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| **The antibiofilm effects of pH-sensitive nitric oxide donors for the treatment of chronic wound infections** |
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| **Background:** Bacterial biofilms are a major global health concern, responsible for approximately 80% of all recorded chronic and recurrent microbial infections. One example of a clinically significant biofilm-associated infection is the chronic wound. Biofilms in chronic wounds disrupt the normal healing process, giving rise to infection-related morbidity and mortality. Many novel antibiofilm agents have been reported in the literature, notably nitric oxide (NO) gas which has shown efficacy in biofilm dispersal and wound healing but has limited applicability due to its short half-life. Here, the antibiofilm effects of NO donor molecules from the N-diazeniumdiolate (NONOate) family, propylamine propylamine NONOate (PA-NO) and spermine NONOate (SP-NO), which release NO in an acid-catalyzed manner, has been investigated. |
| **Methods:** The Griess assay was used to confirm NO release from candidates NONOates and investigate the pH-dependence of decomposition, investigating pH levels 5.5, 6.5, and 7.5. To assess the antibiofilm effects of the NO donor candidates, *Pseudomonas aeruginosa* (PAO1) biofilms were grown for 24 hours in 96-well microplates. Biofilms were treated with 250 µM PA-NO and SP-NO at pH levels 5.5, 7.5, and 8.5, for 60- and 120-min. Biofilms were then stained with crystal violet solution and absorbance was measured to represent total biomass. Antibiofilm efficacy was assessed at both 37 °C and, the more wound-relevant temperature, 32 °C. |
| **Results:** The Griess assay showed decreasing amounts of NO detected as the pH of the NONOate solution was increased. No biofilm dispersal was observed after 60-min treatment, at all pH levels assessed; however, extending the treatment time to 120 min showed significant biomass reductions for 250 µM PA-NO, and SP-NO, at 37 °C and pH 7.5. Assessing biofilm dispersal at 32 °C also showed 250 µM PA-NO and SP-NO to show the most significant biomass reductions at pH 7.5, compared to other pH environments. |
| **Conclusions:** The work presented here highlights the potential for NONOates to be used as an antibiofilm therapy in chronic wound infections. For the first time, the antibiofilm effects of NONOates have been assessed in wound-relevant conditions, assessing pH as a variable, and investigating a lower temperature that is more consistent with those reported *in vivo*. Future work should investigate the temporal dispersal events via microscopy and consider the potentiation of antibiotic efficacy.  |