The Dynamics of Growing Biofilm

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with lots of help from

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Biofilms

Biofilm fouling of fiber filter

Plaque on teeth

The Power of Mathematics
The Excitement of Biology
Some of the Interesting and Important Questions

• How do gels grow?
  
  *P. Aeruginosa* (on catheters, IV tubes, etc.)

  Mucus secretion (bronchial tubes, stomach lining)

  Colloidal suspensions of cells (cancer cell growth)

  Gel Morphology (The cellular shape of sponges)

• Why are gels important?

  Protective capability

  High viscosity (low washout rate) - for drugs, acid protection
Biofilm formation in *Pseudomonas Aeruginosa*

Wild type  Biofilm mutant  Mutant with autoinducer

Heterogeneous structures
The Dynamics of Growing Biofilm

• **Quorum Sensing:**
  – What is it?
  – How does it work?

• **Heterogeneous structures:**
  – How do these cells use polymer gel (EPS) for locomotion?
  – What are the mechanisms of pattern (structure) formation?
  – Why is polymer gel so effective as a protective environment?
Quorum Sensing in *P. Aeruginosa*

**Quorum Sensing**: The ability of a bacterial colony to sense its size and regulate its activity in response.

Examples: *Vibrio fisheri, myxococcus, P. Aeruginasa*

*P. Aeruginosa*

- Major cause of hospital infection in the US.
- Major cause of deaths in intubated CF patients, and IV fed patients.

*P. Aeruginosa* in planktonic (non-colonized) form are non-toxic, but as a biofilm, they are highly toxic and well protected by the polymer gel in which they reside. However, they do not become toxic or begin to form polymer gel until the colony is of sufficient size to overwhelm the immune system. Before this, they cannot be detected by the immune system.
Quorum sensing in *P. Aeruginosa*

- Planktonic
- Loosely Bound
- EPS secreting
“Wall Sensing” in *P. Aeruginosa*

**Wall Sensing:** The ability of bacteria to differentiate in response to Contact with a wall (the substratum).

Planktonic  
Loosely Bound  
EPS secreting
The Dynamics of Growing Biofilm

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The Biochemistry of Quorum Sensing
An ODE Model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R$</td>
<td>LasR</td>
</tr>
<tr>
<td>$A$</td>
<td>3-oxo-C12-HSL</td>
</tr>
<tr>
<td>$P$</td>
<td>Dimer complex</td>
</tr>
<tr>
<td>$L$</td>
<td>LasI</td>
</tr>
<tr>
<td>$S$</td>
<td>RsaL</td>
</tr>
<tr>
<td>$r$</td>
<td>lasR mRNA</td>
</tr>
<tr>
<td>$l$</td>
<td>lasI mRNA</td>
</tr>
<tr>
<td>$s$</td>
<td>rsaL mRNA</td>
</tr>
</tbody>
</table>
Modeling Biochemical Reactions with ODE’s

Bimolecular reaction \[ A + R \xleftrightarrow{} P \]

\[
\frac{dP}{dt} = k_+ AR - k_P
\]

Rate of production \ Rate of degradation

Production of enzyme from mRNA \[ l \xrightarrow{} L \]

\[
\frac{dL}{dt} = k_l l - K_L L
\]

Rate of production \ Rate of degradation

Production of mRNA \[ P \xrightarrow{} l \]

\[
\frac{dl}{dt} = \frac{V_{\text{max}} P}{K_l + P} - k_{-l} l
\]
The full system of differential equations for the biochemistry:

\[
\begin{align*}
\frac{dP}{dt} &= k_{RA} RA - k_P P, \\
\frac{dR}{dt} &= -k_{RA} RA + k_P P - k_R R + k_1 r, \\
\frac{dA}{dt} &= -k_{RA} RA + k_P P + k_2 L - k_A A, \\
\frac{dL}{dt} &= k_3 l - k_1 L, \\
\frac{dS}{dt} &= k_4 s - k_s S, \\
\frac{ds}{dt} &= V_s \frac{P}{K_s + P} - k_s s, \\
\frac{dr}{dt} &= V_r \frac{P}{K_r + P} - k_r r + r_0, \\
\frac{dl}{dt} &= V_l \frac{P}{K_l + P} \frac{1}{K_s + S} - k_l l + l_0.
\end{align*}
\]
Modeling diffusion across cell membrane

\[
\rho \left( \frac{dA}{dt} + k_{RA} RA - k_P P - k_L L + k_A A \right) = \delta(E - A)
\]

\[
(1 - \rho) \left( \frac{dE}{dt} + k_E E \right) = \delta(A - E)
\]

where \( \rho \) is the cell volume fraction.

