FLUXION

APPLICATION NOTE

Biofilm Compound Profiling

Dose response assay using *P. fluorescens* biofilms

Introduction

Eradication of biofilms is an issue of importance across a variety of scientific, medical, and engineering disciplines. Biofilms are often refractory to many antibiotics and disinfectants to which planktonic bacteria are susceptible. A chief aim of much of biofilm research is to explore the effectiveness of anti-biofilm compounds as well as the discovery of novel inhibitors of biofilm formation or exactors of biofilm dispersal.
The BioFlux System (Figure 1) is a microfluidic platform designed to run automated shear flow protocols for cell biology and microbiology experiments in high throughput. This application note reports compound profiling results for a number of compounds found either to disrupt biofilm communities by eliciting a change in communication within the biofilm, causing dispersal, or to kill cells using a variety of mechanisms (Table 1).

Figure 1: The BioFlux 200 System for live cell assays under controlled shear flow.

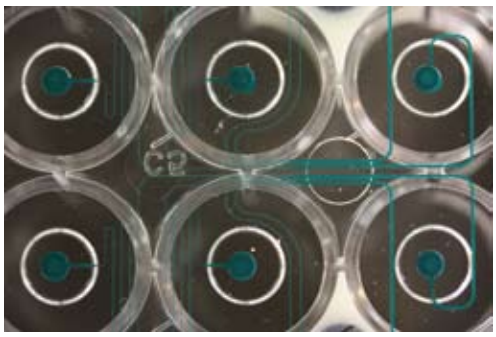

Figure 2: BioFlux Plate channels as viewed from beneath the well plate. Microfluidic flow cells are integrated into the bottom of an SBS-standard well plate. Each fluidic channel runs between pairs of wells and has a central viewing window for observation.

Methods

All channels (Figure 2) were primed from the outlet ports (waste wells) with growth media (M63, sodium citrate) until the microfluidic path was completely filled with liquid. Biofilm growth was initiated under shear flow. Briefly, *P. fluorescens* cells were seeded into the microfluidic channels and allowed to attach for 1 hour. Shear force was applied for 24 hours at 30°C. Treatments, diluted in growth media, were added to each channel. Shear force application was resumed for 24 hours uninterrupted at 30°C. The biofilms were then observed for growth by microscopy. Growth was also evaluated before and after compound addition by sampling the effluvium and plating a dilution of the sample on TSA plates.
Viability staining was performed according to manufacturer's directions using the BacLight Live-Dead Assay kit (Invitrogen, Carlsbad, CA). Intensity measurements were quantitated using the BioFlux 200 analysis module.

Results and Discussion

A variety of compounds were tested on established *P. fluorescens* biofilms grown under shear (Table1). One class of compounds consisting of sodium nitroprusside (SNP) and ferric ammonium citrate (AFC) is known to affect quorum sensing in the related species of bacteria, *P. aeruginosa*, resulting in biofilm dispersal. Consistent with previously published data (Barraud et al 2006), SNP alone was not sufficient to completely disrupt the biofilm or to kill planktonic cells, however when combined with the antibiotic tobramycin, which is only effective on planktonic bacteria, there was a marked decrease in biofilm viability and the viability of the bacteria in the effluent. However, contrary to established data (Musk et al 2005), the viabilty of the biofilms treated with AFC increased in a dose dependent manner with a concommitant increase in runoff bacteria. One possibility to account for this is a difference in iron-dependent gene expression between *P. fluorescens* and *P. aeruginosa*.
The second class of compounds, antibiotics, was shown to be effective against *P. aeruginosa* biofilms grown in microtiter dishes (Olson et al 2002; Reid et al 1994). Consistent with those studies, all treatments with all compounds resulted in decreases in biofilm viability and viable bacteria in the effluent when compared to the media control.

