

The chemical and antimicrobial activity within 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 diabetes¹
- Nitric oxide (NO) is a potent antimicrobial² and antibiofilm³ molecule produced by the mammalian innate immune system in response to microorganisms, that has unrealised potential in wound care⁴
- A novel NO-generating dressing (NOGD), that produces NO within, showed superiority over standard of care in a randomised controlled clinical trial (RCT) in DFUs⁵, and has demonstrated antibiofilm activity *in vitro*⁶
- NOGD chemistry is designed to support healing by absorbing or donating fluid as the wound requires, stimulating fluid flux, and maintaining low pH, while imparting inhibitory properties towards microorganisms
- This chemistry is also harnessed to activate the generation of NO within the dressing to provide additional sustained antimicrobial action

Objective

The aim of these *in vitro* studies is to demonstrate that the combination of water activity, osmolarity, and pH are inhibitory to microorganisms, and together activate further rapid antimicrobial activity within a nitric oxide-generating wound dressing

Results

- Water activity: NOGD water activity was 0.71, CL water activity was 0.94, and APL water activity was 0.64; therefore, 90% of the water activity was attributable to the APL
- Osmolarity: 6,300 mOsmols/L for NOGD compared to osmolarity of wound fluid of approx. 290 mOsmols/L⁷
 - Defined water activity (osmotic potential) is inhibitory towards bacteria and fungi (yeast and mold)⁸
 - High osmolarity stimulates fluid flux, causing wound fluids including microorganisms within these fluids, to be drawn into the dressing
- pH: NOGD buffered the alkaline solution immediately, then lowered it to pH 5-6 over 48 hours (Fig 1)
 - This low pH is inhibitory towards microorganisms⁹
 - Low pH activates rapid, continual generation of NO, via nitrite acidification, in the dressing
- Antimicrobial activity: NOGD achieved a >4 log₁₀ reduction for all 8 challenge microorganisms by 24 hours, which was maintained at 48 hours (Table 1), exemplified for antibiotic-resistant *P. aeruginosa* in Fig 2
 - Fluid flux and low pH activates generation of broad-spectrum, sustained antimicrobial NO within the dressing

