INHIBITION OF BACTERIAL BIOFILMS IN MICROFLUIDIC CHAMBERS

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Bacterial biofilms are a frequent source of infection, colonizing catheters, prosthesis and other medical equipment. Because biofilms are resilient to adverse environmental conditions and protect bacteria against antibiotic treatment, they have proven particularly refractory to treatment; constituting a major health problem in the clinical setting. For these reasons, there is ample interest in developing compounds able to prevent their formation. Conjugative plasmids are known to increase biofilm formation rates, probably due to the adhesive capabilities of the conjugative pilus. Here, we investigate the ability of 2-hexadecynoic acid (2-HDA), a compound known to inhibit bacterial conjugation, to prevent biofilm formation. For this purpose, we fabricated a microfluidic device able to undergo continuous flow, which we investigated two flow velocities: at 8 μl/min and 10 μl/min, and monitored biofilm formation in continuous time. Image analysis of 22-h time lapse videos revealed the inhibitory effect of 2-HDA on plasmid-mediated biofilm formation in the bacterium E.coli as well as a reduction of the biofilm due to the flux velocity.

1 Introduction

Biofims are one of the most resilient forms of bacterial colonization. Bacteria growing in biofilms are more resistant to antibiotic treatment, and very hard to eliminate due to their protective matrix. They constitute a major clinical problem. [1]

Conjugative plasmids often encode antibiotic resistances. They also promote biofilm formation, this combination makes plasmid-induced biofilms particularly dangerous. [2]

Bacterial conjugation can be inhibited with specific compounds (COINs) that affect the conjugative pilus. It is currently unknown whether conjugation inhibition can also ameliorate biofilm formation. In previous studies, the 2-hexadecynoic acid (2-HDA) has shown to be effective against biofilm formation in static environments. [3] Here, we will study this fatty acid in a continuous flow environment.

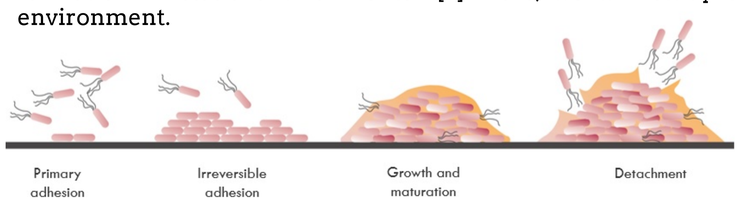

Fig. 1 Stages of biofilm formation [1]

Fig. 2. 2-Hexadecynoic acid molecule [4]

2 Objectives

The overall goal of this project is ***to test whether conjugation inhibitors may be used to prevent biofilm formation.*** For this purpose, we intended to:

1- *Develop an experimental setup to test biofilm formation* under continuous and regulatable flow in microfluidic chambers.

2- *Test whether conjugative plasmids induced biofilm formation* in this setting.

3- *Test whether conjugation inhibitors may be used to prevent biofilm formation.*

3 Methods

Stages

1- Chip Design

-Photolythography

2- Microfabrication

-PDMS (polymer)

3- Bacteria Preparation

Strain: BW25113 E.coli

- R100-1
- R100-1 + 2-HDA
- Control group

All were stained with Crystal Violet 0.01% w/v

4-Microfluidics

- 22 h on a continuous flow at 8 and 10 μl/min

5-Data Analysis

-Photographs taken every 2 minutes

- Integral Density in ROI the chip measured and plotted against time, taking into account the grey scale

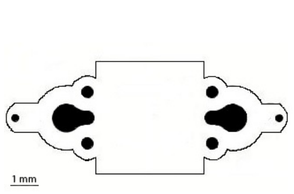

Fig. 3. Redesigned microfluidic chip design with scale [5] **Fig 4.** Wafer used in microfabrication to make the chips

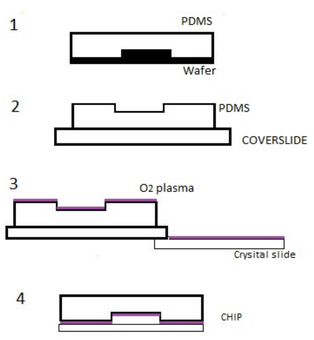

Fig 5 . Diagram of the phases in microfabrication

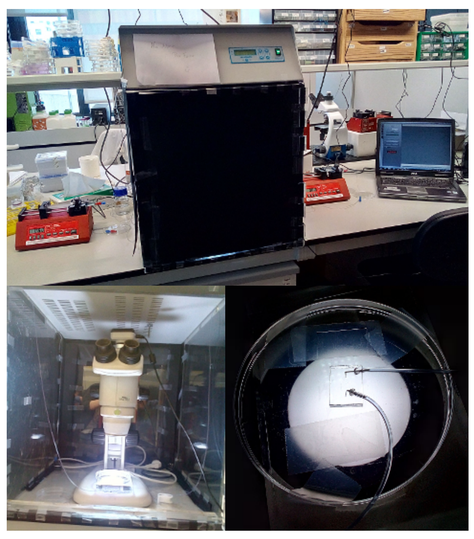

Fig. 6, 7 and 8 Microfluidic setup used in the experiment with all of the materials used to do so

