Animal Model of Chronic Lung Infection to *P. aeruginosa*: Are Agar and Alginate beads interchangeable?

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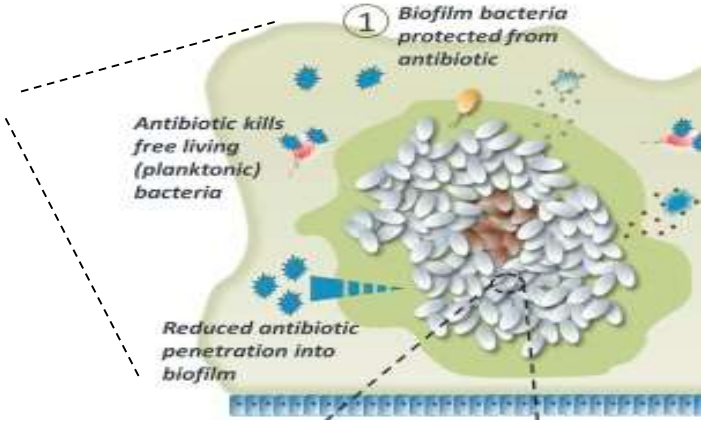
12th International Conference and Workshop on BIOLOGICAL BARRIERS

Are agar and alginate beads interchangeable?

Background

Chronic infections



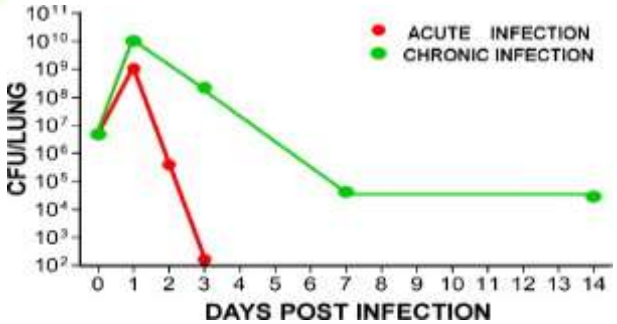
Clusters of *P. aeruginosa* biofilms (<50-100 µm wide) are found embedded in the conducting airways mucus, often surrounded by immune cells such as Neutrophils

Lung models of chronic infections

Models required that P. aeruginosa is embedded in polymer (e.g., agar, agarose, or alginate) to create an interface with the host to prevent rapid bacteria clearing or host death

Agar: Neutral polysaccharide

Alginate: Polyanionic polysaccharide



Sophie Moreau-Marquisa *et al.* Pseudomonas aeruginosa biofilm formation in the cystic fibrosis airway. Pulmonary Pharmacology & Therapeutics 21 (2008) p. 595

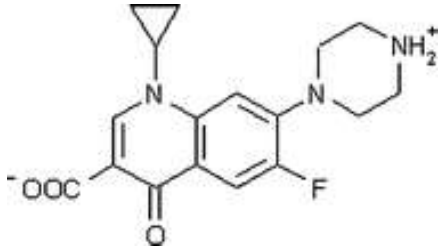
Bjarnsholt, Thomas, et al. Pseudomonas aeruginosa biofilms in the respiratory tract of cystic fibrosis patients. Pediatric pulmonology 44.6 (2009): 547-558.

Are agar and alginate beads interchangeable?

hypothesis

Depends on the physicochemical characteristics and concentration of the antibiotic tested?

Ciprofloxacin (neutral pH7.4) vs Tobramycin (cation)

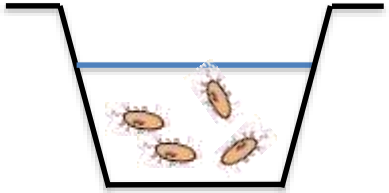


Planning

- Perform time-kill experiments in the presence of TOB or CIP using bioluminescent PAO1 (PAO1::luxCDABE – gift from P. Plésiat) grown planktonically, 
  as adherent biofilm, 
  entrapped in agar or Ca-alginate beads 

- Measure the diffusion coefficient of both drugs in agar and Ca-alginate gels

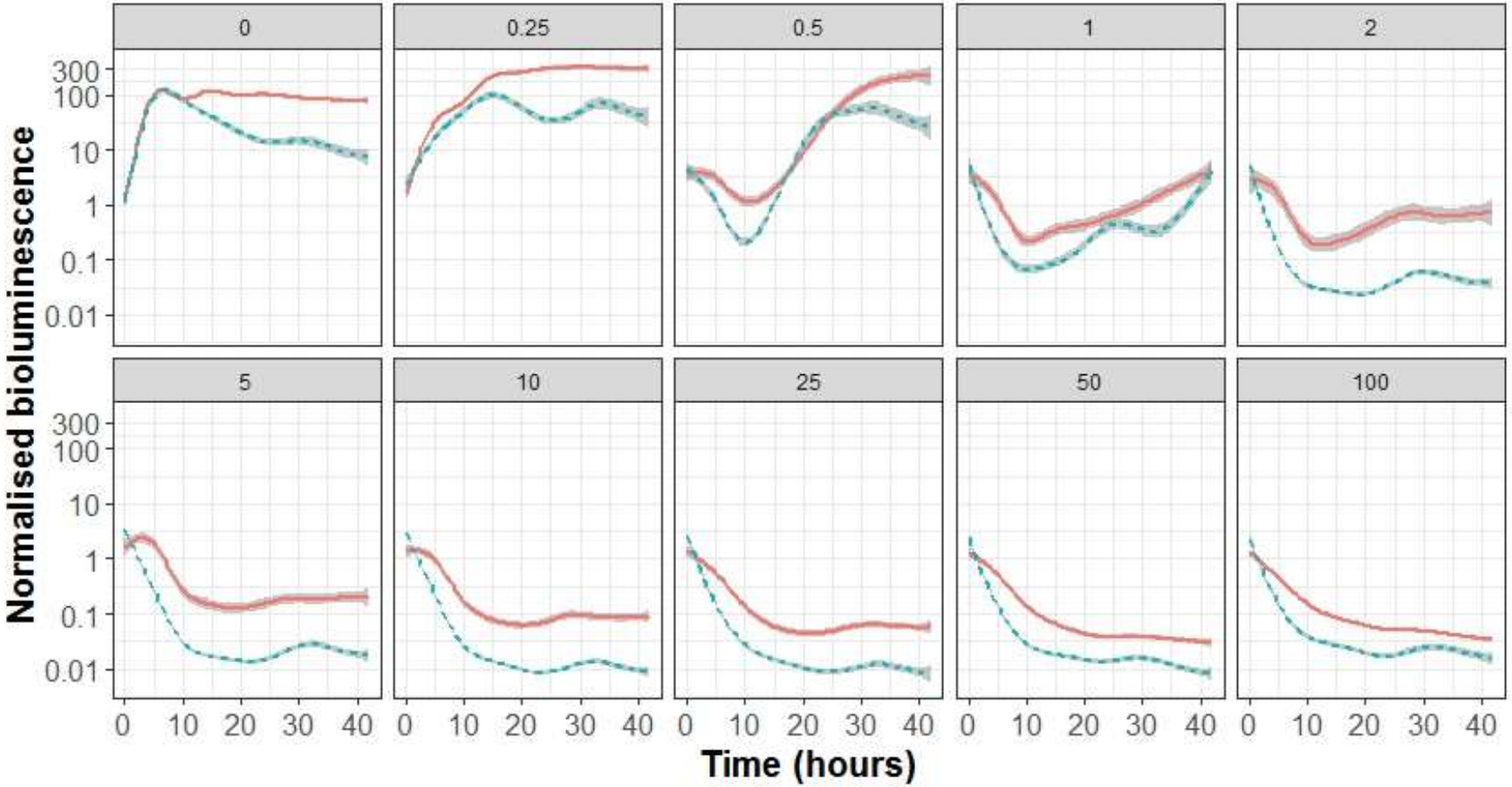
Time-kill curve
planktonic vs 24 hours old biofilm



