NOVEL MINIMALLY CROSSLINKED POLYMER AEROGELS FOR THE TREATMENT OF INFECTED CHRONIC WOUNDS

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Introduction

Biofilms are communities of bacteria encased in a self-produced polymer matrix and they are largely responsible for a majority of recalcitrant infections. In chronically infected wounds the presence of a biofilm often results in failure of antibiotic treatment, and significantly delayed wound healing (Schultz *et al*., 2017). This leads to complications such as increased pain, decreased mobility, and poorer mental health for patients (Renner and Erfurt-Berge, 2017).

To form biofilms bacteria use a complex communication system known as quorum sensing (QS) in which they produce and detect small signalling molecules in order to coordinate their behaviour and, thus, form a biofilm. While QS is used by bacteria to improve their ability to infect a host, it presents a potential target for new treatments and antimicrobial compounds (Brackman *et al.*, 2011). One such group of compounds are known as furanones. Furanones are a family of naturally occurring chemicals found primarily in marine and terrestrial plants. Their structure closely resembles that of the signalling molecules used by bacteria such as the wound pathogen *Pseudomonas aeruginosa* for quorum sensing. This allows furanones to interfere with the process of quorum sensing, limiting the ability of affected bacteria to form a biofilm, and resulting in the failure of the bacteria to successfully infect a wound or to render an established infection significantly easier to treat with traditional antibiotics (Proctor *et al.*, 2020).

Objectives

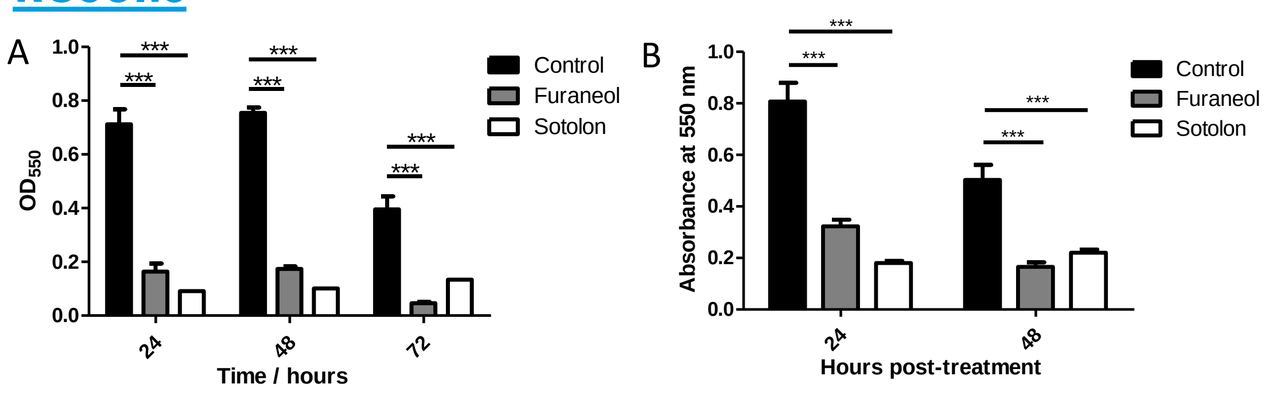
This work had two primary objectives:

- To assess the ability of two naturally occurring furanones to inhibit biofilm formation and aid in reducing established biofilm biomass.
- To develop and assess a novel polymeric drug delivery system for the delivery of furanones to chronically infected wounds.

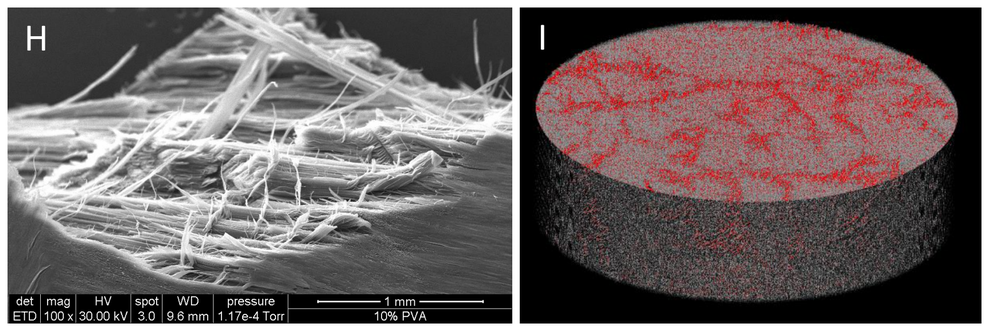
Methods

- Antibiofilm potential of furanones was tested using a standard biofilm biomass assay (O'Toole, 2011) and fluorescent cell viability staining assay (Murray *et al*., 2017) was used.
- Minimally crosslinked polymer aerogels were produced using a rapid, one-step, lyophilisation method.
- To assess furanone loaded aerogels a novel chronic wound biofilm model was developed. This model consists of a nutrient medium consisting of porcine muscle lysate, blood, proteins, and salts.

