Biofilms: What are they and why do we care?

Guest speakers: Mark Webber & Freya Harrison
Moderator: Laura Piddock

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- Connect people
- Knowledge sharing
Antimicrobial Encyclopaedia

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Today’s speakers

Biofilms: What are they and why do we care?

Mark Webber
Group Leader
Quadram Institute (UK)

Freya Harrison
Principal Investigator
University of Warwick (UK)

Moderator:
Laura Piddock
Scientific Director
GARDP
Mark Webber has been a group leader at the Quadram Institute in Norwich UK, since the start of 2017. His research group studies the molecular mechanisms of antibiotic resistance with focus on understanding how, where, when and why bacteria evolve antibiotic resistance.

A particular interest of his group is bacterial biofilms and how bacteria adapt to antimicrobial pressure within them. Their work employs a variety of molecular microbiology, functional genomic and bioinformatic approaches to study bacterial survival and resistance mechanisms. Mark has published over 100 articles relating to antimicrobials and has acted as an editor for various journals.
Biofilms; the good the bad and the ugly

Mark Webber

Quadram Institute, Norwich, UK
mark.webber@quadram.ac.uk
@ma_webber
What are biofilms and why should we care about them?
What are biofilms?

- Bacteria readily form communities of aggregated cells
- Cells forming a biofilm produce an extracellular matrix
- Often multispecies
- Found pretty much everywhere (wet, dry, biotic, abiotic)
- Clinically very important – IPC* focusses on biofilms, and cause in vivo and device associated infections
- Also industrially very important
- Significantly different properties compared to cells grown in liquid – a distinct lifestyle
- Lots of heterogeneity within a biofilm in cell behaviour
- Usually highly tolerant of antibiotics

*IPC – Infection, prevention and control
The structure of a biofilm varies with conditions

https://www.biofilms.ac.uk/biofilm-image-gallery/
How does a biofilm form?

- A generalised lifestyle describes:
  - initial colonisation of a site
  - commitment to a sessile lifestyle
  - production of biomass and matrix
  - release of cells allows colonisation of new environments
Antimicrobial resistance and biofilms
Why are biofilms so hard to kill with antibiotics?

A combination of mechanisms are commonly relevant. Environmental and bacterial cues (quorum sensing etc) affect all these mechanisms.

- Altered physiology
- Persister cells
- Low permeability
- Dormancy
- HGT*
Why are biofilms so hard to kill with antibiotics?

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- Altered physiology
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Matrix matters, but not for all drugs....

Why are biofilms so hard to kill with antibiotics?

A combination of mechanisms are commonly relevant. Environmental and bacterial cues (quorum sensing etc) affect all these mechanisms.
The challenge of persister cells

- Bacterial populations are heterogeneous and contain persister cells
- Biofilms have particularly high fractions of persister cells
- These are often insensitive to a wide range of antibiotics

Do biofilms care about low levels of antibiotics?
Biofilm evolution model

- Simple
- Adaptable
- Multiple lineages
- Multiple organisms
- Multiple stressors

In the absence of drug the model rapidly selects for increased biomass production

(Trampari et al. npj Biofilms and Microbiome 2021)
Biofilms rapidly evolve resistance

- *Salmonella* biofilms exposed to *sub-lethal* cefotaxime, azithromycin or ciprofloxacin
- Resistance emerged in all cases
- Patterns were similar to planktonic controls although there were differences in rates and cross resistance
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Biofilms rapidly adapt to antibiotics

**MICs of:**
- Azithromycin
- Cefotaxime
- Chloramphenicol
- Ciprofloxacin
- Kanamycin
- Nalidixic acid
- Tetracycline
- Triclosan

**Log2 fold change in resistance profile (compared to wild type average)**
Biofilms rapidly adapt to antibiotics

BUT….. Resistance comes at a cost to biofilm formation

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Log2 fold change in resistance profile (compared to wild type average)

Log2 fold change in biofilm forming ability (compared to wild type average)
Biofilms rapidly adapt to antibiotics

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\text{MICs of:}
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BUT….. Resistance comes at a cost to biofilm formation

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Log2 fold change in resistance profile (compared to wild type average)

Exposure to azithromycin, cefotaxime, and ciprofloxacin shows a significant change in MICs over time, indicating adaptation to antibiotics.
Analysis of *Salmonella* mutants:

- Each drug selected distinct mutations
- Biofilm and planktonic lineages segregate
- Similar numbers of mutations for each drug
- No ‘universal’ mechanism of resistance was seen
- But some targets repeatedly seen: *acrB, ramR, envZ*
Azithromycin as an example

- Macrolide antibiotic
- Good activity
- Stops protein synthesis
- Has to get into the cell to be active
- Important for treatment of *Salmonella*
Novel mechanism of azithromycin resistance

(Trampari et al. in revision)
Novel mechanism of azithromycin resistance

(Trampari et al. in revision)
Novel mechanism of azithromycin resistance

(Trampari et al. in revision)
Why does developing resistance impair biofilm?