- CIP concentrations: 0.0625 to 50 µg/mL
  0.25 to 100 x MIC
- TOB concentrations: 0.125 to 100 µg/mL
  0.25 to 100 x MIC

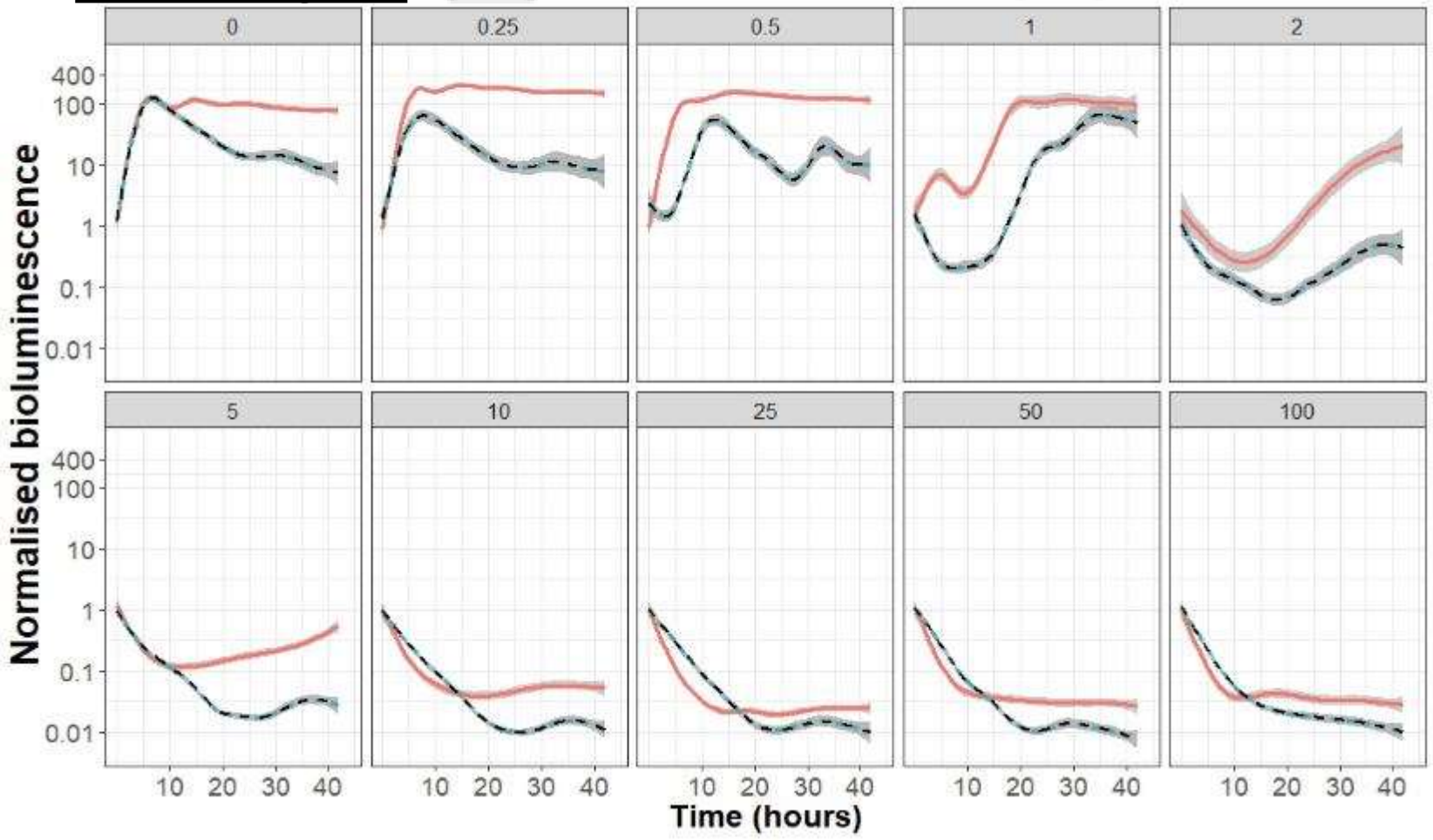
Ciprofloxacin

planktonic biofilm



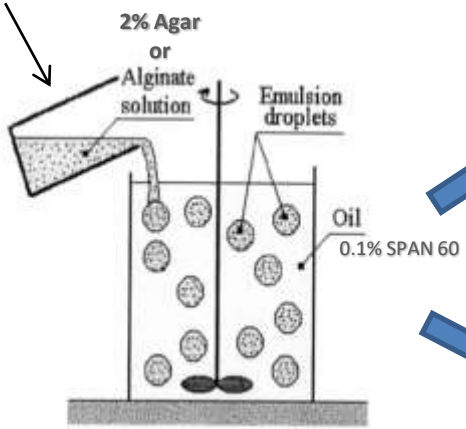
Tobramycin


planktonic
biofilm

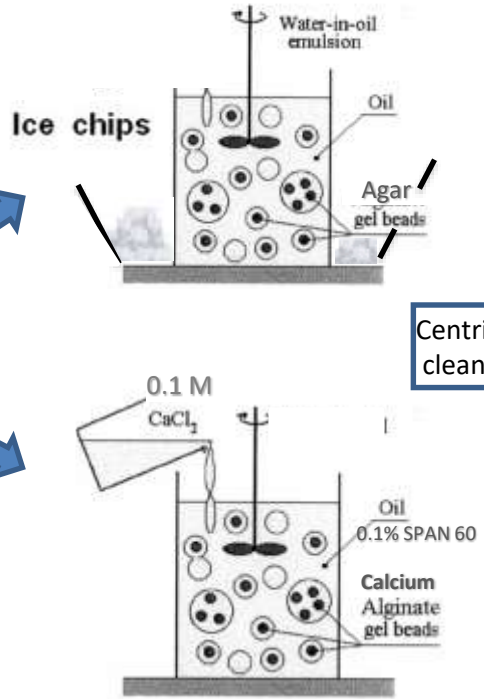

0
0.25
0.5
1
2
5
10
25
50
100
Normalised bioluminescence
400
100
10
1
0.1
0.01
10
20
30
40
Time (hours)

