Animal models of biofilm infections.

Faculty:
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Program

• Agenda 10.00-12.00 am
• Introduction (CLM)
• *Pseudomonas aeruginosa* Biofilm infections - Lung infection models and wound model (CLM)
• Lunch 12.00 am - 1.00 pm
• Peritoneal implant model and Filler model (TBJ)
• Inflammation/adaptive immunology (PØJ)
• Final remarks. Evaluation of course (NH)
Animal models of *Pseudomonas aeruginosa* biofilm infections.

1) Lung infection model
2) Chronic wound model
3) Peritoneal implant model
4) Filler model
Usefulness of animal models

Testing of hypothesis generated from observations in the patients

Pre-clinical testing of agents showing promising *in vitro* effect
The Law (Denmark)

- Animal experiments on vertebrates allowed if the aim is significant, specified and scientific.
- Permission given to individual persons with sufficient education/background, after having passed a special animal experimental course and test.
- Permission from a council with a professional chairman (Judge) and ten members appointed by the Ministry of Justice.
- Only allowed if other methods cannot replace the experiments.
- Files approved for all individual experiments.
- Subject to unannounced visits from authorities.
- Annual report from the council in an anonymized form.
- Approximately 300,000 animals used/year in Denmark.
- Mortality not allowed as an intended end point.
- Animal welfare increasing attention.
- Fanatic anti-animal experiment groups not a problem in Denmark, so far.
- Some rules not invented by animal facilities!!
Acute lung infection

Ware LB, Matthay MA. NEJM 2000

Resolution

Craig A et al IAI 2009

Moser Hamburg 2011
Chronic biofilm infection in CF—no resolution.

- Adaptive immune response accelerates inflammation and contributes to pathogenesis:
  - Skewing of Th1/Th2 balance
  - Immune complex disease
  - Paradox persistent acute type inflammation (PMNs)
  - High number of necrotic PMNs
  - Progressive loss of lung function
  - Fibrosis

- The type of adaptive immune response influenced by the inflammatory response.

- Provides possible treatment targets.
Cystic fibrosis. Multiorgan disease

- Malabsorption, pancreatic insufficiency
- Male infertility
- Hepatic insufficiency
- Diabetes mellitus
- Allergy
- Kidney insufficiency
- Chronic endobronchial infection

Mucoid biofilm of P. aeruginosa in an alveolar surrounded by severely inflamed tissue (PMNs, pneumonia). Autopsy (BS242/74) of a CF girl (MLM) who died due to chronic P. aeruginosa lung infection and 21 precipitating antibodies against P. aeruginosa. HE stain x 40
Pro’s

• Numerous inbred strains
• Prolonged infection possible
• Inflammation CF-like
• Vast immunological tools available
• CF mice available (and ENaC mice)

Con’s

• Important histological differences
• Natural resistant to *P. aeruginosa*
• (No spontaneous lung infection in CF mice)
• Short lifespan
Dominated by rat and mouse models

The lung infection models

– Embedded models ("Classical")
  • Agar (Cash 1979)
  • Agarose
  • Seaweed alginate
  • Mucoid model in native alginate ("de Novo")
  • Adaptation model
    – Planktonic models
      • Nebulizing chamber
      • Nasal application/inflation
      • Drinking model
    – Tube model ("Japanese")
  – Xenograft model
Bacteria
Bead machine
Alginate
Agar- or alginate beads is necessary for chronic infections — the Cash model

Seaweed alginate beads 60 μm (30-110 μm)
Alginate beads with *Pseudomonas aeruginosa*
Confocal microscopy picture of alginate beads.

Courtesy Thomas Bjarnsholt.
Challenge

Anesthesia: Midazolam and Phentany + fluanison
Post challenge
Characteristics.
The seaweed alginate model (Pedersen SS, et al. APMIS 1990)

- A clinical alginate producing isolate PAO 579 (provided by Govan, Edinburgh). $10^{7.8}$ CFU/ml, 0.1 ml/rat.
- Intratracheal infection with beadtipped needle
- Sacrificed rats 4 weeks after challenge.
- Compared to the agar model.
- Macroscopic pathology:
  - Grey nodules (abscesses) and pleural adhesions.
- Histological pathology:
  - Pronounced PMN dominated inflammatory response. Beads with bacteria.
- Quantitative bacteriology:
  - Positive bacteriology in 10/12 rats.
- Antibody production:
  - Significantly higher number of precipitating antibodies in the alginate group.
Characteristics mice.
(Using PAO 579. $10^9$ CFU/ml, 0.04 ml/mouse)

- Course of infection highly dependent on mouse strain.
Resistant versus susceptible mice

Observations

Mortality %

- 100 -
- 80 -
- 60 -
- 40 -
- 20 -
- 0 -

- BALB/c (n=89)
- C3H/HeN (n=85)
- C57BL/6 (n=25)

C Moser 2004
Characteristics mice.
(Using PAO 579)

• Course of infection highly dependent on mouse strain.
• Shorter duration of the infection (1-2 weeks).
Quantitative bacteriology

Fig. 1. Graph showing percentage of mice in which *P. aeruginosa* could be cultured from lung homogenates on the day of sacrifice.

