Growing Mature Biofilms for Antimicrobial Screening, Host-pathogen Interactions, and Adhesion

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ABSTRACT

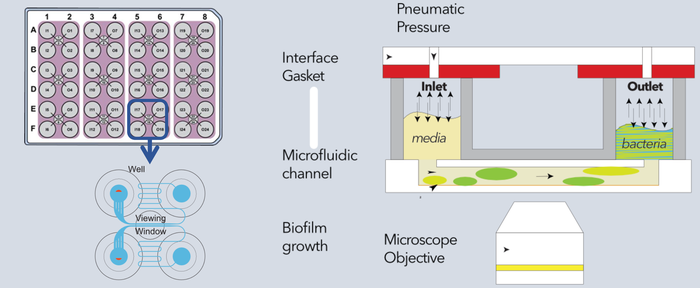
Biofilm assays, such as compound screening and host-pathogen interaction assessment, are often conducted in microtiter plates. Despite their throughput and convenience, biofilms grown in well-plates do not fully mature, thereby reducing the quality and relevance of higher content biofilm data, including morphology, compound penetration, and analysis of mixed species biofilm interactions. Here, we present the BioFlux shear flow system, a microfluidic flow cell array that enables the growth of biofilms in precisely controlled conditions, including shear stress, temperature, and environmental gas. P. fluorescens, P. aeuruginosa, P. gingivalis (under anaerobic conditions), and E. coli were grown under shear flow conditions. An antibiotic panel against P. fluorescens was used to generate compound profiling data using biofilm viability and viability of planktonic cells data streams. Interactions between P. aeruginosa with airway epithelial cells were imaged in real-time. Finally, microbial adhesion to extracellular matrix proteins under flow was assayed. Using this high-throughput approach enabled full maturation and attachment of biofilms for physiologically relevant analysis.

INTRODUCTION

- Pathogenic microorganisms often form adherent biofilms to survive in their natural settings
- Biofilms grown in static conditions do not fully mature and are poor representations of physiological biofilms
- Conventional parallel plate flow chambers are complex and low-throughput, increasing the difficulty of drug discovery and development

METHODS

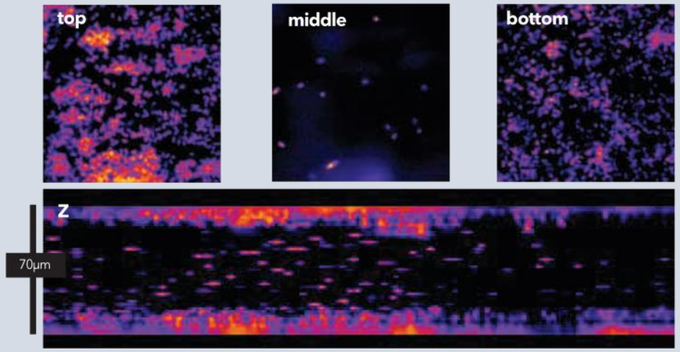
BioFlux Microfluidic Device for Biofilm Assay



Microfluidic channels were primed with media from the outlet well and seeded with bacterial cells from an overnight culture. Media 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 by microscopy over time.

RESULTS

High-content Imaging Under Shear Flow



A Pseudonmonas fluorescens biofilm was grown in minimal media for 24-hours and stained using BacLight Viability Stain (Invitrogen). The live biofilm was imaged on a Nikon TE2000U inverted microscope using Nikon LiveScan Swept Field confocal with 40XELWD objective corrected for the 180 micron thickness of the bottom of the microfluidic 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. Data for this study were acquired at the Nikon Imaging Center at UCSF.

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- Mimic the natural environment of biofilms
- Create physiologically relevant models of chronic infection
- Increase antimicrobial testing throughput