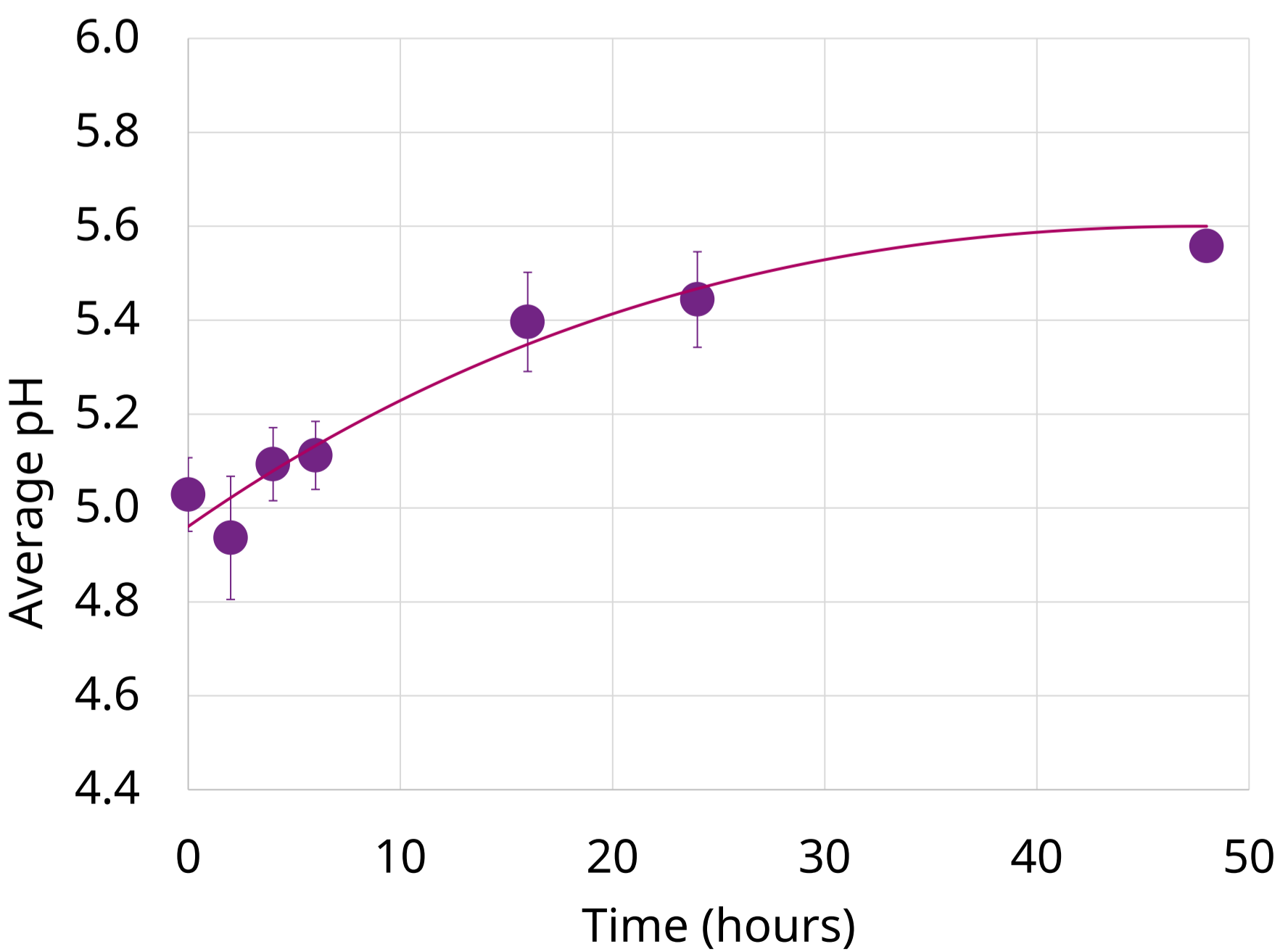


Figure 1. pH of NOGD after challenge with pH 7.4 solution

Challenge organism	0 hours		24 hours		48 hours	
	Control mean log/dsg	NOGD mean log/dsg	Control mean log/dsg	NOGD mean log ↓/dsg	Control mean log/dsg	NOGD mean log ↓/dsg
<i>P. aeruginosa</i>	6.21	6.07	10.79	4.47	10.59	4.47
MRSA	6.17	6.06	10.06	4.46	9.74	4.46
<i>S. pyogenes</i>	6.61	6.61	7.88	5.01	6.70	5.01
<i>K. pneumoniae</i>	6.42	6.31	10.18	4.71	10.12	4.71
VRE	6.47	6.44	9.46	4.84	9.54	4.83
<i>A. baumannii</i>	6.30	6.28	10.17	4.68	10.09	4.67
<i>C. krusei</i>	6.26	6.34	8.10	4.74	8.09	4.74
<i>A. brasiliensis</i>	6.10	6.06	6.15	4.46	6.30	4.46

Table 1. Antimicrobial activity of NOGD against 6 bacteria, a yeast, and a mold. N=3 for control, N=9 for NOGD. dsg=dressing; ↓=reduction

Methods

Test dressings

- NOGD were tested in all experiments
- Water activity was measured for NOGD's two component layers: (i) Carrier Layer (CL) containing nitrite solution (NO precursor); (ii) Absorbent Protective Layer (APL) of hydrated superabsorbent polymer backed with polyurethane film
- For antimicrobial assays, control was a non-antimicrobial gelling fiber dressing

Challenge microorganisms

1. Antibiotic-resistant *Pseudomonas aeruginosa* (NCTC 8506)
2. Methicillin-resistant *Staphylococcus aureus* (MRSA) (ATCC® BAA-1556™)
3. *Streptococcus pyogenes* (NCTC 10872)
4. *Klebsiella pneumoniae* (NCTC 13465)
5. Vancomycin-resistant *Enterococcus faecalis* (NCTC 12201)
6. *Acinetobacter baumannii* (NCTC 13421)
7. *Candida krusei* (NCPF® 3876) (yeast)
8. *Aspergillus brasiliensis* (NCPF® 2275) (mould)

Protocols

- Water activity was measured with a tunable diode laser water activity meter
- Osmolarity was calculated from solute concentrations in NOGD components
- pH was measured by probe after challenging with pH 7.4 solution
- Antimicrobial activity was measured using a modified direct inoculation method¹ with simulated wound fluid preconditioning (48 hours):
 - Dressings were inoculated with 1x10⁶ CFU microbial suspension, sealed, and incubated at 35±3°C for 0-48 hours
 - Dressings were stomached in neutralising broth and viable microorganisms enumerated by plating on agar
 - Testing was performed in triplicate, and for 3 different batches of NOGD (i.e., N=9, and n=3 for control, at each time point)

