Bacterial Signaling Systems As Antimicrobial Targets

Michael Givskov
Professor, Director
Costerton Biofilm Center
Faculty of Health and Medical Sciences
University of Copenhagen, Denmark

Research Director
Singapore Centre on Environmental Life Sciences Engineering (SCELSE)
Nanyang Technological University
Singapore

CBC collaborates closely with the Asian Biofilm Center SCELSE
Pathogens live in biofilms in the environment and in the host

Biofilms carry striking similarities among microbes:

• Shield pathogens from external stress: in the environment (e.g. disinfection, predation) and in the host (e.g. antibiotics, phagocytosis).

• Biofilm infections often become chronic with development of destructive inflammatory conditions
Biofilm infections

• are associated with implants and medical equipment including artificial hips, knees, heart valves, stents, vascular prostheses, pacemakers and artificial hearts.

• are associated with distinct infectious disease states including chronic wounds, cystic fibrosis, corneal, and urinary tract infections.
Biofilms in chronic infections are 5-100 μm in diameter.

In vivo biofilms

Chronic wound

In vitro
Patients often acquire biofilm infections under conditions of critical illness, insertion of medical devices and implants, or otherwise impairment of the primary host defenses.
Biofilms are heterogeneously distributed in 3 dimensions

Number of *Pseudomonas aeruginosa*

<table>
<thead>
<tr>
<th>@Position</th>
<th>Wound N = 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>920±9%</td>
</tr>
<tr>
<td>3</td>
<td>300±13%</td>
</tr>
<tr>
<td>6</td>
<td>8200±8%</td>
</tr>
<tr>
<td>9</td>
<td>800±10%</td>
</tr>
<tr>
<td>12</td>
<td>15±5%</td>
</tr>
</tbody>
</table>
Tissue fillers
Adverse reactions to tissue fillers caused by biofilms
Biofilms are “hard to see” & “hard to kill”

• Bacteria in biofilms attain the highest levels of resistance to the current antibiotics, and almost unlimited capacity to evade host immunity.
An antibiotic is a substance that inhibits the growth of a microorganism.
Drivers of novel antibiofilm technologies:

- Global resistance is a rapidly emerging crisis
- Few new antimicrobials are being developed
- Novel targets are necessary
- Mechanism of action must not lead to resistance
Chemical biology: the quest for novel targets and antimicrobials

• Antimicrobials for Biofilm Control.
• Synergistic actions with conventional antibiotics?
Chemical biology platform

Chem. Libraries:

**Natural products**
- Bioassay-guided fractionation
- Organic chemistry
- Structure identification
- Biofilm models
- Animal biofilm models

**Synthetic compounds**
- Virtual docking
- High-throughput screening platform
- Biol. screens
Our strategy:

• To force biofilm bacteria into an “unprotected” mode with chemical signals.

• Bacteria are subsequently killed by the immune system and/or conventional antibiotics.
Targets and concepts

Cell-cell signaling (Quorum Sensing)

- Chemicals that jam QS switch-off immune protection, virulence and reduce inflammation and antibiotic resistance
- PMNs can prey on the biofilm

Secondary messenger c-di-GMP signaling

- Chemicals that jam c-di-GMP signaling cause dispersal and can dismantle biofilms
- PMNs prey on dispersed cells
CLASSICAL TARGETS FOR DISRUPTING THE PSEUDOMONAS AERUGINOSA QS SYSTEM

The QS controllers

QS controlled traits

Biofilm traits

Virulence factors

Shielding
Rhamnolipids
eDNA
Efficient QS blockers

Figure 1. Bacterial autoinducers and inhibitors. (A) Examples of bacterial autoinducers belonging to distinct structural classes. (B) Examples of synthetic QS inhibitors in P. aeruginosa (11–15). Approximate IC₅₀ values (from different reporter assays) are listed below the compounds. Efficacies of 11 and 12 were reported by Smith et al.,¹¹,¹² 13 and 15 by Geske et al.,¹³ and 14 by Hentzer et al.¹⁴

Givskov et al 1996

Hentzer et al 2003

D. pulchra
Furanone C-30 is an almost perfect example of a chemical biology approach to block QS

A successful chemical biology approach to control virulence and shielding through the Quorum Sensing systems.

QS inhibitors do not kill *P. aeruginosa* per se but promote rapid clearance.
delivering the “proof of concept” that blockage of quorum sensing with an experimental drug could function as a biofilm antimicrobial in vivo.
Ajoene – the major bioactive QSI compound

Ajoene content in garlic bulbs: 600-700 μg/g

Antimicrobial effects in vivo

Pulmonary infection (mouse) model

25 μg ajoene g⁻¹ BW 
every 24 h 
2 days prophylactic treatment 
day 2 post-infection

12.5 μg ajoene g⁻¹ BW 
every 24 h 
2 days prophylactic treatment - 
day 1 post-infection

P. aeruginosa, 
PAO1

Early clinical isolate CF438 treated with 12.5 μg/ml ajoene

Clinical isolate

Byt billede:
Ny slide og klik på ikon, 
indsæt billede
11 genes were significantly down-regulated by ajoene 
All the genes are QS regulated

Number of genes significantly down-regulated by other QSIs
- Furanone C-30: 163 genes (85% of the QS-controlled genes)
- Patulin: 157 genes (49% of the QS-controlled genes)
- Penicilllic acid: 300 genes (34% of the QS-controlled genes)
Horseradish

Iberin

- Iberin has never been isolated from horseradish before
- Cabbage, broccoli, radish are known to contain iberin

- Horseradish is a well known producer of allyl isothiocyanate.

