RESEARCH GROUP: NICHE & Pharmaceutical Science & Practice

Project Title:

Hydrogel-based delivery of halogenated furanones for Inhibition of quorum sensing and biofilm development in the wound pathogen *Pseudomonas aeruginosa*

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Level:

Ph.D.

Background to the project :

Chronic wounds are a persistent and problematic burden in primary healthcare. Biofilms formed by the opportunistic pathogen *P. aeruginosa* contribute to bacterial colonisation in both acute and chronic wound infections (Schaber *et al*., 2007). Such *P. aeruginosa* biofilms lead to influx of high numbers of neutrophils, resulting in persistent inflammation and resultant impairment of wound healing (Fazli et al., 2011). During pathogenesis, *P. aeruginosa* grows primarily as biofilms that provide protection from host defences and from antibiotics (Singh et al., 2000). It is established that cell-to-cell communication plays a crucial role for maturation of biofilms, and their development into a complex three-dimensional architecture. Many host-associated bacteria use chemical signals to monitor their own species population density and coordinate traits such as virulence, toxin production, invasion, etc, in a phenomenon known as quorum sensing (Fuqua et al., 1997). In Gram negative bacteria capable of quorum sensing, a variety of N-acyl homoserine lactones (AHLs) are employed as the primary signalling compounds (Eberl, 1999) (Figure 1).

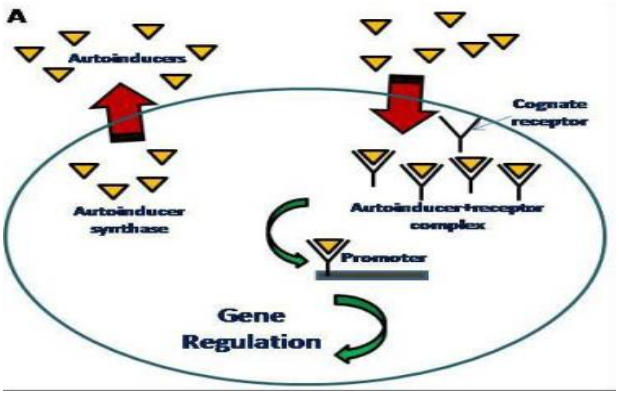

Figure 1: During QS, autoinducers [▼] are produced and diffused freely out of the cell. When the concentration of the autoinducers reach the threshold value, a positive feedback loop will be formed that causes more autoinducers to be synthesized. The autoinducers produced will bind to their cognate receptor [Y] to form an autoinducer-receptor complex [Y] which will then binds to the target promoter that lead to QS gene regulation

Interestingly, and of utility in this work, is the fact that certain structurally similar compounds possess **AHL-antagonistic activity**: for example the halogenated furanones produced by the marine red alga *Delisea pulchra* interfere with the *N*-acylated homoserine lactone (AHL) regulatory system in several Gram-negative bacteria (Koh et al., 2013). As a consequence, it is a rational approach to assess the potential of these furanone compounds as quorum sensing inhibitors. The development of novel **non-antibiotic drugs** to attenuate of bacterial virulence will enable easier pathogen eradication (Givskov et al., 1996), and it is in wound healing and management specifically that we see a potential benefit of such an approach. We hypothesise that delivery of QS antagonists will allow prevention of biofilm development in our *in vitro* biofilm and wound models:

Confirmation of our hypothesis, *vis a vis* prevention of biofilm development by hydrogel delivery of QS antagonists has clear implications for antibiotic stewardship in wound management and the work to be carried out in this PhD project specifically aligns with the recent UKRC themes in the area of antimicrobial resistance and antibiotic stewardship, specifically “reducing unnecessary antimicrobial use” and “accelerating therapeutics”.

Objectives of the research project :

Quorum sensing (QS), a cell density-based intercellular communication system, which plays a key role in regulation of the bacterial virulence and biofilm formation, represents a promising target for developing new strategies against *P. aeruginosa* infection. The overall aim of this work is to demonstrate that disruption of QS in the model organism *P aeruginosa*, can be achieved via chemical effectors, and to show that disruption of QS leads to a reduction in biofilm biomass *in vitro*. This information will be used to construct new wound management modalities wherein QS antagonists are incorporated into a novel, shear sensitive hydrogels that, upon release of those antagonists, will enable disruption of *P. aeruginosa* biofilm.

Year 1. Hydrogel development and drug loading

Objective 1 – The first objective of this work is to develop methods of analysis for furanone compounds, using both spectroscopic and liquid chromatography methods. Sigma supply these compounds at reasonable cost, and this completion of this objective will result in robust methods for measurement of these molecules in biologically relevant matrices in later parts of the project.

