In vitro models for the study of the activity of anti-infective agents against biofilms

Françoise Van Bambeke, PharmD, PhD

Pharmacologie cellulaire et moléculaire
Louvain Drug Research Institute
Université catholique de Louvain,
Brussels, Belgium

<www.facm.ucl.ac.be>
Biofilms in human infections

Biofilms are associated to 65\textsuperscript{a}-80\textsuperscript{b} % of human infections and can colonize virtually all organs …

<table>
<thead>
<tr>
<th>Sites of Primary and Secondary Biofilm Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SITES OF PRIMARY INFECTION:</strong></td>
</tr>
<tr>
<td>Subvenous catheter</td>
</tr>
<tr>
<td>Mouth</td>
</tr>
<tr>
<td>Artificial hip implant</td>
</tr>
</tbody>
</table>

ear  
nose  
throat  
throat  
throat  
mouth & teeth  
eye  
lung  
heart  
heart  
kidney  
kidney  
gall bladder  
gall bladder  
pancreas  
pancreas  
nervous system  
nervous system  
skin  
skin  
bone  
bone  
implanted medical devices

\textsuperscript{a}CDC 1999; \textsuperscript{b}Lewis et al, Nat Rev Microbiol. 2007; 5:48-56
Antibiotics and biofilms in clinical practice

When and how should we treat biofilms in chronic sinusitis?

Jain R, Douglas R.

Treatment failure is not rare...
How to find a solution?

→ Appropriate models…
In vitro static models

- Pegs
- Multiwell plates
# Antibiotic activity: planktonic vs. biofilm cultures

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal inhibitory concentration</td>
<td>MIC</td>
<td>The lowest concentration of an antibiotic that inhibits the visible growth of a planktonic culture after overnight incubation.</td>
</tr>
<tr>
<td>Minimal biofilm inhibitory concentration</td>
<td>MBIC</td>
<td>The lowest concentrations of an antibiotic that resulted in an OD650 difference at or below 10% (1 Log difference in growth after 6 h of incubation) of the mean of two positive control well readings.</td>
</tr>
<tr>
<td>Minimal bactericidal concentration</td>
<td>MBC</td>
<td>The lowest concentration of an antibiotic producing a 99.9% CFUs reduction of the initial inoculum of a planktonic culture.</td>
</tr>
<tr>
<td>Biofilm bactericidal concentration</td>
<td>BBC</td>
<td>The lowest concentration of an antibiotic producing a 99.9% reduction of the CFUs recovered from a biofilm culture compared to growth control.</td>
</tr>
<tr>
<td>Minimal biofilm eradication concentration</td>
<td>MBEC</td>
<td>The lowest concentration of an antibiotic that prevents visible growth in the recovery medium used to collect biofilm cells.</td>
</tr>
<tr>
<td>Biofilm prevention concentration</td>
<td>BPC</td>
<td>Same as MBIC but bacterial inoculation and antibiotic exposure occur simultaneously.</td>
</tr>
</tbody>
</table>

*Macià et al, Clin Microbiol Infect. 2014; 20(10):981-9*
Determining antibiotic activity against planktonic bacteria

**MIC (minimal inhibitory concentration)**

**MBC (minimal bactericidal concentration)**

- Control
- 0.5 μg/mL
- 1 μg/mL
- 2 μg/mL
- 4 μg/mL
- 8 μg/mL

**MIC**

**MBC**
Static models: Calgary Biofilm Device

Determination of Minimal Biofilm Eradication Concentration (MBEC)

Comparing antibiotic activity: planktonic / biofilm cultures

Ampicillin and levofloxacin vs. *H. influenzae* from middle ear fluid

Activity against biofilm $<<$ activity against planktonic bacteria

*Takei et al, J Infect Chemother 2013; 19:504–9*
PK/PD studies: the principles

Pharmacokinetics
conc. vs time

Pharmacodynamics
conc. vs effect

PK/PD
effect vs time
Static models: 96-well polystyrene plates

appropriate dyes
to evaluate biomass or bacterial load
Quantifying biomass and metabolic activity in biofilms

Quantifying biomass and metabolic activity in biofilms

**biofilm mass**

**crystal violet**


**Gram(+) bacteria**

**metabolic activity**

**Gram(-) bacteria**

resazurin

resorufin

fluorescein diacetate

fluorescein
diacetate


CFU counting vs. RF fluorescence

An example for S. aureus

relation between fluorescence and bacterial inoculum for S. aureus

30 min. incubation

log resorufin fluorescence

log CFU/ml

log OD620 nm

CFU & RF signal proportional

sensitivity depending on incubation time

Pharmacodynamic model for antibiotic activity

An example with young biofilm of *S. aureus*

![Graph showing vancomycin concentration vs % control value](image)

- **CT**
- **RF**
- **CV**

- **C<sub>25-50-75</sub>** « rel. potency »
- **Emax** « efficacy »

*Bauer, Siala et al, Antimicrob Ag Chemother. 2013;57:2726-37*
**S. pneumoniae** biofilms - influence of maturity

**moxifloxacin**

**maximal efficacy \( \uparrow \) with maturity**

---

Vandevelde et al, Antimicrob Ag Chemother. 2014; 58:1348-58
S. pneumoniae biofilms - influence of maturity

Vandevelde et al, Antimicrob Ag Chemother. 2014; 58:1348-58
Comparison of PD parameters for different drugs

*S. pneumoniae*

Vandevelde et al, Antimicrob Ag Chemother. 2014; 58:1348-58
Study of drug combinations

P. aeruginosa exposed to ciprofloxacin + clarithromycin

Macrolides are synergistic with fluoroquinolones on preformed biofilms

See also poster by Mustafa et al.
How to explain this “apparent” resistance or tolerance?