Characteristics mice.
(Using PAO 579)

- Course of infection highly dependent on mouse strain.
- Shorter duration of the infection (1-2 weeks).
- Macroscopic abscesses are seldom.
Mouse model of chronic *P. aeruginosa* lung infection
Characteristics mice.
(Using PAO 579)

- Course of infection highly dependent on mouse strain.
- Shorter duration of the infection (1-2 weeks).
- Macroscopic abscesses are seldom.
- Similar histopathology.
Histopathology

- Severe inflammation
- Both central and in the periphery
- Alginate area with biofilm like structures
Characteristics mice.
(Using PAO 579)

• Course of infection highly dependent on mouse strain.
• Shorter duration of the infection (1-2 weeks).
• Macroscopic abscesses are seldom.
• Similar histopathology.
• Antibody production, and activation of cellular immunity.
Activation of CD4+ cells
Use of the model

- Vaccination studies (HK Johansen, O Ciofu)
- Immune modulation (C Moser, HK Johansen)
- Antibiotic resistance (O Ciofu)
- Immune responses (C Moser, PØ Jensen)
- Treatment studies (C Moser, Z Song, T Bjarnsholt, N Hoffman)
- Host-Pathogen interactions (C Moser, H Wu, T Bjarnsholt, PØ Jensen, S Prakhabar)
Improvements in care.

- Liquid post challenge (1 ml isotonic NaCl 37°C s.c.).
- Maintenance of body temperature.
- Analgetic treatment of surgical wound (bupivakain).
- Euthanasia strategy.
Evaluation of experiments.

1) Mortality/euthanasia
2) Quantitative bacteriology
3) Pathology
Number of animals?
(sample size).

Total number of animals in two groups.

$\delta = \text{clinically relevant difference.}$

$SD = \text{standard deviation.}$

Usually set power $(1-\beta)$ to 0.80, and $\alpha$ to 0.05.

Practical statistics for medical research.
Euthanasia

1) Sparing severely ill animals from further suffering.
2) What characterizes the animals that survived as compared to the once that died?

Can we conclude?
Evaluation/end points
Whole blood
Quantitative bacteriology
Macroscopic pathology

• Broad estimate
• Length and width
• Lung pathology index
• Weight of lung
• Relative affected area
Macroscopic pathology

- Front and dorsal picture taken.
- Transferred to Visiomorph (developed by Visiopharm (www.visiopharm.com))
- Affected area circled.
- Number of pixels registered (=15,889).
Macroscopic pathology

- Total area circled.
- Number of pixels registered (=24,076).
- Relative affected area: 15,889/24,076 = 0.66.
- Both sides registered and mean calculated.
Histopathology
Histopathology explained
Histopathological evaluation

• By a pathologist
  – x2-400, 5-10 fields
• Type
  – Acute type (PMN dominated)
  – Chronic type (MN dominated)
  – PMN/MN type
• Granulomas, microabscesses, athelectasis etc.
• Degree
  – 0 (no inflammation) -> +++ (heavy inflammation)
• Evaluated blindly
• Semiquantitative
• Reproducible
Histopathology

- Type PMN (dominated by polymorphonuclear neutrophilic granulocytes).
- Degree +++ (extensive inflammation with necrosis and microabscesses)
  - x100
  - x400
Histopathology

- Type MN (dominated by mononuclear cells)
- Degree + (mild focal inflammation)
  - x100
  - x400
Broncho-alveolar lavage
Modifications of the model

- Embedment in native alginate
- Adaptation model
- Niche model
Mucoid *P. aeruginosa*
(Hoffmann N, et al. IAI 2005)

- Stabile mucoid phenotype.
- Cultured for 28h, 37°C.
- Centrifuged, and resuspended in 2 ml ox broth.
- Adjusted to 1x10⁸ CFU/ml (1x10⁹ for BALB/c mice) in crude or purified native alginate.
  - Crude: culture supernatant
  - Purified: supernatant heated to 80°C for 30 min. Precipitated with 99% ice-cold ethanol. Resuspended in sterile 0.9% saline.
- Mice challenged intra-tracheally with a bead-tipped needle.
  - CF-mouse: *cftr*<sup>tm¹Unc-TgN(FABPCFTR)</sup> (Jackson Lab.)
  - BALB/c mouse (M&B Lab.)
Quantitative bacteriology

Mucoid *P. aeruginosa* embedded in crude native alginate induced higher mortality of lung infected BALB/c mice as compared to the non-mucoid isolate (p<0.05).

