Examination of biofilms grown in microfluidic flow cell arrays for compound screening, host pathogen interactions, and microbial adhesion

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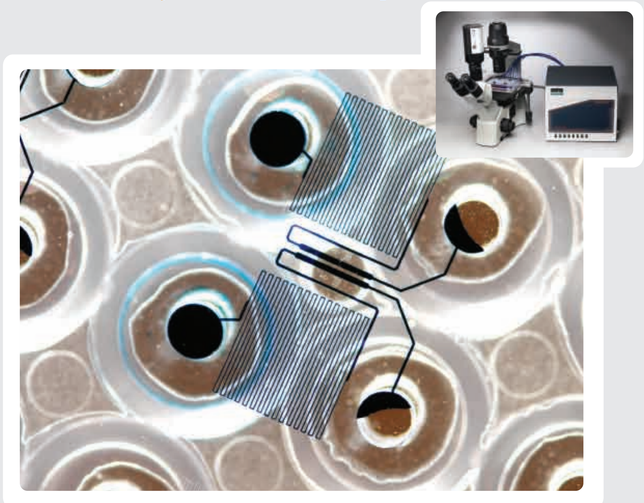
Abstract

Higher-throughput biofilm assays often employ microtiter plates for compound, mutant and conditional screening. Despite their throughput and convenience, well-plate assays have difficulty producing higher content biofilm data, such as morphology, compound penetration, analysis of growth under flow and analysis of mixed species biofilm interactions. We present a microfluidic flow cell array called the BioFlux system which incorporates high content flow-based biofilm experiments integrated into a standard well-plate format. This approach enables up to 24 simultaneous flow-based biofilm experiments per plate. Many biofilm-forming species benefit from the use of shear flow either as an environmental stimulant or for the addition of fresh media or compounds. Conventional parallel plate flow chambers provide shear flow, but their complexity and low throughput limit their utility. The use of a microfluidic flow cell array addresses the need for flow as well as the experimental throughput to carry out replicate experiments and controls simultaneously. We grew bioflims from several bacteria species including: *P. fluorescens, P. aeuruginosa, P.gingivalis* (under anaerobic conditions) and *E. coli* under shear flow within the system. We used an antibiotic panel against *P. fluorescens* to generate compound profiling data using biofilm viability and viability of planktonic cells data streams. The system was also used to study interactions of *P. aeruginosa* with airway epithelial cells under low flow conditions in real time. Microbial adhesion to extracellular matrix proteins under flow was also explored.

System Description



The BioFlux™200 system is:

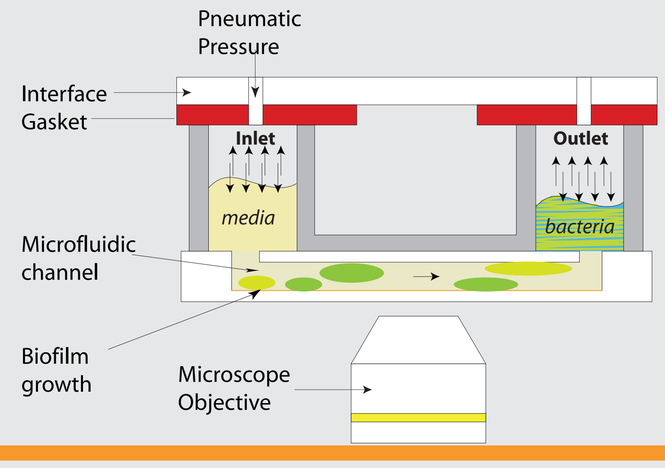
A BioFlux plate (above) multi-well plate with up to 24 shear controlled microfluidic channels, a.k.a. micro-sized flow cells, coupled to the wells. All reagents and cells are loaded in the wells to be introduced into the microfluidic channels.

The BioFlux controller (inset) delivers physiological shear flow. It is controlled by the BioFlux software, which also supports imaging and image analysis.