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Channel	Drug	[Conc]	Mode of action
1	Control		
2	Sodium Nitroprusside (SNP)	1000nM	Nitric oxide pathway dispersal of biofilms
4	SNP	500nM	
5	SNP + tobramycin (tob)	500nM /100µM	tob inhibits 30S kills only planktonic cells
6	SNP + SDS	500nM/0.05%	SDS - cell lysis
7	SNP + tob	500nM/500µM	
8	Ferric ammonium citrate (FAC)	100µM	repress gene expression
9	FAC	200µM	for biofilm formation
10	FAC	500µM	
11	Enrofloxacin (Enro)	2 µg/ml	binds gyrase
12	Enro	4 µg/ml	antibiotic
13	Enro	8 µg/ml	
14	Ciprofloxacin (Cipro)	5 µg/ml	binds gyrase
15	Cipro	50 µg/ml	antibiotic
16	Cipro	100 µg/ml	
17	Gentamicin (Gent)	256 µg/ml	30S ribo/trans
18	Gent	512 µg/ml	antibiotic
19	Gent	1024 µg/ml	
20	Control (10% ethanol)		
22	Erythromycin (Eryc)	64 µg/ml	50S ribo/trans
23	Eryc	256 µg/ml	antibiotic
24	Eryc	512 µg/ml	

Table 1: A panel of anti-microbial compounds were tested in multiple concentrations on a single 48-well BioFlux Plate (24 experimental channels).

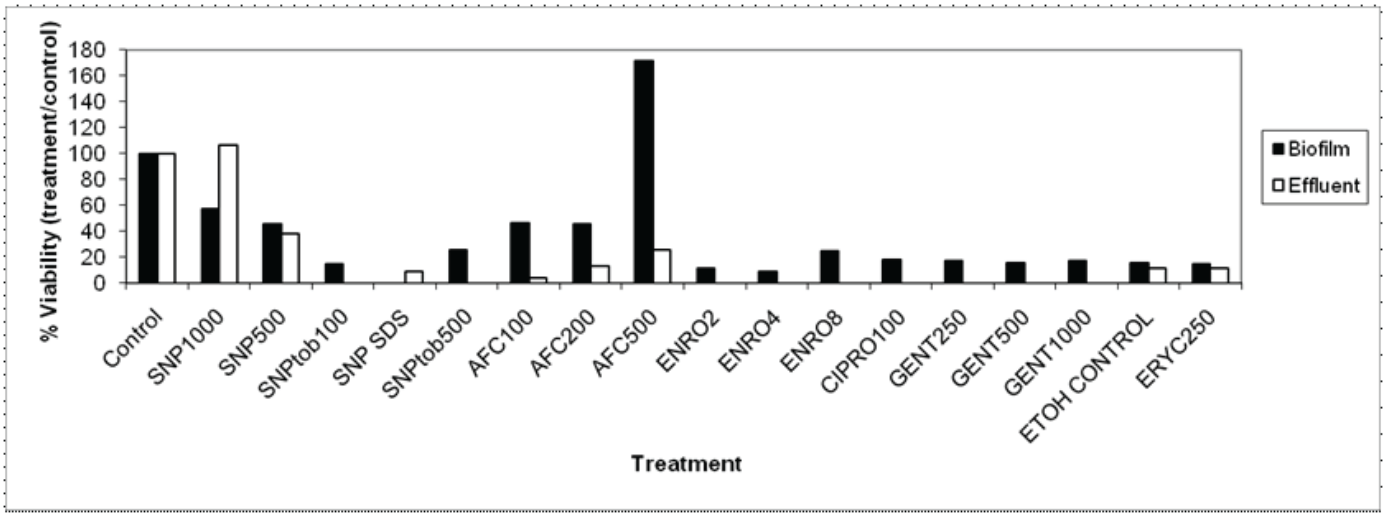

Figure 3: Relative viability of P. fluorescens after treatment with a variety of anti-biofilm compounds. Biofilm viabilty was measured using the BacLight Live/Dead assay. Relative Viability within the biofilm (black bars) was calculated by the following formula (RV=green fluorescence/red fluorescence); all calculated viabilities were compared to the control channel. Growth of bacteria in the effluent (white bars) was measured by comparing colony counts from before and after treatment; all treatments compared to the control.