- We have previously seen an inverse relationship between expression of \textit{ramA} and biofilm formation.
- Invoking \textit{ramA} to gain resistance has a cost to biofilm.

Baugh \textit{et al.}, 2014, Holden \textit{et al.}, 2020
Conclusions

Biofilms are everywhere!

Biofilms matter!

Biofilms are drug resistant as a result of multiple factors

Biofilms do care about low concentrations of drugs

There is no universal mechanism of AMR in biofilms

Understanding fundamental biology is important in developing ways to control biofilms
Acknowledgments
Freya Harrison is a Principal Investigator in the School of Life Sciences at the University of Warwick, UK with an interest in how the development of biofilm infection is influenced by interactions between infecting bacteria, and between bacteria and their environment.

Freya’s research group builds and employs high-validity models of biofilm infections to understand how specific host environments can alter bacterial physiology and result in highly antibiotic-resistant phenotypes. Her group also uses bespoke models to test the activity of new antibacterial agents, including natural products derived from historical infection remedies. This ethnopharmacological approach to antimicrobial drug discovery is fueled by collaborations that bridge the traditional sciences and humanities divide.
Building & using models of lung and wound biofilms

Dr Freya Harrison

Harrison Lab @ Warwick

Lab alumna

Dr Blessing Anonye, University of Central Lancashire

Funders

- Medical Research Council
- Diabetes UK
- National Biofilms Innovation Centre
- Biotechnology and Biological Sciences Research Council
Biofilm in cystic fibrosis (CF) lungs & diabetic foot infections (DFI)

- **Bacteria**
- **Biofilm matrix**
- **Eukaryotic nuclei**

*Oates et al. J Diabetes Res 6:153586*

*P. aeruginosa eukaryotic nuclei*

*Bjarnsholt et al. Pediatr Pulmonol 44:547*
Biofilm in CF & DFI: similarities

- Normal defence / clearance mechanisms are compromised
- Site of infection is biochemically abnormal
- Infection lasts for months, year or even decades, despite antibiotic treatment
- Even if isolated bacteria from swabs etc. are susceptible in standard *in vitro* tests
- Some pathogens in common (ESKAPE), often biodiverse
- Huge health and economic burden
- New biofilm-busting therapies desperately needed!
Biofilm in CF & DFI: differences

- Biofilm biology is highly context-specific

- Opportunistic pathogens flexibly adapt their physiology to the unique environments of a diabetic ulcer or the CF lung – this affects antibiotic sensitivity
Biofilm in CF & DFI: differences

- Biofilm biology is highly context-specific

- Opportunistic pathogens flexibly adapt their physiology to the unique environments of a diabetic ulcer or the CF lung – this affects antibiotic sensitivity

- Most candidate antibacterial compounds ultimately fail to translate to clinical use – especially when biofilms are considered

- We need context-specific biofilm models
  - For drug discovery
  - For better prescribing
  - For better understanding of AMR evolution

- My lab uses high-validity models of chronic wound and CF lung biofilm in our work on the fundamental microbiology of common biofilm pathogens and for discovery of novel therapeutics
Ex vivo CF lung model & synthetic chronic wound model

More info: freyaharrison.weebly.com/publications

*SCFM – Synthetic cystic fibrosis sputum
Ex vivo CF lung model & synthetic chronic wound model

Recover biofilm cells for plating & other downstream assays; assay supernatant for secreted products etc.

More info:
freyaharrison.weebly.com/publications

An in vitro model of bacterial infections in wounds and other soft tissues

MARIA WERTHÉN,1 LINA HENRIKSSON,1 PETER OSTRUP JENSEN,2 CLAUS STERNBERG,3
MICHAEL GIVSKOV3 and THOMAS BJARNSHOLT1,2

Peptone water + fetal bovine serum + collagen

P. aeruginosa, 48h in wound model
P. aeruginosa in chronic wound biopsy
Why using a tailored biofilm model is important in drug/target discovery

- **Avoid false positives**: drugs or formulations that look efficacious *in vitro*, but fail *in vivo*
  - And use your model to **find adjuvants** that could overcome this (e.g. aid biofilm penetration)

- **Avoid false negatives**: drugs or formulations that fail *in vitro* testing, but prove efficacious *in vivo* in at least some contexts
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A tale of two models...