→ importance of PK/PD parameters
PK/PD parameters in biofilms

- **pharmacokinetics**
  - diffusibility through the matrix
  - bioavailability within the biofilm
  - access to bacteria
  - efflux out of bacteria

- **pharmacodynamics**
  - bacterial responsiveness (metabolic activity of bacteria)
  - antibiotic expression of activity (local environment [O₂, pH, ..])

---

Janssen, Nature 2009
PK/PD parameters in biofilms

- diffusibility through the matrix
- bioavailability within the biofilm
- access to bacteria
- efflux out of bacteria
Importance of antibiotic concentration inside biofilms for activity

S. aureus biofilms


Activity in biofilm is correlated to antibiotic penetration
How to help antibiotic to reach their target?

→ disruption of the matrix
IacA and polysaccharide synthesis in *S. aureus*

*Ica A is involved in N-acetylglucosamine homopolymer synthesis*

Importance of *icaA* expression and PNAG abundance for antibiotic activity in biofilms

*S. aureus* biofilms

<table>
<thead>
<tr>
<th>strain</th>
<th><em>icaA</em> expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC33591</td>
<td>1</td>
</tr>
<tr>
<td>2011S027</td>
<td>1.8 ± 0.5*</td>
</tr>
<tr>
<td>2003/1083</td>
<td>4.0 ± 0.6 *</td>
</tr>
<tr>
<td>2009S025</td>
<td>2.5 ± 0.5*</td>
</tr>
<tr>
<td>2005/104</td>
<td>4.2 ± 0.4*</td>
</tr>
<tr>
<td>2005/179</td>
<td>6.0 ± 0.9*</td>
</tr>
<tr>
<td>2009S028</td>
<td>4.1 ± 0.2*</td>
</tr>
<tr>
<td>2003/651</td>
<td>16.3 ± 0.7*</td>
</tr>
</tbody>
</table>

Fluoroquinolone activity in biofilm is inversely correlated with *icaA* expression
The antifungal caspofungin as an inhibitor of polysaccharide synthesis

Candida albicans

Glucan synthase

Staphylococcus sp.


Adapted from Arnold, Kucer’s 6th edition

Candida albicans

Adapted from Arnold, Kucer’s 6th edition

Siala et al, in the press
Inhibition of IcaA by caspofungin increases fluoroquinolone penetration in biofilms

S. aureus biofilms

CAS reduces PNAG in the matrix

CAS inhibits IcaA activity

CAS increases fluoroquinolone concentration in biofilms

Siala et al, in the press
Inhibition of IcaA by caspofungin increases fluoroquinolone efficacy in biofilms

S. aureus biofilms

Fluoroquinolone-CAS combinations are highly active!
Improving drug delivery thanks to vectors

Ribeiro et al., Pharmacology & Therapeutics 2016; 160:133–144
PK/PD parameters in biofilms

- **pharmacodynamics**
  - bacterial responsiveness
    (metabolic activity of bacteria)
  - antibiotic expression of activity
    (local environment [O$_2$, pH, ..])
Why do bacteria feel well and antibiotics feel bad in biofilm?

→ environment suboptimal (≠ broth)
Environmental pH

**S. aureus + delafloxacin**

**Biofilm pH**

* Labelling with Seminaphthorhodafluor-4F 5-(and-6) carboxylic acid (C-SNARF-4)

**Influence of pH on delafloxacin MIC**

* Variance analysis
  Prob: 0.120

Biofilm pH may influence antibiotic intrinsic activity

Specific phenotypes in biofilms: persisters

Coulon, Bioassays 2014; 36: 991–6
How to help antibiotics waking up bacteria?

→ anti-persister compounds
Antipersisters + antibiotics

*S. aureus + ADEP4 + antibiotics*

Coulon et al, Nature 2013; 503: 365–70

Antipersisters allow antibiotics to eradicate biofilms

Targets ClpP, core unit of a major bacterial protease complex.
In vitro dynamic models

- permanent fluid stirring
- unidirectional flow replacement
- constant conditions
What does constant flux to the story?

→ closer from in vivo situation
Dynamic models: bioreactors

CDC reactor:
- constant mixing by stirring
  → kinetic experiments with change in medium composition over time
- high shear stress

Study of antibiotic activity - mimicking human exposure

*S. aureus biofilms*

**Simulated regimens:**
DAP (10 mg/kg once daily) / LZD (600 mg twice daily)

**human pharmacokinetic profile**
Study of antibiotic activity - mimicking human exposure

S. aureus biofilms

Simulated regimens:
DAP (10 mg/kg once daily) / LZD (600 mg twice daily)

Planktonic cultures

Biofilm (CDC reactor)

Combination more useful against biofilm than planktonic bacteria

Conclusion: PK/PD in biofilms: what did we learn?

Painting of the establishment of the State University of Ghent in 1817 when the city was under Dutch rule
Conclusion: PK/PD in biofilms: what did we learn?

- Many methods to evaluate biomass / bacterial survival
  - no real consensus on the best options

- Many models to grow biofilms *in vitro*
  - comparison between studies difficult
  - more relevant model?

- Antibiotic activity on biofilms <<<< planktonic bacteria
  - no or limited effect on the matrix
  - determining PK parameters: diffusion / bioavailability
  - determining PD parameters: expression of activity / bacterial responsiveness
Perspectives: PK/PD in biofilms: where do we go?
PK/PD in biofilms: Where do we go?

Increasing antibiotic activity against biofilms

→ dispersing the matrix
  o matrix constituents?
  o nanovectors to improve delivery

→ modifying environmental factors
  o metabolism within biofilm?
  o factors affecting antibiotic activity?

→ modifying bacterial metabolism
  o reversion from persister phenotype
Acknowledgments