4 Results

$8\mu l/min$

At 8μl/ min the 2-HDA reduces the plasmid induced biofilm formation

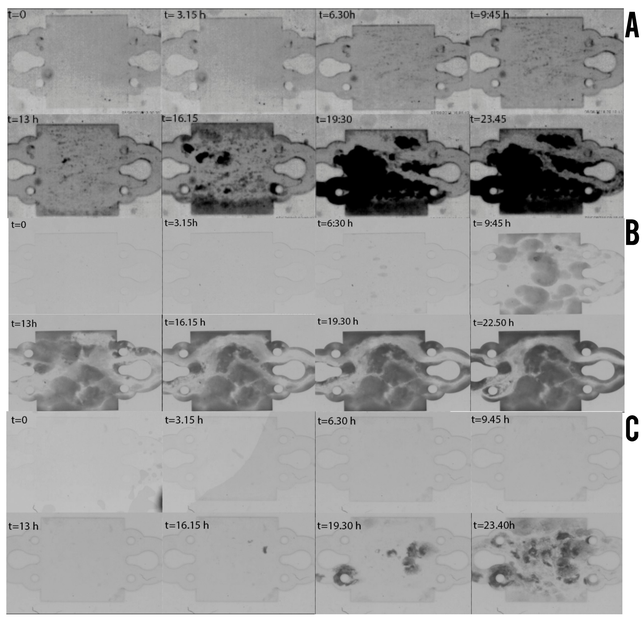

Fig. 9 Compilation of photographs that compare the evolution of the biofilm formation at 8 μl/min under the three conditions

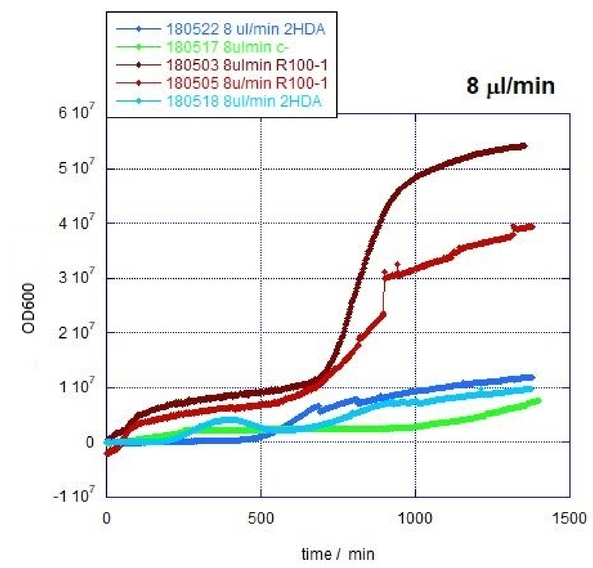

Fig.10 . Optical density plot against time (in minutes) of each of the experiments done at 8 μl /min. The red lines are the bacteria with the plasmid, the blue with the plasmid and conjugative inhibitor and the green is the control group.

A- BW25113 +R100- 1
B- BW25113 + R100-1 + 2-HDA
C-BW25113

$10\ \mu l/min$

At 10μl/min the 2-HDA abolishes the biofilm formation induced by the plasmid

Fig. 11 Photographs of the evolution of the bacterial biofilms at 10 μl/min under the three conditions

Fig. 12 Plot of the Optical density of the BW25113 experiments done at 10 μl/min. The purple lines are the bacteria with the plasmid, the grey lines are the bacteria with the plasmid and conjugative inhibitor and the green are the control groups,

5 Conclusions

- Microfluidic chambers offer a reproducible and highly regulatable method for testing biofilm formation

- 2-HDA blocks plasmid-induced biofilm formation

-Flow velocity is a critical factor regulating biofilm formation. Higher flows act synergistically with the inhibitor.

With this information, we can continue studying biofilm prevention strategies with COINs in a more natural environment, thus providing a medical solution to major diseases.

The control advantages that microfluidics provide let it be simple to change the conditions to see which are ideal for biofilm removal.

6 References

[1] G. Sharma, S. Sharma, P. Sharma, D. Chandola, S.Dang, S.Gupta and R.Gabrani (2016) *Escherichia coli biofilm:development and therapeutic strategies* Journal of Applied Microbiology **121**, 309-319

[2] J.M Ghigo (2001)*Natural conjugative plasmids induce bacterial biofilm development* Nature **412**, 442-445

[3] M. Getino, D.J. Sanabria-Ríos, R. Fernández-López, J. Campos-Gómez, J. M. Sánchez-Lópes, A. Fernández, N.M. Carballeira, F. de la Cruz (2015) *Synthetic Fatty Acids Prevent Plasmid-Mediated Horizontal Gene Transfer* Mbio **6**, 5, 1-8

[4] National Center for Biotechnology Information. PubChem Compound Database, CID=151047, https://pubchem.ncbi.nlm.nih.gov/compound/151047 (accessed June 20, 2018). Modify Date: 2018-06-09

[5] J.L. Song, K.H Au, K.T.Huynh and A. I Packman (2014) *Biofilm Responses to Smooth Flow Fields and Chemical Gradients in Novel Microfluidic Flow Cells* Biotechnology and Bioengineering **111**, N° 13, 598-607