Polymer beads preparation

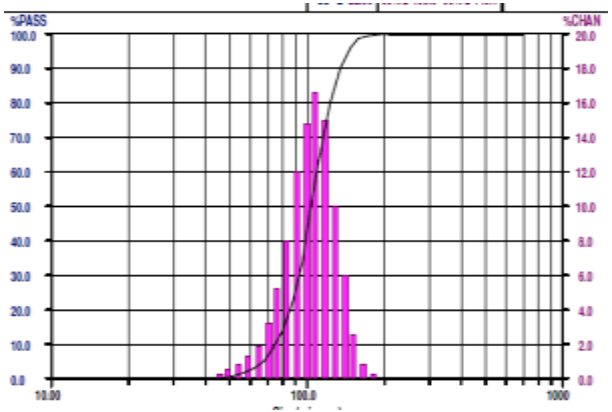
10 mL with PA01 at
OD600 = 0.06



Agar: 48 °C
Alginate: Room temperature



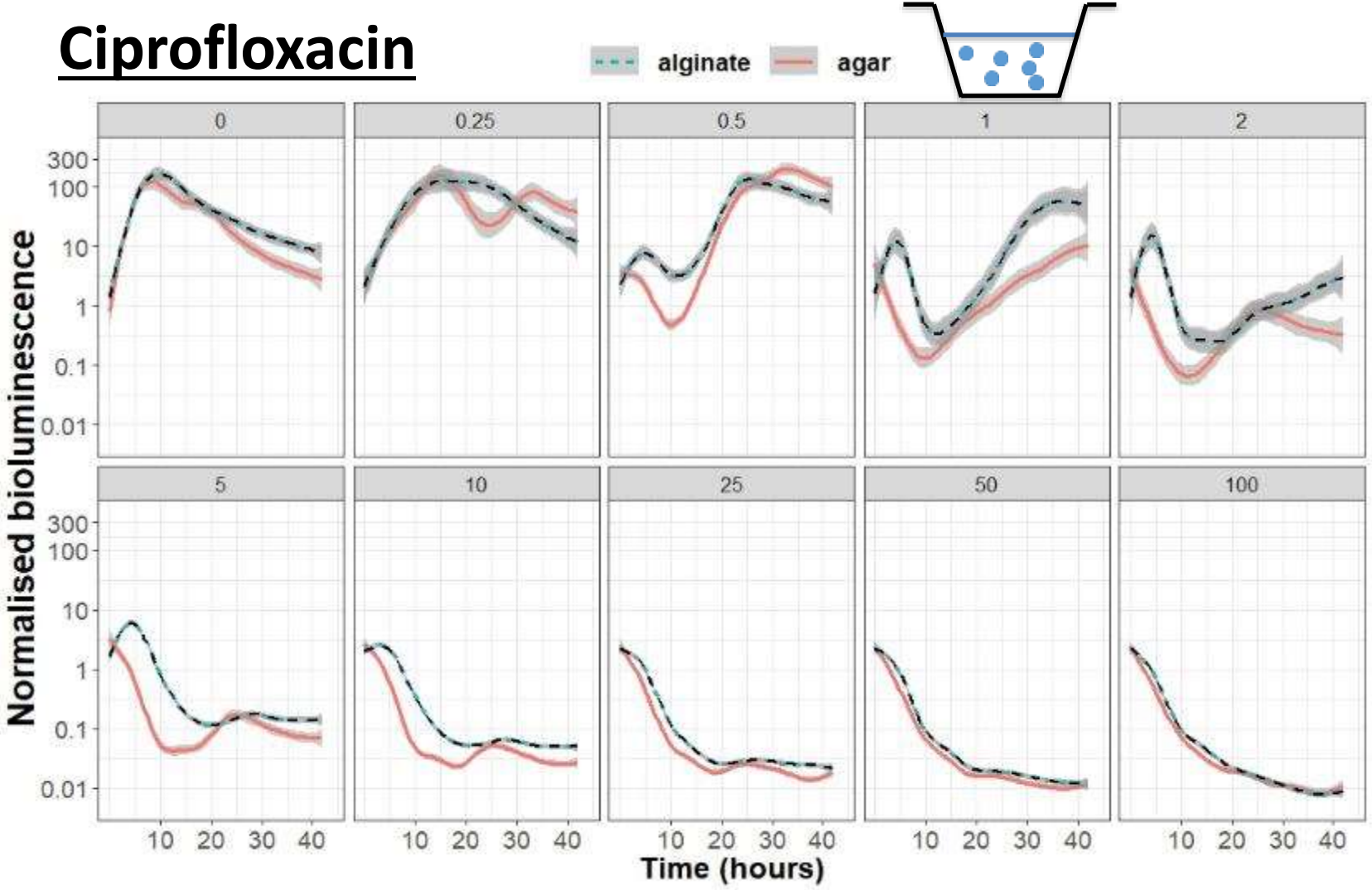
Centrifugation and
cleaning steps



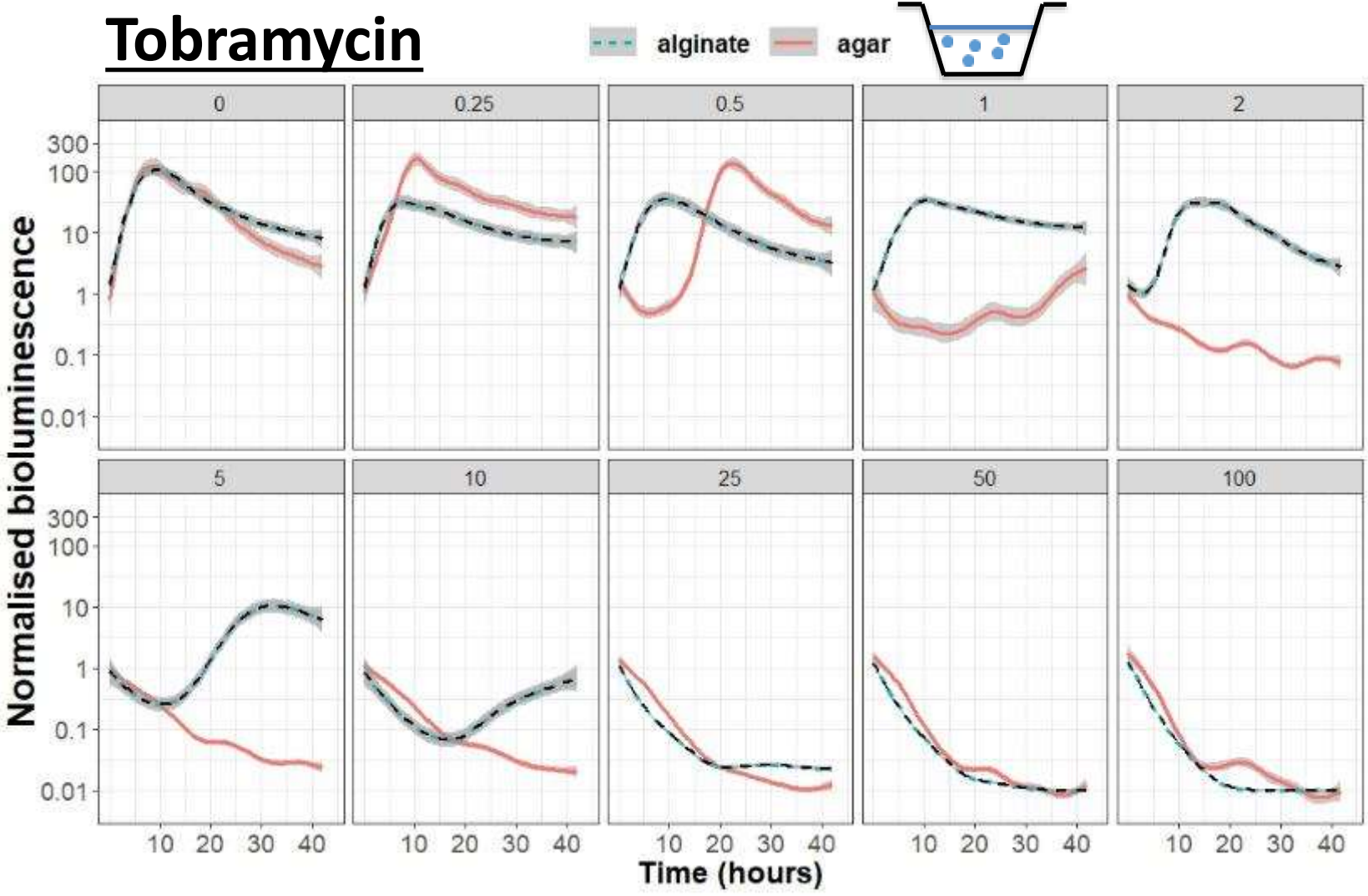
Summary		Percentiles	
mv	= 111.2	10% = 76.51	60% = 111.2
mn	= 94.75	20% = 86.77	70% = 117.3
ma	= 106.1	30% = 93.73	80% = 124.4
cs	= 0.057	40% = 99.77	90% = 135.5
sd	= 22.56	50% = 105.6	95% = 145.1

GROWCOTT, E. J., COULTHARD, Alex, AMISON, Richard, et al. Characterisation of a refined rat model of respiratory infection with Pseudomonas aeruginosa and the effect of ciprofloxacin. Journal of Cystic Fibrosis, 2011, vol. 10, no 3, p. 166-174