Story 1: Using the *ex vivo* lung model to better understand the physiology and AMR of *P. aeruginosa* biofilm in CF

Story 2: How we’ve used the CF and chronic wound models in our work on antibacterial natural product preparations (avoiding both false positives and false negatives!)
Biofilm and antibiotic-tolerant phenotype of *P. aeruginosa* in CF lung model

**Probing efficacy of colistin for treating *P. aeruginosa* in the lung model**

As you might expect, lung-grown biofilms could survive concentrations >> MBEC* as measured in a Calgary biofilm device using SCFM.

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*MBEC – Minimal biofilm eradication concentration*
Biofilm and antibiotic-tolerant phenotype of *P. aeruginosa* in CF lung model

Probing efficacy of colistin for treating *P. aeruginosa* in the lung model

Dr Andrew Edwards, Akshay Sabnis (Imperial) Sweeney (2020) *Microbiology* 166:1171

Only 12-19% of the supplied dose can enter the biofilm matrix!
Biofilm and antibiotic-tolerant phenotype of *P. aeruginosa* in CF lung model

But reduced penetration is not the full story...

Jenny Littler
Unpublished data
Biofilm and antibiotic-tolerant phenotype of *P. aeruginosa* in CF lung model

Transcriptome in the model: RNAseq of 5,829 genes in *P. aeruginosa* PA14 over 7 days of infection

Niamh Harrington & Jenny Littler
Biofilm and antibiotic-tolerant phenotype of *P. aeruginosa* in CF lung model

Transcriptome in the model: RNAseq of 5,829 genes in *P. aeruginosa* PA14 over 7 days of infection

A snapshot at 48h

DEGs* in lung biofilm vs. SCFM in vitro

DEGs in SCFM around lung vs. SCFM in vitro

1007

289

90

↓ Quorum sensing, phenazines, Type VI secretion
Changes in genes associated with abx/AMP* resistance

*DEGs – Differentially expressed genes, abx – antibiotics, AMP – Antimicrobial peptides

Niamh Harrington & Jenny Littler
Use the right biofilm model for drug discovery, and use it early in testing

One of our candidate ancientbiotics: ‘Bald’s eyesalve’
Use the right biofilm model for drug discovery, and use it early in testing

Planktonic killing of soft tissue pathogens in synthetic wound fluid (SWF)

**Gram-negatives**
- *Pseudomonas aeruginosa* PA14
- *Stenotrophomonas maltophilia*

**Gram-positives**
- *Staphylococcus aureus* Newman
- *Staphylococcus aureus* USA300

Peptone water
Fetal bovine serum

Dr Blessing Anonye (UCLan)
Use the right biofilm model for drug discovery, and use it early in testing

Do we see killing of biofilms in synthetic chronic wound?

**Gram-negatives**

- Pseudomonas aeruginosa PA14
- Serratia marcescens
- Enterobacter cloacae
- Acinetobacter baumannii

**Gram-positives**

- Staphylococcus aureus Newman
- Staphylococcus aureus USA300
- Staphylococcus epidermidis
- Streptococcus pyogenes

Dr Blessing Anonye (UCLan)
Use the right biofilm model for drug discovery, and use it early in testing

The synthetic chronic wound biofilm model revealed the need for >1 active molecule!

- Explains most bactericidal activity in planktonic culture
- Is not a good drug candidate
Use the right biofilm model for drug discovery, and use it early in testing

The synthetic chronic wound biofilm model revealed the need for >1 active molecule!

But allicin cannot explain activity of Bald’s eyesalve in SCW biofilm...

- Explains most bactericidal activity in planktonic culture
- Is not a good drug candidate

[Graph showing bacterial counts with different treatments]

Jessica Furner-Pardoe
Use the right biofilm model for drug discovery, and use it early in testing.
Use the right biofilm model for drug discovery, and use it early in testing.
Use the right biofilm model for drug discovery, and use it early in testing

What we, as microbiologists, agree are priorities in antibacterial R&D

- Chronic biofilm infections – extensive & unpredictable AMR
- Better diagnostic / R&D testing of agents to treat these
- Novel agents to treat biofilm infection

How we’re trying to address these

- Developing and using high-validity *ex vivo* and *in vitro* models of biofilm infection
- Context specific – match physicochemical environment of pathogens (CF, wounds)
- Aid in drug/adjuvant discovery
- Evolution of resistance in different infection models
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Thank you for joining us