Does this model exhibit quorum sensing?

Two ways to proceed:
1) Numerical simulation (but few of the 22 parameters are known)
2) Qualitative analysis
Quasi-steady state analysis: Assume that all “fast” variables are in quasi-steady state

\[
\frac{dR}{dt} = V_R \frac{P}{K_R + P} - k_R R + R_0,
\]

\[
\frac{dA}{dt} = V_A \frac{P}{K_A + P} + A_0 - d(\rho) A,
\]

\[
P = \frac{k_{RA} R A}{k_p}, \quad d(\rho) = \frac{k_A}{\rho} + \frac{\delta}{\rho} \left( \frac{k_E (1 - \rho)}{\delta + k_E (1 - \rho)} \right).
\]
An even simpler model

Set $R=A$ (which is not correct), to find

$$\frac{dA}{dt} = V_A \frac{A^2}{K_A + A^2} + A_0 - d(\rho)A, \quad d(\rho) = k_A + \frac{\delta}{\rho} \left( \frac{k_E (1 - \rho)}{\delta + k_E (1 - \rho)} \right).$$
A Simple Model for Wall Sensing

Cells are immobile, so internal variables $A$ and $V$ do not diffuse, but extracellular autoinducer $E$ diffuses (rapidly) so

\[ \frac{\partial V}{\partial t} = G(V, A), \quad V \in \mathbb{R}^7 \]

\[ \frac{\partial A}{\partial t} = F(A, V) + d(E(x = L) - A), \]

\[ \frac{\partial E}{\partial t} = D_E \frac{\partial^2 E}{\partial x^2} - k_E E + d \delta(x - L)(A - E)(= 0) \]
A Simple Model for Wall Sensing - cont’d

We can solve for $E(x=L)$ assuming a quasi-equilibrium, to find

\[
\frac{\partial V}{\partial t} = G(V,A), \quad V \in \mathbb{R}^7
\]

\[
\frac{\partial A}{\partial t} = F(A,V) - d \frac{p}{1 + p} A,
\]

\[
p = \frac{\sqrt{\mu D}}{d} \left(1 + \tanh\left(\sqrt{\frac{\mu}{D}} L\right)\right)
\]

Since $p$ decreases as $L \to 0$, this has a *chance* of up-regulating for small $L$. 
A Proper (PDE) Model

Cells are immobile, so internal variables $A$ and $V$ do not diffuse, but extracellular autoinducer $E$ diffuses so

\[
\frac{\partial V}{\partial t} = G(V, A), \quad V \in \mathbb{R}^7
\]

\[
\frac{\partial A}{\partial t} = F(A, V) + \frac{\delta}{\rho} (E - A),
\]

\[
\frac{\partial E}{\partial t} = \nabla^2 E - k_E E + \frac{\delta}{1 - \rho} (A - E),
\]

on the domain $\Omega$, subject to the (Robin) boundary condition

\[
n \cdot D \nabla E + \alpha E = 0, \quad \text{on } \partial \Omega
\]
Autoinducer with fixed colony size, variable density
Autoinducer concentration; fixed density, variable size colony
Heterogeneous Structures

• How do these cells use polymer gel for locomotion?
• What are the mechanisms of pattern (structure) formation?
• Why is polymer gel so effective as a protective environment?
A Hydrogel Primer

• What is a hydrogel?
  A tangled polymer network in solvent

• Examples of biological hydrogels:
  Micellar gels
  Jello (a collagen gel ~ 97% water)
  Extracellular matrix
  Blood clot
  Mucin - lining the stomach, bronchial tubes, intestines
  Glycocalyx - lining epithelial cells of blood vessels
  Sinus secretions

• Other examples of gel-like structures
  Cell colonies - colloidal suspensions
  Gel morphology - sponges, jellyfish
Fibrin Network (Blood Clot)
A Hydrogel Primer - II

• Function of a biological hydrogel
  Decreased permeability to large molecules
  Structural strength (for epithelial cell walls)
  Capture and clearance of foreign substances
  Decreased resistance to sliding/gliding
  High internal viscosity (low washout)

• Important features of gels
  Usually comprised of highly polyionic polymers
  Often exhibit large volumetric changes
    eg. Highly compressed in secretory vesicle and expand rapidly
    and dramatically on release
  Can undergo volumetric phase transitions in response to ionic
    concentrations (Ca++, H+), temperature, ..
  Volume is determined by combination of attractive and
    repulsive forces:
    -- repulsive electrostatic, hydrophobic
    -- attractive, hydrogen binding, cross-linking
How gels grow

• Polymerization/deposition
  (blood clots)