Discussion

- The water activity of NOGD provides high fluid absorption in exuding wounds, donates fluid in drier wounds, and the high osmolarity and superabsorbent polymer creates a fluid flux
- Water activity of 0.71 is known to be inhospitable to all microorganisms⁸
- NOGD immediately buffered an alkaline solution and reduced it to an acidic pH, creating a state inhibitory towards microorganisms and conducive to healing
- On application of the dressing, fluid flux pulls nitrite into the superabsorbent polymer, and the acidic pH activates the production of NO within the dressing
- NOGD demonstrated antimicrobial activity over a minimum of 48 hours, with the combined effects of these chemical features resulting in the eradication of 8 challenge microorganisms

Conclusion

The chemical and antimicrobial activity of NOGD provides an environment that is both beneficial to the wound, and powerfully antimicrobial, to support wound healing

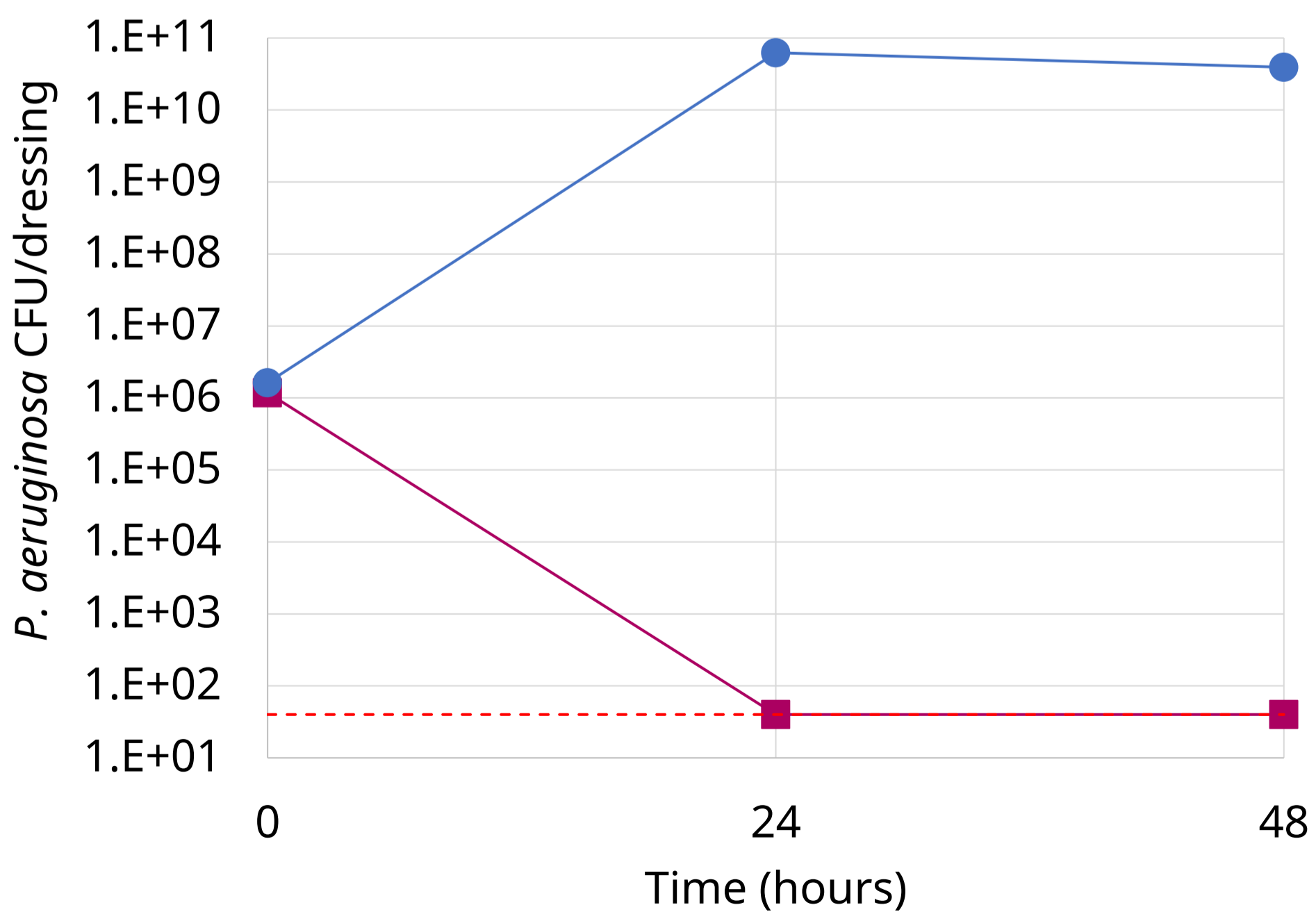


Figure 2. Antimicrobial activity of NOGD against antibiotic-resistant *P. aeruginosa*. (●) control (N=3); (■) NOGD (N=9). LOD=limit of detection

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