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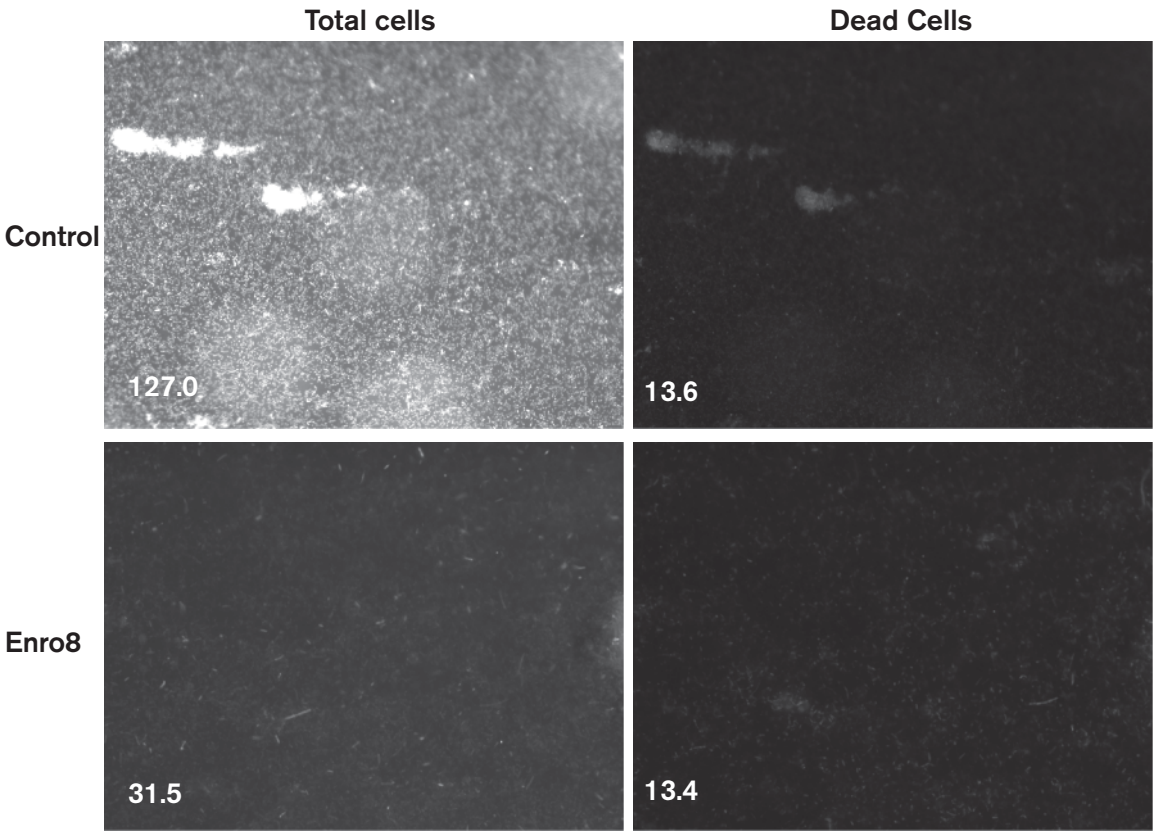

Figure 4: Representative micrographs from the live-dead assay. Relative intensity values are indicated in each micrograph.

Summary

The BioFlux 48-well plate (24 experimental channels) was used to simultaneously assess 9 anti-biofilm compounds with multiple doses for each. Two metrics were explored to evaluate changes in the biofilm after treatment: growth of bacteria in the effluent from the biofilm, and viability staining. Both of which were valid for assessing the effects of treatments. The BioFlux system represents a higher throughput and biologically relevant platform for anti-microbial screening. Further throughput can be gained by running multiple plates from the same instrument, generating up to 96 biofilms simultaneously and making anti-biofilm compound library screening and mutant library analysis under shear within reach.

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