• Secretion

Interesting facts:
- Diffusion coefficient can vary substantially as a function of Ca++
- Expansion does not occur in distilled water.
Modeling Biofilm Growth

A two phase material with polymer volume fraction $\vartheta$

$$\frac{\partial \vartheta}{\partial t} + \nabla \cdot (V_n \vartheta) = g_n$$  \hspace{1cm} \text{Network Phase (EPS)}

$$\frac{\partial (1 - \vartheta)}{\partial t} + \nabla \cdot (V_s (1 - \vartheta)) = 0$$  \hspace{1cm} \text{Solute Phase}

$$\frac{\partial b}{\partial t} + \nabla \cdot (V_n b) = g_b$$  \hspace{1cm} \text{Bacterial Concentration}

$$\frac{\partial (1 - \vartheta)u}{\partial t} + \nabla \cdot ((1 - \vartheta)(V_su - D \nabla u)) = g_u$$  \hspace{1cm} \text{Resource}
Force Balance

Solute phase
\[ h_f \cdot \mathcal{G} (1 - \mathcal{G}) (V_n - V_s) - (1 - \mathcal{G}) \nabla p = 0 \]
(solute-network friction) (pressure)

Network phase
\[ \eta \nabla \cdot (\mathcal{G}_n (\nabla V_n + \nabla V_n^T)) - h_f \cdot \mathcal{G} (1 - \mathcal{G}) (V_n - V_s) - \nabla \psi (\mathcal{G}) - \mathcal{G} \nabla p = 0 \]
(viscosity) (solute-network friction) (osmosis) (pressure)

Incompressibility
\[ \frac{\partial \mathcal{G}}{\partial t} + \nabla \cdot (\mathcal{G}_n (V_n - g_h V_s) \frac{\partial (1 - \mathcal{G})}{\partial t} + \mathcal{G} \nabla \cdot (V_s (1 - \mathcal{G})) = 0 \]

Remark: Models of this form for the network phase have been used by lots of people (Dembo and He, Lubkin and Jackson, Wolgemuth, Oster, Mogilner, …)

Remark 2: A better model might keep track of Ca++ and Na+ concentrations as well
Osmotic Pressure

What is the meaning of the term \(-\nabla \psi(\mathcal{G})\) ?

(osmosis)

In some formulations \(\psi(\mathcal{G}) = F'(\mathcal{G})\) where \(F(\mathcal{G})\) is the Free Energy

\[\psi'(\mathcal{G}) \begin{cases} > 0 \text{ gives expansion (swelling)} \\ < 0 \text{ gives contraction (deswelling)} \end{cases}\]

\[\psi(\mathcal{G}) = kT(k_1(1-\theta)\ln(1-\theta) + \chi\theta(1-\theta))\] From Flory-Huggins theory
Phase Separation

Double-welled potentials give phase transitions and phase separation, similar to Cahn-Hilliard.

\[ \psi(\mathcal{I}) \]

\[ \mathcal{I}(x) \]

To maintain an edge, \( \psi(\mathcal{I}) \) must be of the form \( \psi(\mathcal{I}) = \mathcal{I}^2 G(\mathcal{I}) \) (This is a theorem)
Movement by Swelling

QuickTime™ and a Animation decompressor are needed to see this picture.
Propulsion by Swelling

QuickTime™ and a
Animation decompressor
are needed to see this picture.
No gel; no swell

QuickTime™ and a
Animation decompressor
are needed to see this picture.
Heterogeneous Structures

• How do these cells use polymer gel for locomotion?

• What are the mechanisms of pattern (structure) formation?

• Why is polymer gel so effective as a protective environment?
“Limited Resource” Instability

Remark: This instability does not occur in a resource rich environment.
Channeling

A Pouiselle flow
Channeling
Channeling
Conclusions

• Quorum Sensing:
  – What is it? A biochemical switch
  – How does it work? Extracellular autoinducer fails to diffuse away.

• Heterogeneous structures:
  – How do these cells use polymer gel for locomotion? Expansion by swelling
  – What are the mechanisms of pattern (structure) formation?
    • Limited resource fingering
    • Friction driven channeling
    • Others? (Is sloughing related to a Kelvin-Helmholz instability?)
  – Why is EPS so effective as a protective environment?
    • Restricted pore size, chemical reactivity???
More Questions (than answers)

Why does P. Aeruginosa attack only immune-compromised individuals? (burn victims, post-surgery)

Why does P. Aeruginosa find a ready host in CF patients? (Decreased diffusion???)

What is it about the CFTR mutation in CF patients that makes their mucin so thick?

Is there cross talk between P. Aeruginosa and other pathogens and if so, how?