Allyl isothiocyanate

69 common food products and plants were screened for QSI activity
**ADDITIONAL AND COMPLEMENTARY TARGETS FOR QS INHIBITION**

SCELSE has designed a new series of synthetic derivatives with improved activities.

SCELSE has developed a series of Au containing chemical compounds.

Auranofin

**SCELSE has designed a new series of synthetic derivatives with improved activities**
Exposure of neutrophils (PMNs) and macrophages to biofilms signals through the QS system to produce rhamnolipids that kill the immune cells.

This is a fundamental immune protection mechanism.
As a consequence of disrupting the biofilm biology, the bacteria are no longer protected and become accessible to cellular immunity.

QS is on

Rhamnolipid shield up

Biofilm

QS is off

Rhamnolipid shield down

Neutrophils (PMNs)

Flow chamber for in vitro biofilm growth
Bioassay guided fractionations and isolation procedures showed that the PMN kill was caused by QS controlled rhamnolipid production.

Rapid necrotic killing of polymorphonuclear leukocytes is caused by quorum-sensing-controlled production of rhamnolipid by *Pseudomonas aeruginosa*

Peter Ø. Jensen, Thomas Bjarnsholt, Richard Phipps, Thomas B. Rasmussen, Henrik Calum, Lars Christoffersen, Cleus Moser, Paul Williams, Tacjana Prestler, Michael Givskov and Niels Halby


No rhamnolipids
Macrophages containing *P. aeruginosa rhlA-gfp*.

This cascade can be blocked with Carboxyl PTIO.
NO induced (implant model)

SYTO62

GFP

Merged

6 h

24 h
**cdrA-gfp reduced (implant model)**

- **SYTO62**
- **GFP**
- **Merged**

6 h

24 h
pqS-A-gfp induced (implant model)
rhlA-gfp induced (implant model)
No induction of *rhlA-gfp* with Carboxyl PTIO
Combination of QS inhibitors with conventional antibiotics

- QS inhibitor, + tobramycin
- QS inhibitor, - tobramycin
+ QS inhibitor, + tobramycin

Red is dead
Green is alive

Flow chamber for in vitro biofilm growth

Reducing the eDNA shield increases tobramycin efficacy
Spatio-temporal distribution of live and dead bacteria in tobramycin-treated \textit{lasR, rhlR} QS negative biofilms that were grown with or without exogenous DNA.
Live and dead bacteria in tobramycin-treated *P. aeruginosa* biofilms that were grown with or without addition of lysed PMNs (corresponding to $10^4$ PMNs/ml added to the medium after two days of cultivation)

QS inhibitors and tobramycin treatments show synergy *in vivo*

The vicious cycle !!!

• Virulence factors recruit neutrophils and macrophages, which upon contact with the rhamnolipid containing biofilm matrix lyse, releasing inflammation mediators.

• This incapacitates cellular immunity, increases inflammation, ultimately leading to tissue destruction.

• Rhamnolipid driven destruction of neutrophils and macrophages release large quantities of DNA that eliminate the antimicrobial effects of aminoglycosides and antimicrobial peptides.
QS inhibitors work as antimicrobials on biofilms because they can reinstate proper anti-microbial action of the immune system.

QS inhibitors prevent the vicious cycle.
Cell-cell signaling (Quorum Sensing)

Chemicals that jam QS switch-off immune protection, virulence and reduce inflammation and antibiotic resistance

PMNs can prey on the biofilm

Secondary messenger c-di-GMP signaling

Chemicals that jam c-di-GMP signaling cause dispersal and can dismantle biofilms

PMNs prey on dispersed cells
New natural compounds discovered that lower c-di-GMP

\[ pYhjH \]

pJN105 based
Low copy number
Broad-host-range

Arabinose induction of the PDE depletes c-di-GMP pool

Uninduced = ~ 15 pmol/mg protein
Induced = below LC/MS/MS detection limit.
ABC-Target 2: c-di-GMP signaling

C-di-GMP reducing treatment – disperse biofilm cells

- Induce yhjH
- Dispersing chemical
pYhjH
Proof of concept: c-di-GMP depletion eradicates biofilms *in vivo*

A herbal c-di-GMP lowering compound (to be published)

c-di-GMP pmol/mg prot. in wspF

Bacteriology of implants

Bacteriology of spleens

Placebo
Herbal compound injected
Dispersed bacteria can be easily killed

- Dispersed cells are highly sensitive to iron stress and antibiotics.
- The combination of a biofilm-dispersing agent, with tobramycin kills a large part of the dispersed cells.
- The combination of a biofilm-dispersing agent, an iron chelator and tobramycin eradicates all dispersed cells.
TYPICAL REACTION-RELEASE PRODUCTS FROM IMPLANT SURFACE

Lipase release:
QS and QSI molecules
Incl. double warhead tech.

- Concept explored on biocompatible polymers
- Developed polymers that respond to enzymatic activity
- Flexible scaffold
• Prof. Staffan Kjelleberg
• Prof. Stephan Schuster
• A/Prof. Liang Yang
• A/Prof. Shu Sin Chng
• Dr. Yang Liu
• Song Lin Chua
• Salido May Margarette
• Sean Yang En Tan
• Joey Kuok Hoong Yam

• Prof. Tim Tolker-Nielsen
• Prof. Thomas Bjarnsholt
• Prof. Thomas Eiland Nielsen
• Prof. Niels Høiby
• A/Prof. Jens Bo Andersen
• Dr. Tim Holm Jakobsen
• Dr. Louise Dahl Hultqvist
• Dr. Morten Rybtke
• Dr. Maria Alhede
• Dr. Morten Alhede
• Dr. Peter Ø. Jensen
• Dr. Hong Wu
• Dr. Henrik Almblad
• MSc. Julie Dora Dona Groizeleau