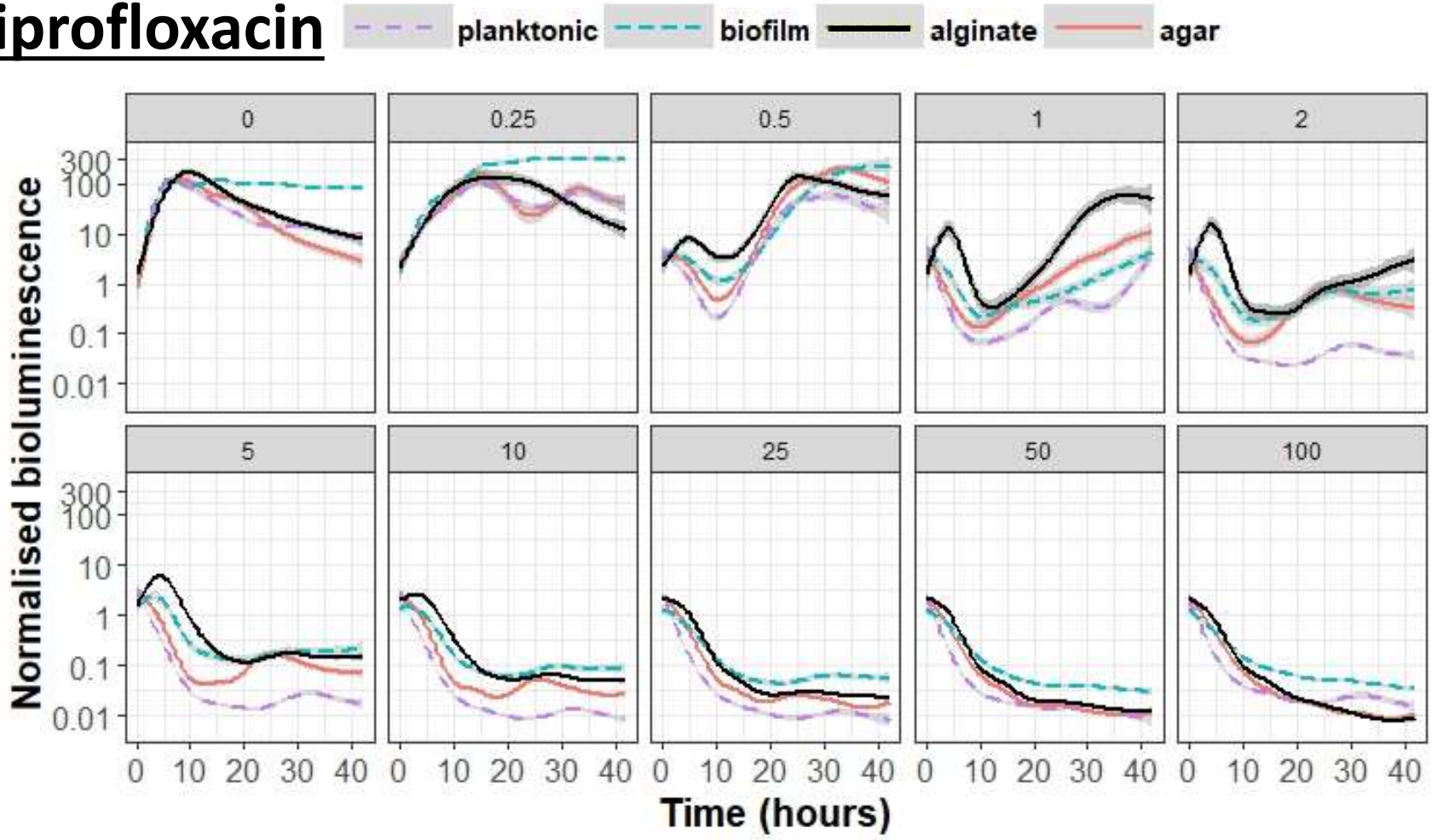
Ciprofloxacin



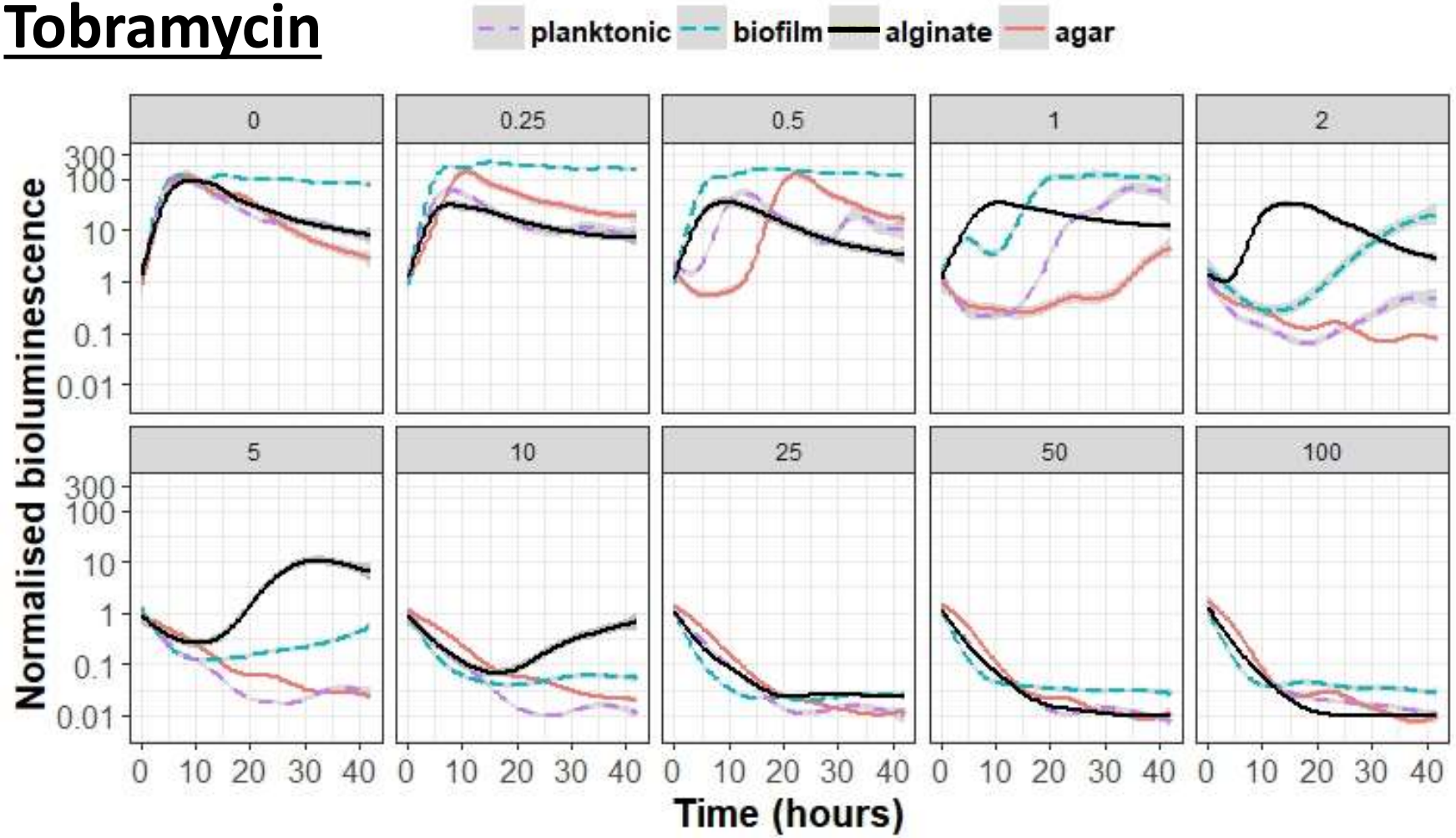
Tobramycin


alginate
agar
0
0.25
0.5
1
2
5
10
25
50
100
300
100
10
1
0.1
0.01
Normalised bioluminescence
10
20
30
40
Time (hours)