Macroscopic pathology

<table>
<thead>
<tr>
<th>Pathology (no. of mice in scoring groups/total no. of mice challenged [%]) at day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt 1</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>Score</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

- See Table 2, footnote a. Asterisks indicate probabilities as follows: *P < 0.01 compared to day 13; **P < 0.01 compared to the mucoid strain NH57388A +QS; ***P < 0.001 compared to BALB/c mice; ****P < 0.001 compared to the nonmucoid strain NH57388B -QS; *****P < 0.001 compared to the mucoid strain NH57388A +QS.

- Day 13, in this case.

- Score 1, normal; 2, swollen lungs, hyperemia, small aplectasia; 3, pleural adhesion, atelectasis, multiple small abscesses; 4, large abscesses, large atelectasis, and hemorrhages.

Histopathology

Microscopy of CF-mouse lung (HE and Alcian blue)

Confocal microscopy of freeze microtomy sections of mice-lungs

Mucoid CF-mouse

Saline

Non-mucoid BALB/c-mouse

CF-patient

Alveoles without infection

CF-sputum

Adaptation model.

- Mouse models of chronic *P. aeruginosa* lung infections are limited to 2 (-3) weeks!
- 20-30 years of chronic infection.
- ≥ 10,000 days (~120,000 bacterial generations) of mutual exposure to the inflammatory responses and bacterial virulence factors.
- ≥ 100 antibiotic i.v. courses (≥1,400 days) of antibiotic exposure.
Biofilm development of wild type PAO1 and sequential isolates from patient no 1. Flow chambers were inoculated with *gfp*-tagged wild type and *P. aeruginosa* isolates grown on Casamino Acids minimal media. CLSM images were acquired at 1, 3, 5 and 7 days after inoculation.

Survival

van Gennip and Moser 2010
Quantitative bacteriology

van Gennip and Moser 2010
## Histopathology

<table>
<thead>
<tr>
<th>Groups</th>
<th>Type</th>
<th>Degree</th>
<th>Athelectasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/1980</td>
<td>7 PMN/MN*</td>
<td>4 +++**</td>
<td>5 mice§</td>
</tr>
<tr>
<td></td>
<td>0 MN</td>
<td>3 +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 NI</td>
<td>0 -</td>
<td></td>
</tr>
<tr>
<td>II/1988</td>
<td>7 PMN/MN*</td>
<td>2 +++++***</td>
<td>4 mice§§</td>
</tr>
<tr>
<td>(n=7)</td>
<td>0 MN</td>
<td>5 +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 NI</td>
<td>0 -</td>
<td></td>
</tr>
<tr>
<td>III/1997</td>
<td>2 PMN/MN</td>
<td>0 ++</td>
<td>No mice</td>
</tr>
<tr>
<td>(n=10)</td>
<td>0 MN</td>
<td>2 +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8 NI</td>
<td>8 -</td>
<td></td>
</tr>
<tr>
<td>IV/1999</td>
<td>2 PMN/MN</td>
<td>0 ++</td>
<td>1 mice</td>
</tr>
<tr>
<td>(n=11)</td>
<td>0 MN</td>
<td>2 +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9 NI</td>
<td>9 -</td>
<td></td>
</tr>
<tr>
<td>V/2003</td>
<td>3 PMN/MN</td>
<td>1 ++</td>
<td>1 mice</td>
</tr>
<tr>
<td>(n=8)</td>
<td>1 MN</td>
<td>3 +</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 NI</td>
<td>4 -</td>
<td></td>
</tr>
</tbody>
</table>

*Significantly more mice with a PMN involving (= acute type) inflammation as compared to group III and IV (p<0.05). **Significantly higher degree of inflammation as compared to group III, IV and V (p<0.02). ***Significantly higher degree of inflammation as compared to group III and IV (p≤0.0002). §Significantly more mice with athelectasis as compared to group III, IV and V (p<0.05). §§Significantly more mice with athelectasis as compared to group IV and V (p<0.05).
G-CSF and MIP-2 decreases.