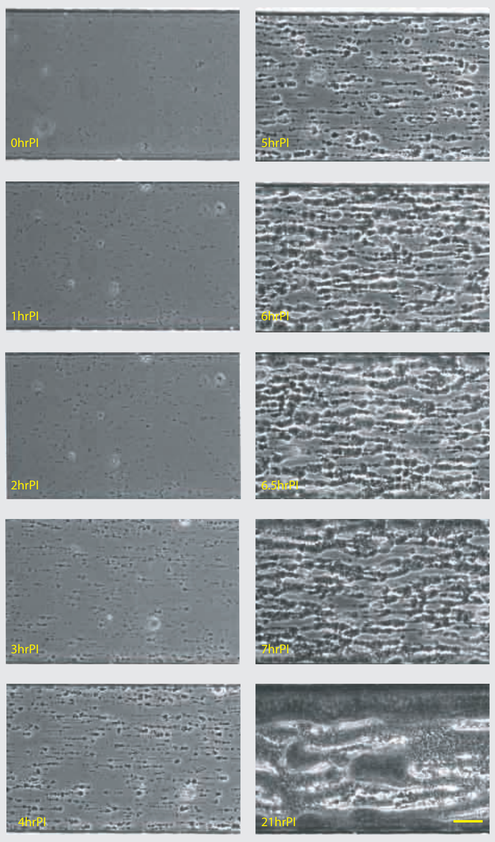
Experimental Methods

Microfluidic channels were primed with media from the outlet well and seeded with bacterial cells from an overnight culture. Medium was introduced from the inlet wells for the duration of biofilm growth. Cells were grown under continuous shear on a heated stage. Biofilms were observed over time by microscopy.

Data were captured using an inverted microscope and BioFlux Imaging software. Fluorescent signals were measured using the analysis module in the BioFlux software. .



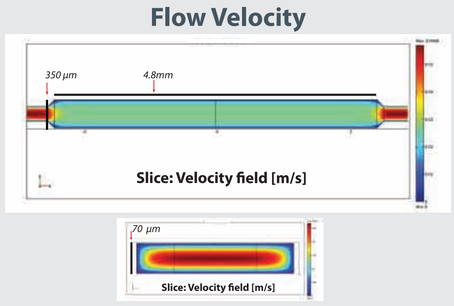
Growth of Biofilms under Shear Flow



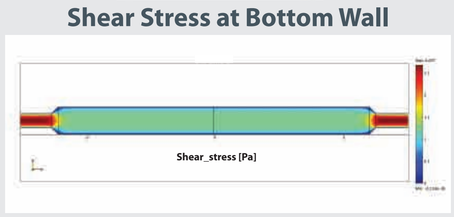
A time-lapse observation of *P. fluorescens* biofilm grown in rich media under shear flow.

In Silico Flow Profiling in the BioFlux

Flow Velocity

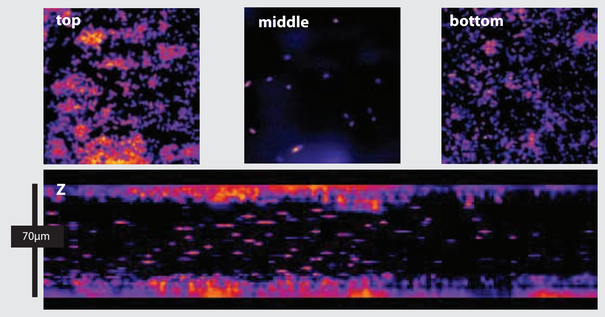


Shear Stress at Bottom Wall



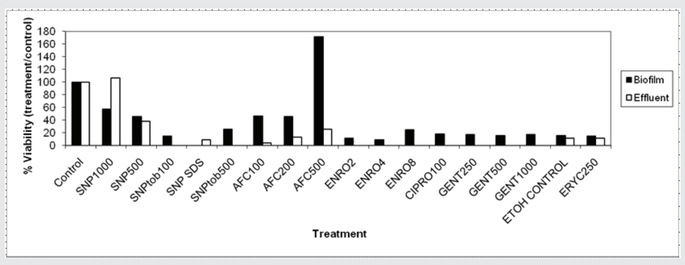
In silico flow profiling was performed for the BioFlux channel in the viewing window region using the program COMSOL (www.comsol.com) multiphysics for values in the middle of the delivered flow range. Data are shown for the velocity within the viewing window top down view as well as for the cross-sectional area. The simulated flow, except for the extreme edges, of the channel is observed to be uniform. The shear stress maximum is found at the bottom wall; the shear stress in the middle of the channel's x-sectional area is found to be zero.

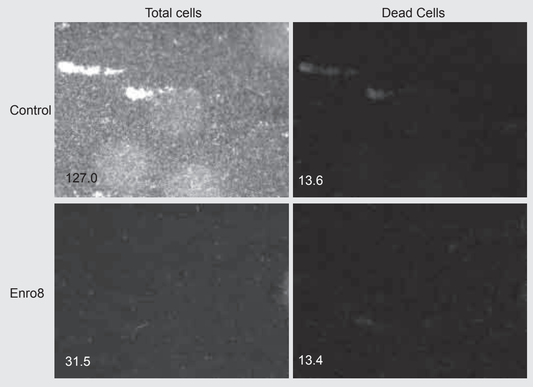
High Content Imaging within the Microfluidic Channel



A *Pseudomonas fluorescens* biofilm was grown in minimal media for 24 hours and stained with BacLight Viability Stain (Invitrogen). The live biofilm was imaged on a Nikon TE2000U inverted scope with Nikon Live-ScanSwept-Field Confocal with 40X ELWD objective corrected for the 180 micron thickness of the bottom of the BioFlux plate. Data were collected using a Photometrics Cascade camera and NIS-Elements software in 1.5 micron slices over a Z-distance of 70 microns. This distance covers the complete height of the BioFlux microfludic channel in the viewing window region of the plate. Data for this study were acquired at the Nikon Imaging Center at UCSF.