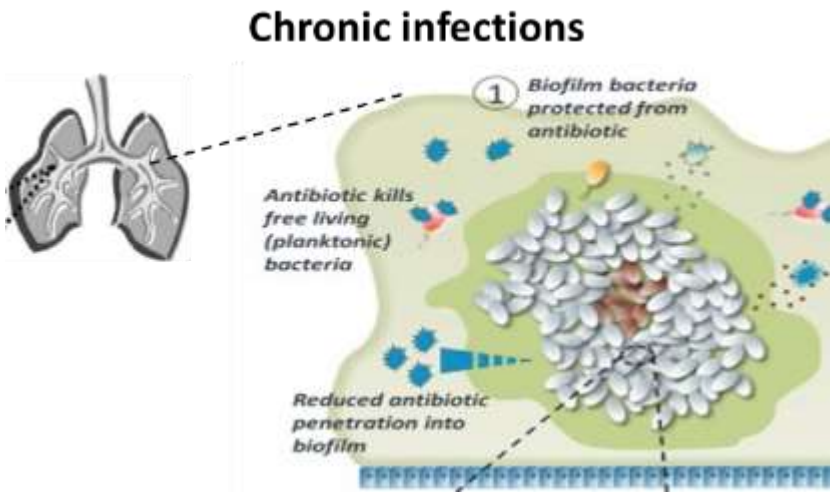
Ciprofloxacin



Tobramycin


planktonic
biofilm
alginate
agar
0
0.25
0.5
1
2
5
10
25
50
100
Normalised bioluminescence
300
100
10
1
0.1
0.01
0
10
20
30
40
Time (hours)

In airway, *P. aeruginosa* biofilms are embedded in mucus



Clusters of *P. aeruginosa* biofilms (<50-100 µm wide) are found embedded in the conducting airways mucus, often surrounded by immune cells such as PMNs

Alginate beads dispersed in mucus simulating media could be an interesting in vitro model to mimic in vivo chronic infections

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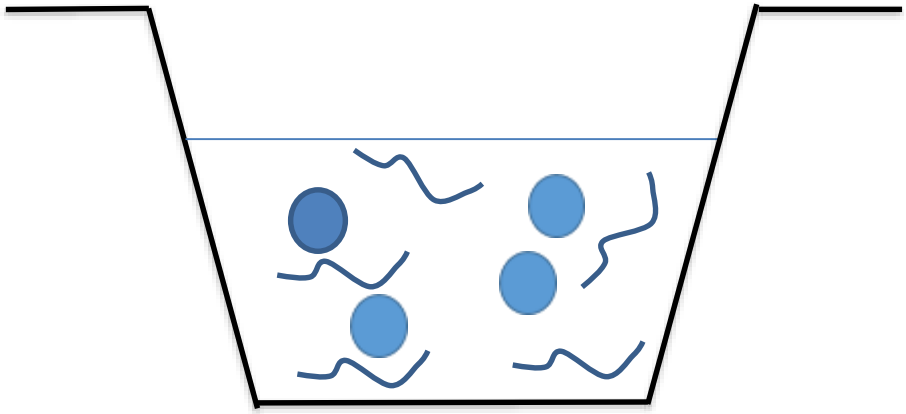
83 (2017) p 113-17

Pseudomonas aeruginosa Aggregate Formation in an Alginate Bead Model System Exhibits *In Vivo*-Like Characteristics

Majken Sønderholm,[a] Kasper Nørskov Kragh,[a] Klaus Koren,[b]
Tim Holm Jakobsen,[a,d] Sophie E. Darch,[c] Maria Alhede,[a] Peter Østrup Jensen,[d]
Marvin Whiteley,[c] Michael Kühl,[b,e] Thomas Bjarnsholt[a,d]