van Gennip and Moser 2010
Phenotypic analysis of non-mucoid *Pseudomonas* Isolates

- **Patient Isolates**
- **Chronic infection**
- **Hypermutability**
- **Colony phenotype**
- **Motilities**
- **Detection of AHL signals**
- **Virulence production**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Isolates name/year</th>
<th>Chronic period (years)</th>
<th>Hypermutability</th>
<th>Colony phenotype</th>
<th>Motilities</th>
<th>Detection of AHL signals</th>
<th>Virulence production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>14889a/1980</td>
<td>8</td>
<td>nHp</td>
<td>R</td>
<td>Swim</td>
<td>3-O-C12-HSL</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>19193a/1984</td>
<td>12</td>
<td>nHp</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>476a/1988</td>
<td>16</td>
<td>nHp</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>15278a/1994</td>
<td>22</td>
<td>Hp</td>
<td>S</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>67903b/1999</td>
<td>27</td>
<td>Hp</td>
<td>S</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>1395b/2003</td>
<td>*</td>
<td>Hp</td>
<td>S</td>
<td>+</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Patient 2</td>
<td>374d/1985</td>
<td>7</td>
<td>nHp</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>64691c/1999</td>
<td>21</td>
<td>Hp</td>
<td>S</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Patient 3</td>
<td>1738b/1985</td>
<td>15</td>
<td>nHp</td>
<td>S</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>54514a/1997</td>
<td>27</td>
<td>Hp</td>
<td>S</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Patient 4</td>
<td>19696/1984</td>
<td>5</td>
<td>nHp</td>
<td>S</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>68000d/1999</td>
<td>20</td>
<td>Hp</td>
<td>S</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Patient 5</td>
<td>21168a/1984</td>
<td>6</td>
<td>nHp</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>15357a/1994</td>
<td>16</td>
<td>nHp</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Patient 6</td>
<td>16020/1991</td>
<td>15</td>
<td>nHp</td>
<td>R</td>
<td>+</td>
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<td>++</td>
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<td>65608a/1999</td>
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<td>Hp</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Patient 7</td>
<td>20688a/1984</td>
<td>4</td>
<td>nHp</td>
<td>S</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>3284a/1995</td>
<td>15</td>
<td>Hp</td>
<td>S</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Patient 8</td>
<td>15761/1978</td>
<td>1</td>
<td>nHp</td>
<td>R</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

* Isolate 1395b/2003 was recovered from 3 years after patient was lung transplanted.

* nHp., nonhypermutable, Hp., Hypermutable.

* R, rough irregular colony phenotype, S. Smooth regular colony phenotype

* ++, motility zone ≥ 20mm, +, motility zone ≥ 10mm, -, motility zone ≤ 5mm

* Production of AHLs were detected by inspecting the bioluminescence of monitor strain, +, detectable, -, non-detectable

* ++, high level, +, intermitent level, -, low level

Partial conclusion

For non-mucoid isolates:
1) Initial stages: Ability for **biofilm formation** correlates to pathogenicity.
2) Late stages: Virulence dominated by other factors - hyperproduction of exopolysaccharides?

<table>
<thead>
<tr>
<th>Year</th>
<th>Non-mucoid</th>
<th>Mucoid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Sacrifice day 5, or day 1, 2 or 3.
Survival of mice infected with mucoid or non-mucoid *P. aeruginosa*.

van Gennip and Moser 2010
Quantitative bacteriology

P<0.0001

van Gennip and Moser 2010
Macroscopic pathology (mucoid "adaptive" strain).

- Athlectasis
- Haemorrhages
- Abscesses
- Adherences
- "Airtrapping"
Figure 3. Pathology and colony morphology.
Inflammatory response
Non-mucoid versus mucoid.

G-CSF: PMN mobilizer from the bone marrow

van Gennip and Moser 2010

ESCMID Online Lecture Library © by author
Conclusion adaptation model

• For non-mucoid isolates:
  – Initial stages: Ability for biofilm formation correlates to pathogenicity.
  – Late stages: Virulence dominated by other factors - hyperproduction of exopolysaccharide.

• For mucoid isolates:
  – More CF-relevant pathology.
  – Virulence increases with time.

• Combined:
  – New infection strategy providing a CF-relevant time-perspective (years).
  – Possible to investigate the impact of bacterial adaptation on host responses.
Pulmonary niches or zones

The conductive and respiratory zone of the lungs.
Pulmonary dimensions

Thiberville L et al Eur Resp J 2009

West. Pulm phys and pathophys. Lippincott Williams & Wilkins, 2001
Recognition and recruitment.