Objective 2 – The second objective will be to load a shear-sensitive hydrogel with furanone derivatives and determine the effect on rheological properties. Our group has extensive experience in characterising cross-linked hydrogel formulations and determining the effect on stability and cumulative release profiles. This will enable determination of release kinetics of furanones from hydrogels, thereby demonstrating applicability of hydrogels as a drug delivery vehicle. Use of an *in vitro* cell culture scratch assay will allow us to confirm that furanones do not, themselves, inhibit recovery in an *in vitro* model of wound healing.

Year 2. Biofilm formation and evaluation of QS inhibition.

Objective 3 – The team have extensive experience of growing microbial biofilms on a variety of abiotic surfaces and in measurement of biomass yield/structure, in addition to measurement of QS molecules. Here, we will establish a biofilm model using *P. aeruginosa* and evaluate the effect of direct, surface-mediated delivery of furanone compounds on the architecture, development and viability of biofilms using standard methods (Deligianni et al, 2010). The use of confocal microscopy with GFP/RFP tagged strains will also be employed to assess biofilm development and structure in the presence and absence of QS disruptors.

Objective 4 – running in parallel with objective 3, we will determine the effect of furanone exposure at physiologically relevant levels on biofilm development. We hypothesise that addition of furanones will disrupt QS, this being directly assessed as a reduction in biofilm yield.

Year 3

Objective 5 – functional genomics analysis of biofilm formation and impedance. Strains from the Seattle *P. aeruginosa* PAO1 transposon mutant library are available to the research community through the University of Washington (Held et al., 2012). Mutants available for most nonessential genes, and we will initially focus on *las* and/or *rhl* deficient mutants: these strains are unable to produce quorum sensing molecules (Wagner et al,.2003), and represent excellent tools with which to validate our earlier work. Thus, mutants deficient in QS can be compelled to biofilm production by the addition of exogenous autoinducers [e.g. N-(3-oxododecanoyl) homoserine lactone and N-butyryl homoserine lactone]. The ability to precisely control levels of autoinducers will enable us to induce variable levels of biofilm, with robust measurements using methods developed in Y1/Y2 of the project. We hyopthesise that when QS antagonists are added, a dose response will be observed, enabling subsequent tailoring of drug delivery. To summarise, wild type cells – form biofilm, biofilm repressed by furanones. In Las/rhl mutant cells, no biofilm is produced (negative control). Upon addition of exogenous QS autoinducer molecules – biofilm is formed. Add QS autoinducer molecules + furanones antagonists – no biofilm, as furanones disrupt effect of exogenous autoinducers.

Methods to be used :

Oscillating rheometry will be used mostly in the first year to assess the flow properties of our novel hydrogel system. In particular, this method will enable our group to determine the effect of drug addition to visco-elastic properties. Dissolution testing will be used to monitor furanone release with respect to time. This method is a standard tool used to characterise release and to enable an estimation to be made of the total possible dose during an exposure time. It will also be used to determine the presence is molecular interactions, which are a frequent feature of such formulations.
HPLC and spectroscopic methods will be used to determine drug release and characterise stability, in terms of possible degradation products in the hydrogel.
Microbial growth and Biofilm formation will be assessed either in planktonic culture or on glass slides or plastic surfaces using well established methods that are routinely employed in our labs. From previous work we know that QS molecules are amenable to extraction from culture broths and analysis by LC/ion trap MS, and we have this ability in house.
Viability of cells in biofilms (or those released from biofilms) will be assessed by direct plate counting, and also using the backlight cell viability assay. The world class bioimaging facility at Ulster in addition possesses SEM, ESEM and super-resolution microscopy facilities, and a number of these analysis modalities will enable ultrastructural analysis of natural unimpeded biofilm, and disrupted biofilms

Skills required of applicant :

A 2:1 (Hons.) degree in Pharmacy, Pharmaceutical Science, Biomedical Sciences, Microbiology or closely related discipline is preferable. Given the need to take detailed measurements using the rheological studies, attention to detail and a careful approach to notetaking is essential.

The student will be fully trained, including courses in experimental design, statistical analysis, and critical thinking, as well as in basic microbiology, molecular biology, microscopy/ immunostaining.

Experience in basic microbiology, biochemistry and histology would be an advantage, as would knowledge of/ability in molecular biology, microscopy and immunostaining procedures. The ability to use bioinformatics tools for sequence analysis is desirable, as is experience of working in a research laboratory. An interest in chemistry is desirable but not essential.

References:

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