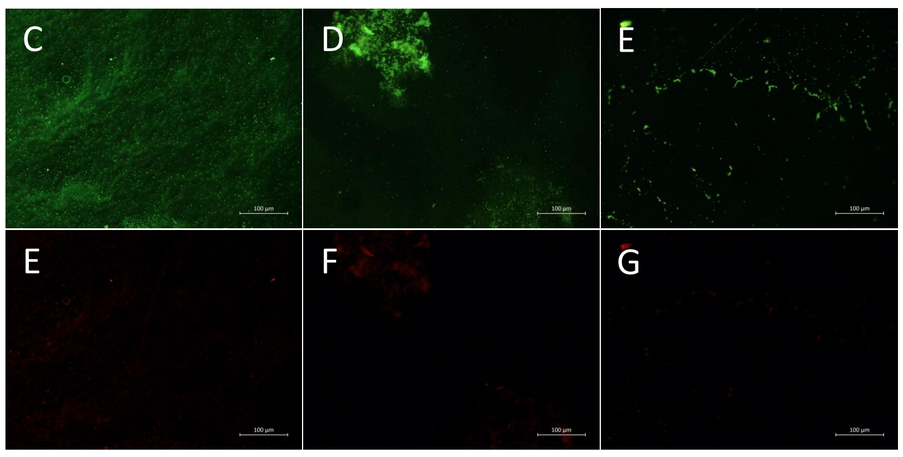
Results



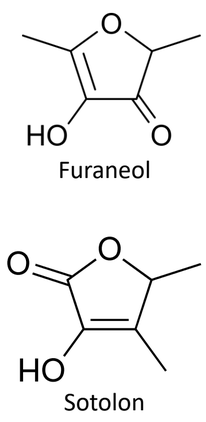
(A) Both Furaneol and Sotolon were shown to have biofilm inhibitory action against *P. aeruginosa* PAO1 when applied at the point of inoculation and this activity was sustained for up to three days showing reductions of up to 88% and 87% respectively. **(B)** Both furanone compounds were able to aid in the dispersal of mature 24h old biofilms with furaneol showing a 67% decrease and sotolon treatment resulting in a 77% decrease in total biofilm biomass. Data represent the mean of three independent replicates. Analysis is by independent T-tests.

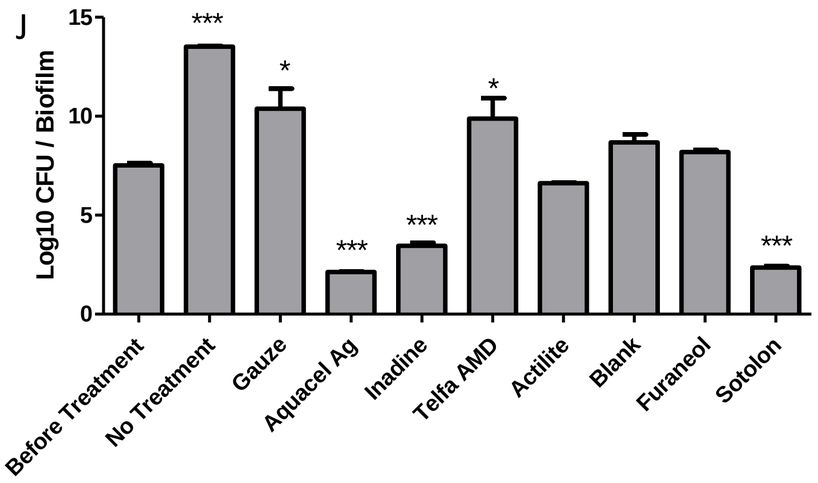


(H) Minimally crosslinked aerogels were shown via scanning electron microscopy to have a uniaxial alignment of their structure with fibres aligned perpendicular with respect to the upper and lower aspect of the aerogel. **(I)** Despite showing alignment of the polymer strands, the material was shown, using micro CT scanning to have a low degree of internal structural homogeneity.



Fluorescent cell viability staining of biofilms showed that untreated biofilms **(C & F)** formed as well populated mono-layers and that both furaneol **(D & G)** and sotolon **(E & H)** treated biofilms showed significantly fewer viable biofilm bound cells (green) without an increase in the proportion of dead bacterial cells (red).





(J) Currently used antimicrobial wound dressings were shown to possess a range of activity against biofilm with industry standard Aquacel AG showing the greatest killing of biofilm bound cells (5.4 log reduction). While furaneol loaded aerogels showed no significant reduction in biofilm bound cells sotolon loaded aerogels showed an efficacy equal to that of Aquacel AG (5.2 log reduction). Data represent the mean of three independent replicates. Analysis is by independent T-tests.

Conclusions

- Furaneol shows excellent efficacy in preventing biofilms from becoming established in early infection.
- Sotolon can be used to disperse established biofilm.
- Minimally crosslinked polymer aerogels are an effective method of applying furanones to chronically infected wounds.
- Furanone loaded aerogels perform as well as, or out perform, currently used antimicrobial wound dressings without introducing the selection pressures that may lead to the development of antimicrobial resistance.

References

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