Effect of mucus on time-kill curves



Alginate beads dispersed in ASM to mimic in vivo chronic infections

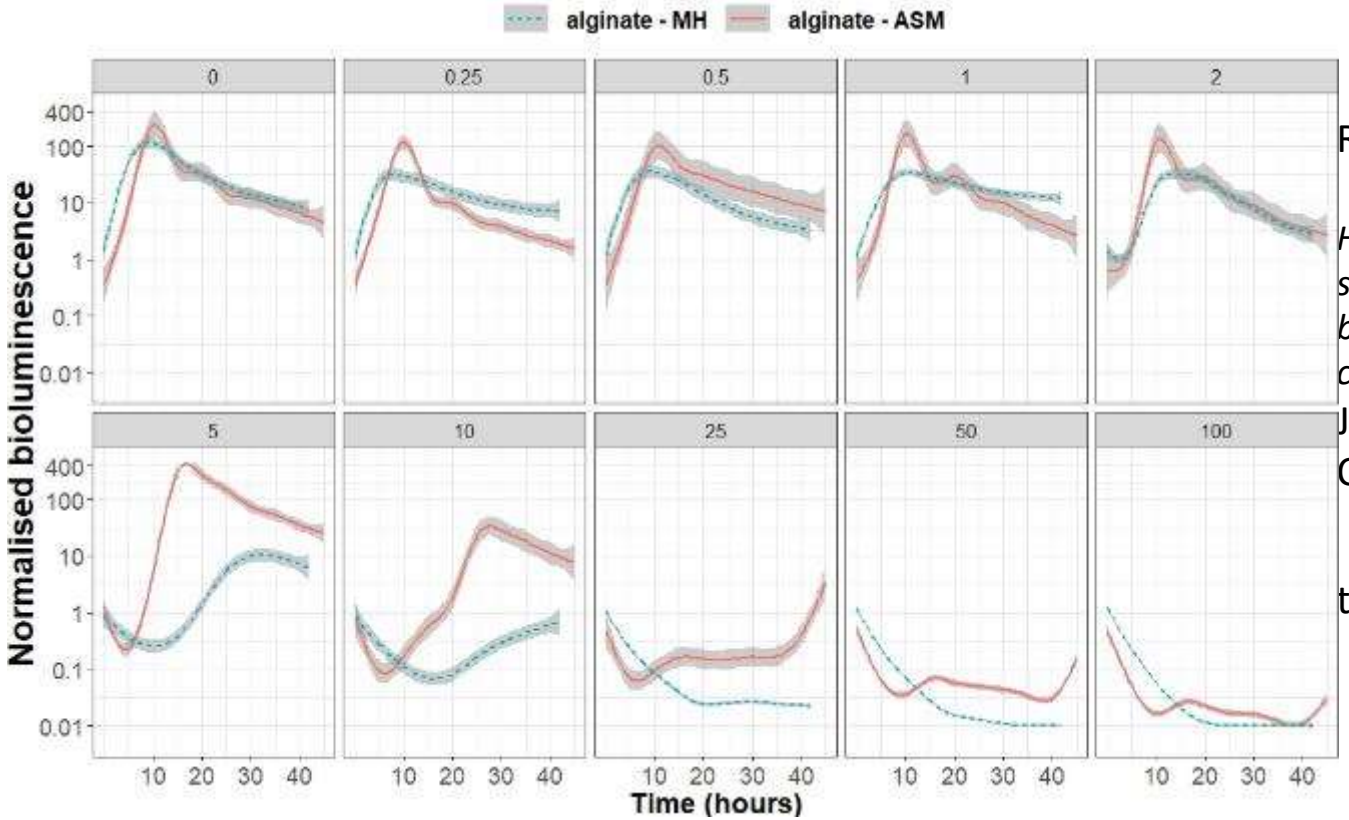
Alginate beads
Bioluminescent PA

Artificial sputum medium (ASM) mimic the sputum of cystic fibrosis

- 5 g/L mucin from pig stomach
- 4 g/L salmon sperm DNA
- 0.5% (v/v) egg yolk emulsion
- 5.9 mg/L of DTPA
- Minerals and amino acids
- pH = 7

Kirchner, Sebastian, et al. Journal of visualized experiments: JoVE 64 (2012).
Sriramulu, Dinesh D., et al. Journal of medical microbiology 54.7 (2005): 667-676.

Effect of mucus on Tobramycin time-kill curves



Results in agreement with Müller *et al.*

Human airway mucus alters susceptibility of P. aeruginosa biofilms to tobramycin, but not colistin.

Journal of Antimicrobial Chemotherapy (2018).

A significant reduction of tobramycin efficacy when *P. aeruginosa* biofilms were grown in the presence of mucus-like medium

Biofilms in the presence of mucus-like medium respond differently to tobramycin

Hypothesis

Difference observed between alginate and agar beads is due to a change in antibiotics diffusion in the beads ?

Stewart, P. S. (1996). Theoretical aspects of antibiotic diffusion into microbial biofilms. Antimicrobial agents and chemotherapy, 40(11), 2517-2522.

TABLE 1. Diffusion coefficients of selected antibiotics in water at 37°C calculated by the Wilke-Chang correlation[a]

Agent	MW (g/mol)[b]	D_{aq} (10^{-6}) (cm^2/s)
Ciprofloxacin	330.4	6.87
Benzylpenicillin	376.4	6.69
Tobramycin	465.5	5.56
Gentamicin	477.6	6.20
Piperacillin	517.6	5.25
Ceftazidime	548.6	5.12
Vancomycin	1,468.3	2.83

[a] Reference 28.
[b] MW, molecular weight.

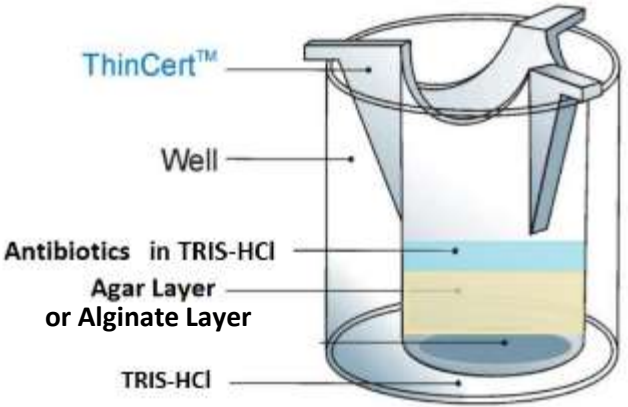
TABLE 2. Comparison of experimentally measured and predicted D_e of selected antibiotics in polymer gels[a]

Agent	T (°C)	Expt D_e (10^{-6}) (cm^2/s)	Predicted D_e (10^{-6}) (cm^2/s)	Conditions
Benzylpenicillin	25	3.38	3.01	10% porcine gastric mucus
Tobramycin	37	3.00	5.03	2% agar
Gentamicin	25	2.52	2.78	10% porcine gastric mucus
	37	2.20	5.61	2% agar
Piperacillin	37	3.70	4.75	2% alginate
	37	4.00	4.75	2% agar
Ceftazidime	25	3.42	2.30	10% porcine gastric mucus
	37	2.90	4.63	2% alginate

[a] The predicted value was determined from equation 5 by using the values of D_{aq} given in Table 1 and was corrected for temperature as appropriate. diffusion coefficients.

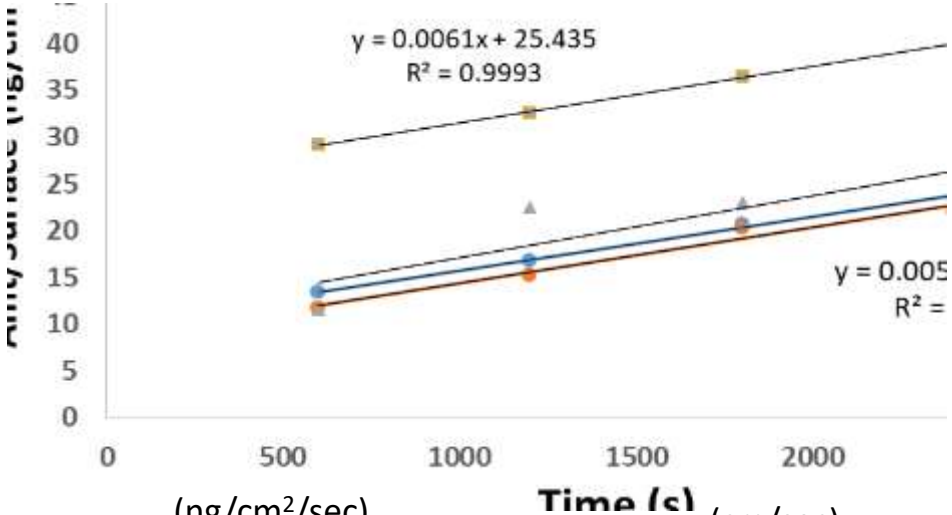
Diffusion in 2% agar and Ca-alginate gels

The basic assumption that the polymer layer is homogeneous with constant thickness and diffusivity



(**less than 5% of the initial amount cross the gel layer**)

- Antibiotics in TRIS-HCl (0.05 M pH7): 10 MIC and 100 MIC



(ng/cm²/sec)

(cm/sec) $P = \frac{J}{C_0}$ (ng/cm³)

(cm/sec) $D = \frac{P}{L}$ (cm)