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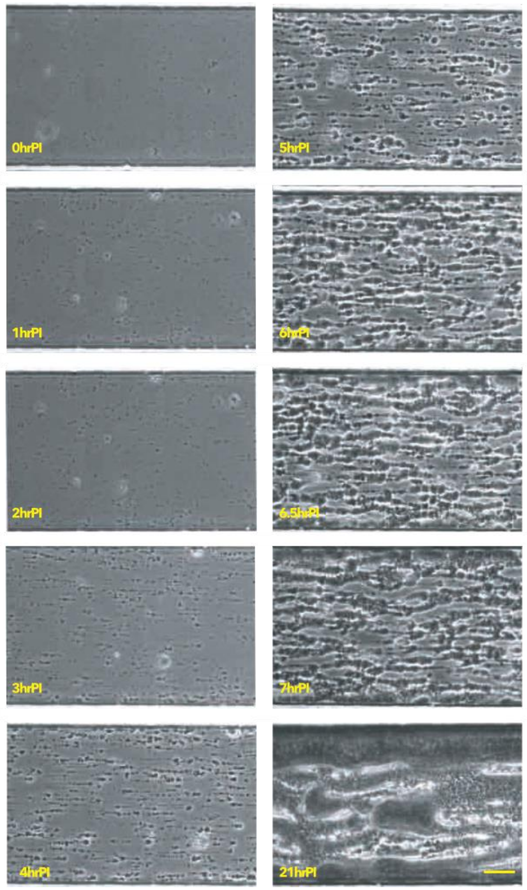
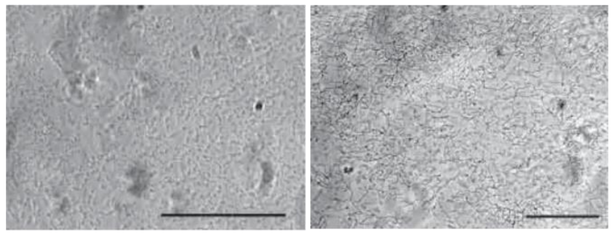
RESULTS

Growth of Biofilms Under Shear Flow



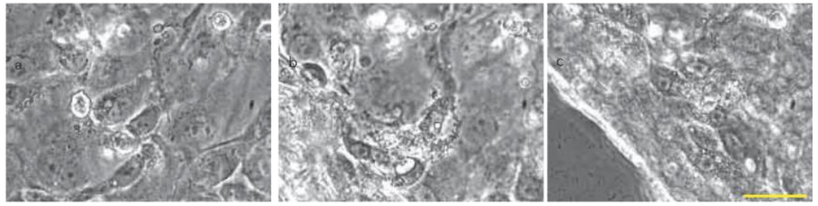
Time-lapse imaging of P. fluorescens biofilm grown in rich media under shear flow conditions.

Anaerobic Oral Bacteria



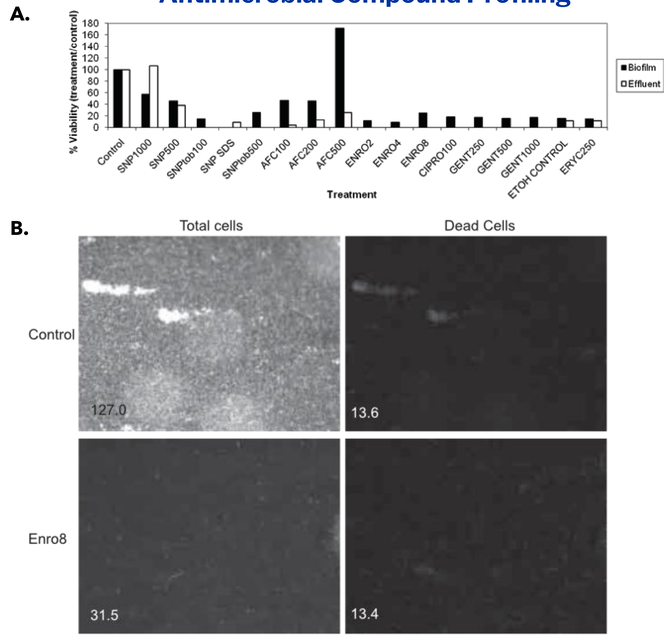
S. gordonii was seeded in BHI and subjected to 2 dyn/cm^2 shear flow for 24-hours (left). P. gingivalis was added to the outlet well and perfused into the channel containing the S. gordonii biofilm. Media was changed to PG media in the inlet well. Flow at 2 dyn/cm^2 was started after a 1-hour incubation at 37°C. The biofilm was examined after 6.5 hours (right). The gas mix used 95% N_2 + 5% CO_2. Scale bar is 50 µm.

Host-pathogen Interactions



Effects of P. aeruginosa attachment on epithelial cell structure and monolayer contraction. Start of flow (left), 1.5-hours post-flow (middle), and 3-hours post-flow (right). Scale bar = 40 µm.

Antimicrobial Compound Profiling



Relative viability of P. fluorescens after treatment with a variety of anti-biofilm compounds. Biofilm viability was measured using Bac Light LIVE/DEAD assay (A). Relative viability (RV=green fluorescence/red fluorescence) within the biofilm (black bars) was calculated; 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 were compared to the control channel. Representative micrographs from the LIVE/DEAD assay (B). Relative intensity values are indicated on each micrograph.

CONCLUSION

- Biofilm formation and function are impacted by environmental mechanical stress induced by shear flow.
- Co-cultures can be used to investigate interactions between multiple species of bacteria in physiologically relevant conditions.
- BioFlux microfluidic plates enable rapid high-throughput antimicrobial compound profiling with high-content imaging.

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