Five distinct lung DC subsets based on surface markers and location.

Type of generated response dependent on:
- Cytokine environment
- Danger signals
- Pathogen recognizing receptors (PRRs)
- Pathogen
- Antigen processing

If harmless antigen with minor additional signals induce Treg.

Craig A et al IAI 2009


In the respiratory zone:
- 100% blood supply
- Short distance from blood to lumen
- Pathogen recognizing receptors (PRR)

In the conductive zone:
- 1% blood supply
- Long distance from blood to airway lumen
- Limited PRR
Challenge of distinct lung niches.
Hypothesis: Localization matters.

Lars Christophersen et al. CEI 2012 in press
Quantitative bacteriology

Lars Christophersen et al. CEI 2012 in press
Large beads

Small beads

L Christophersen et al. CEI 2012 in press
Airways area SB<LB: n.s
Area beads SB<LB: p<0.0001

Airways area SB<LB: p=0.002
Area beads SB<LB: p<0.0001

Not analysed

L Christophersen et al. CEI 2012 in press
Presence of *P. aeruginosa* in the biofilm-like structures

A

B

Large beads

C

D

Small beads
Confocal Laser Scanning Microscopy
Application of the model: Activity of the bacteria.

Large beads

Small beads

Courtesy of Kasper Nørskov Kragh
Inflammation: PMN mobilization by serum G-CSF

Lars Christophersen et al. CEI 2012 in press
Inflammation: PMN mobilization by pulmonary G-CSF

G-CSF Lung homogenate

0 500 1000 1500 2000 2500 3000 3500 4000

pg/ml

0 1 2 3 4 5 6 7

Day

LB
SB
LB-PA
SB-PA

Lars Christoffersen et al. CEI 2012 in press
Inflammation: PMN chemoattraction by pulmonary MIP-2

Lars Christoffersen et al. CEI 2012 in press
Conclusion (bead size direction):
Biofilms in smaller airways, increases inflammation.
Increases lung tissue damage.
Direct antibiotic treatment at smaller airways!
Healing versus non-healing wounds?

- Increased levels of inflammation e.g. TNF-α and IL-1β and proteinases, e.g. MMP-9.
- Destruction of extracellular matrix proteins like fibronectin and growth factors.
- Heterogenic patient populations. Several disposing factors.
- Poor microbiological diagnostics and non-representative sampling.
- ROOM FOR IMPROVEMENTS
Modified from the burned mouse model

*Pseudomonas aeruginosa* in chronic wounds (H Trøstrup, K Thomsen et al. Submitted 2012).

Inoculation of *Pseudomonas aeruginosa* biofilm after 2-4 days
TRANSITION ZONE

NORMAL SKIN

BURNED TISSUE

Henrik Calum 2008
Perspectives

• Can focused treatment of *P. aeruginosa*-biofilms improve wound healing?
• Can modulation of the inflammation improve the course of biofilm infections in chronic wounds?
Whole body plethysmography
What we measure?

• The ”box” flow:

Nasal flow:  
conditioning factor  
(temp., humidity)

Chest flow:  
resistance factor  
(negative pressure)
Parameter derivations

\[
\text{Pause} = \frac{\text{Te} - \text{RT}}{\text{RT}}
\]

\[
\text{Penh (Enhanced pause)} = \frac{\text{PEF}}{\text{PIF}} \times \text{Pause}
\]
Monitorization of biofilm infection in a mouse model (IVIS imaging system)

Precolonized catheters with *P. aeruginosa* Xen 5 were implanted at subcutaneous sites. Makes the bacteria bioluminescent by insertion of a complete *lux* operon.

(Kadurugamuwa, J. Infection and Immunity, vol 71, 882-90, 2003)
E. coli –GFP $10^{11}$ Light box with blue filter optics. Hamamatsu three-chip cooled color charge-coupled device camera.

(Zhao et al. PNAS, vol.98, 9814-18, 2001)
Take home messages

• Biofilm infections are numerous, especially in the hospitals, and is a daily challenge.
• Representative (animal) models are mandatory.
• Several chronic *P. aeruginosa* animal models are available. Constantly being improved and more clinically relevant.
• Frequent contact between the clinical world and the basic science is important.
Acknowledgements

• Rigshospitalet, Department for Clinical Microbiology.
  – Niels Høiby
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  – Frederik Buchwald
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• Copenhagen University, ISIM.
  – Michael Givskov
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  – Elizabeth Fredheim
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