(cm²/sec) diffusion coefficient

Diffusion in 2% agar and Ca-alginate gels

CIP in agar	
average	**1.5 x 10 $^{-6}$ cm/s**
SD	0.2 x 10 $^{-6}$ cm/s
Diff in H_2O at 37°C	6.9 x 10 $^{-6}$ cm^2/s *
% from Diff in H_2O at 37°C	**22 %**

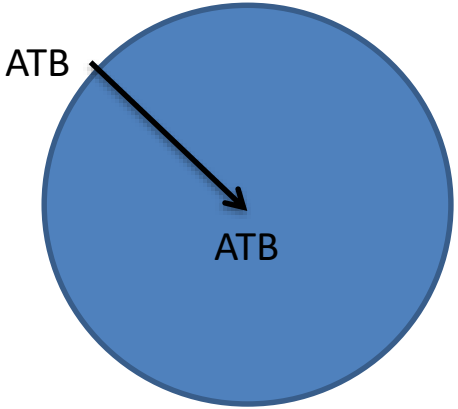
CIP in alginate	
average	**1.4 x 10 $^{-6}$ cm/s**
SD	0.4 x 10 $^{-6}$ cm/s
Diff in H_2O at 37°C	6.9 x 10 $^{-6}$ cm^2/s *
% from Diff in H_2O at 37°C	**21 %**

TOB in agar	
average	**1.6 x 10 $^{-6}$ cm/s**
SD	0.7 x 10 $^{-6}$ cm/s
Diff in H_2O at 37°C	5.6 x 10 $^{-6}$ cm^2/s *
% from Diff in H_2O at 37°C	**28 %**

TOB in Alginate	
average	**0.8 x 10 $^{-6}$ cm/s**
SD	0.5 x 10 $^{-6}$ cm/s
Diff in H_2O at 37°C	5.6 x 10 $^{-6}$ cm^2/s *
% from Diff in H_2O at 37°C	**14 %**

* Stewart, P. S. (1996). Theoretical aspects of antibiotic diffusion into microbial biofilms. Antimicrobial agents and chemotherapy, 40(11), 2517-2522.

Antibiotic diffusion in a polymer sphere



The time required for a solute to attain 90% of the bulk fluid concentration at the center of a spherical matrix is estimated by

$$t_{90} = 0{,}37\frac{R^2}{De}$$

Stewart, Philip S. "Diffusion in biofilms." Journal of bacteriology 185.5 (2003): 1485-1491.

where R is the radius and *De* is the effective diffusion coefficient in the polymer.

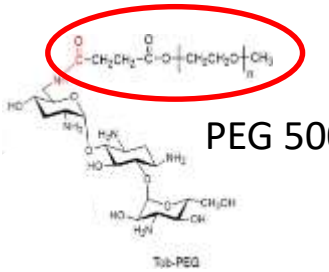
For beads having a diameter of 100 µm

And *De* = 8 x 10^{-7} cm/s

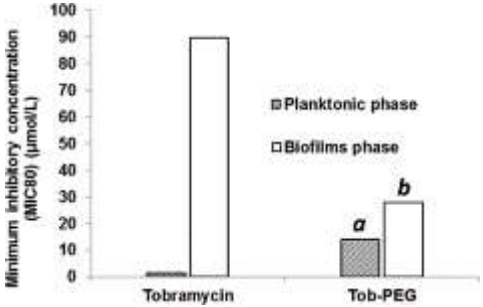
$$t_{90} = 0{,}37 \times \frac{\left(50 \times 10^{-4}\right)^2}{8 \times 10^{-7}} = 12 \text{ seconds}$$

Tobramycin can be expected to penetrate in a matter of seconds or minutes in the Ca-alginate or agar beads

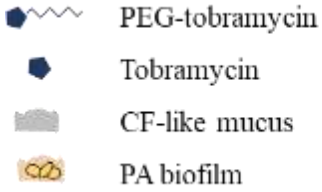
PEGylation of Tobramycin Improves biofilm antimicrobial activity

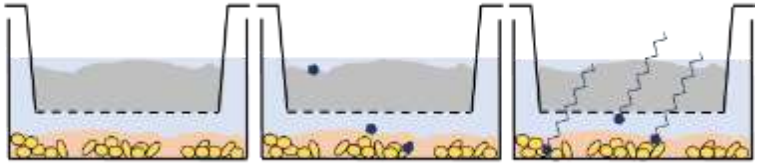


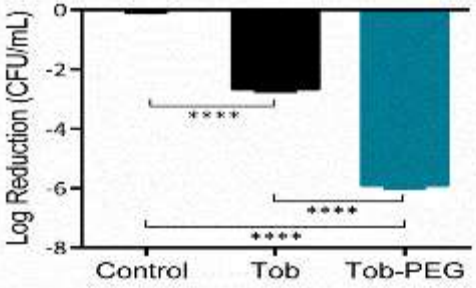
PEG 5000



Du, J., Bandara, H. M. H. N., Du, P., Huang, H., Hoang, K., Nguyen, D., ... & Smyth, H. D. Improved biofilm antimicrobial activity of polyethylene glycol conjugated tobramycin compared to tobramycin in pseudomonas aeruginosa biofilms. Molecular pharmaceutics, 2015, vol. 12, no 5, p. 1544-1553.







Log-reduction of PA biofilms after treatment with TOB-PEG

Bahamondez-Canas, T. F., Zhang, H., Tewes, F., Leal, J., Smyth, H. D. (2018) . PEGylation of tobramycin improves mucus penetration and antimicrobial activity against Pseudomonas aeruginosa biofilms in vitro. Molecular pharmaceutics, 15(4), (2018) p 1643-1652.

Conclusions

Are Agar and Ca-Alginate beads models interchangeable to evaluate ATB efficacy?

It depends on the ATB tested.

Ciprofloxacin readily penetrated both gels. According to diffusion coefficient calculated, both beads were equilibrated with the CIP concentrations in the dispersing broth in few seconds

To evaluate the effect of TOB (cationic ATB) → **Depends on its concentration**

At high (> 10 x MIC) and low (<0.25 x MIC) concentrations → beads are interchangeable

Around MIC (0.25 – 10 x MIC) efficacy of TOB is reduced in Alginate beads compare to Agar ones

Decrease in diffusivity of TOB in Ca-alginate compare to agar gel should not be the main factor explaining the lower sensibility of PA01 to TOB when entrapped in Ca-alginate beads compare to its sensibility when entrapped in agar beads