite, after 24 hours (Fig 2)
  - ➢ This data suggests the NO generated by 0.5 M and 1 M nitrite in the CL is sufficient to kill and prevent MRSA biofilm *in vitro*

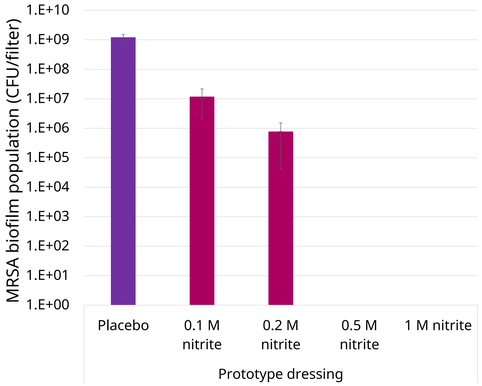

Figure 1. MRSA biofilm kill by dressing prototypes containing nitrite at varying concentrations (N=4). Placebo=CL with no nitrite

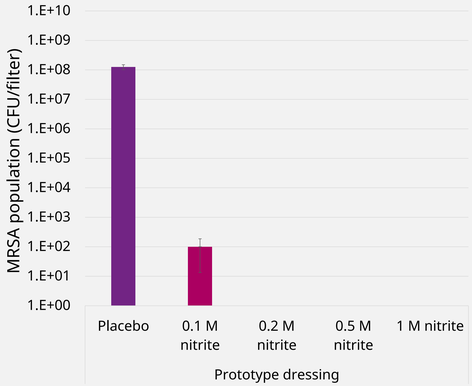

Figure 2. MRSA biofilm prevention by dressing prototypes containing nitrite at varying concentrations (n=4). Placebo=CL with no nitrite

NOGD treatment duration:

- Using NOGD with CL containing 1 M nitrite, MRSA biofilm was reduced by 3 $\log_{10}$ after 2 hours, by >7 $\log_{10}$ after 4 hours, and completely eradicated after 6 hours (Fig 3)
- Biofilm formation was completely prevented after 6 hours (Fig 4)
  - ➢ This data confirms the NOGD can completely kill MRSA biofilm in 6 hours, and prevent its formation in 6 hours, in an NO-dose dependent manner, *in vitro*

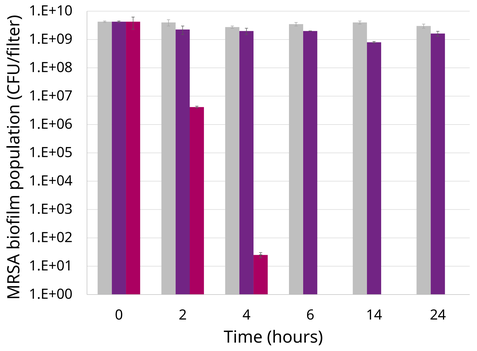

Figure 3. MRSA biofilm kill by NOGD after various treatment durations. (■) control; (■) placebo with no nitrite; (■) NOGD (N=4)

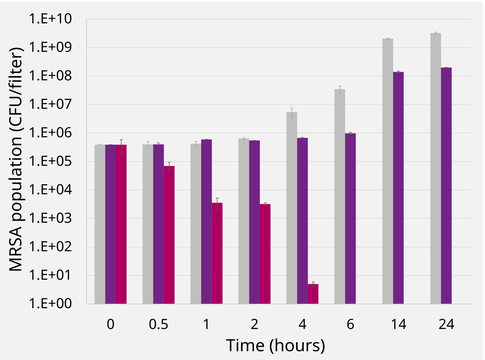

Figure 4. MRSA biofilm prevention by NOGD after various treatment durations. (■) control; (■) placebo with no nitrite; (■) NOGD (N=4)

Discussion

- Biofilm kill and prevention models show that varying the concentration of nitrite used to generate NO, and varying the duration of treatment with the final NOGD design, resulted in a NO dose-response effect on MRSA biofilm kill and prevention
- Nitric oxide, a natural antimicrobial molecule of our innate immune system with intrinsic antibiofilm activity, presents a low risk of antimicrobial resistance[7] and biofilm tolerance[4], unlike many other antimicrobial agents employed in wound care today
- These studies build on significantly superior DFU clinical outcomes[5] and early *in vitro* performance[6] of this novel dressing technology

Conclusion

Nitric oxide within an advanced wound dressing has high potential as an antibiofilm agent in the treatment of hard-to-heal wounds

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