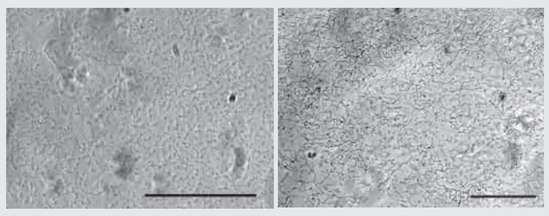
Anti-Biofilm Compound Profiling





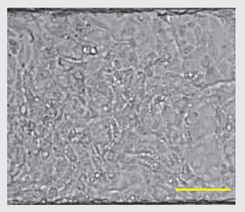
Relative viability of *P. fluorescens* after treatment with a variety of anti-biofilm compounds. (A) Biofilm viabilty was measured using the BacLight Live/Dead assay. Relative Viability within the biofilm (black bars) was calculated by the following formula (RV=green fluorescence/red fluorescence); all 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. (B) Representative micrographs from the live-dead assay. Relative intensity values are indicated in each micrograph.

Growth of Anerobic Oral Bacteria in the BioFlux

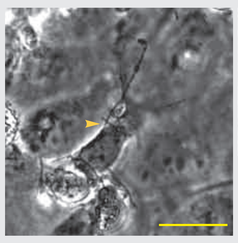


S. gordonii was seeded in BHI and subjected to 2dyn/cm² shear flow for 24 hours (left). *P. gingivalis* was added to the outlet well and perfused into the channel containing the *S. gordonii* biofilm. Media was changed to PG media in the inlet well. Flow at 2 dyn/cm² was started after an hour incubation at 37 degrees. The gas mix used was 95% N_2: 5% CO_2. The biofilm was examined after 6.5hours (right). Scale bar is 50µm.

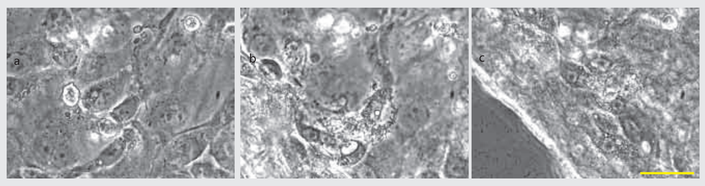
Host Pathogen interactions: *P.aeruginosa* and Airway Epithelium



Airway epithelial cells grown to 100% confluence in the BioFlux plate (scale bar = 100µm).

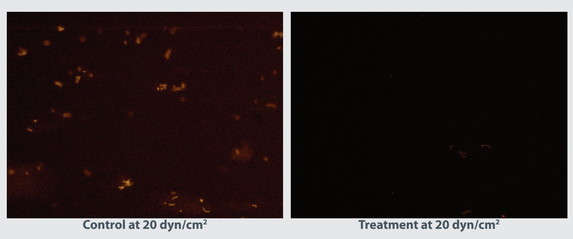


P. aeruginosa attaching under flow to airway epithelial cells (scale bars =20µm).



Cytopathic effects of *P. aeruginosa* attachment to epithelial cells. (a) start of flow (b)1.5 hours post flow (c)3 hours post flow (scale bar= 40 µm)

Microbial Adhesion on ECM Molecules



Control at 20 dyn/cm² | Treatment at 20 dyn/cm²

	WT	mutant
PBS at 1 dyn/cm²	100	100
Treatment at 1	47.2	18.6
PBS at 20 dyn/cm²	100	100
Treatment at 20	0	16.3

*Relative fluorescent intesity of treatment over control intensity expressed in percent.

Bacterial cells were introduced to microfluidic channels coated with Matrigel and allowed to attach for 10 minutes at room temperature. Bacteria were then washed with PBS alone or PBS containing treatment . Images were captured under flow.

Summary

Here, we demonstrate several different applications of the BioFlux system for the study of bacteria under flow, including biofilms, profiling compounds on biofilms, microbial adhesion and host pathogen interactions.

The main advantages to using this system for biofilms under flow is the higher parallelization, media is contained within the plate, and tight and quick shear control.