FLUXION

APPLICATION NOTE

Host Pathogen Interactions

Pseudomonas aeruginosa growth on airway epithelial cells

Introduction

Shear flow conditions enabled by the BioFlux system (Figure 1) can be used to either stimulate physiological conditions (e.g. urinary tract, gastrointestinal tract, respiratory tract, blood stream) or used for introduction of organisms and buffers during uninterrupted microscopic observation. *In vitro* models can be developed for the study of host pathogen interactions that utilize both benefits of this system.

Cystic fibrosis (CF), a genetic disorder affecting cellular ion transport, is complicated by chronic lung infections by a variety of opportunistic bacteria, including *P. aeruginosa*. Study of host-pathogen interactions in CF is germane to discovering how the infections are established and how to prevent them. Here we present a model of airway epithelial cells and *P. aeruginosa* in the BioFlux system.

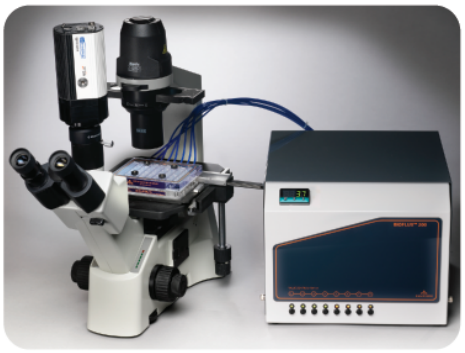


Figure 1: The BioFlux System for live cell assays under controlled shear flow.

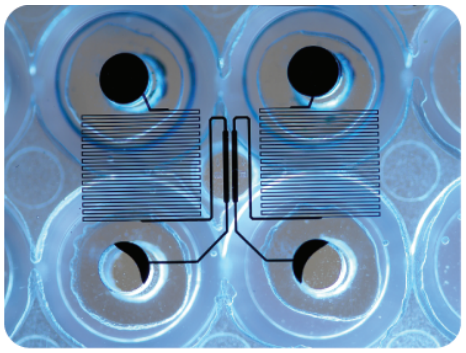


Figure 2: BioFlux Plate channels as viewed from beneath the well plate. Microfluidic flow cells are integrated into the bottom of an SBS-standard well plate. Each fluidic channel runs between pairs of wells and has a central viewing window for observation.

Methods

Culturing airway epithelial cells

Microfluidic channels of the BioFlux plate (Figure 2) were coated with Matrigel (BD Biosciences, Franklin Lakes, NJ) diluted in serum free media 1/50 (v/v) for 1 hour at 37°C. Channels were washed with media for 10 minutes prior to seeding cells. Airway epithelial cells (Calu-3) were seeded into the channels in Eagle's Minimum Essential Media plus 10% serum, 10mM Hepes, and 2mM glutamine. Cells were allowed to settle and attach with no flow for 5-8 hours. Cell feeding was accomplished by initiating gravity flow from the inlet well. Airway epithelial cells are sensitive to high flow rates; high flow rates should be avoided during culturing. Confluent monolayers were achieved after 5-7 days of culturing at 37°C in a 5% C02 atmosphere (Figure 3).

Host-pathogen interactions

Overnight cultures of *P. aeruginosa* were grown in LB media. Bacterial cells were washed three times by centrifugation with CO_2-Independent media (Invitrogen, Carlsbad, CA). Bacteria were introduced into the channels from the waste well at 1 dyn/cm^2 for 10 minutes. Bacteria were incubated for 1 hour at 37°C under gravity flow for attachment. Phase contrast, time-lapse images were captured 3 frames per minute for 1 hour using a QICam camera (QImaging, Surrey, B.C.) on a Nikon TS100 microscope (Nikon, Melville,NY). Flow from the media well at 0.8 dyn/cm^2 was initiated as was imaging for 3 hours at 1 frame per minute.

Results

Cells were observed for attachment, aggregation, and cytopathic effects within the epithelial cell-bacterial interaction model. Bacteria were observed adhering to cells in the monolayer under low shear flow conditions (Figure 4). Aggregation of bacterial cells was also observed (Figure 4, yellow arrow). Cytopathic effects and the contraction of the monolayer were observed later in the data collection period, perhaps due to size of inoculum or due to type III secretion (Figure 5) underlining the importance of controlling number of bacteria introduced under such conditions as well as experimental conditions for experiments of a longer duration.

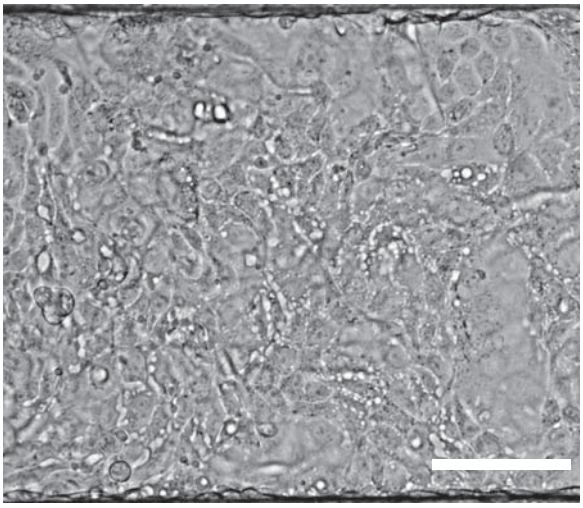
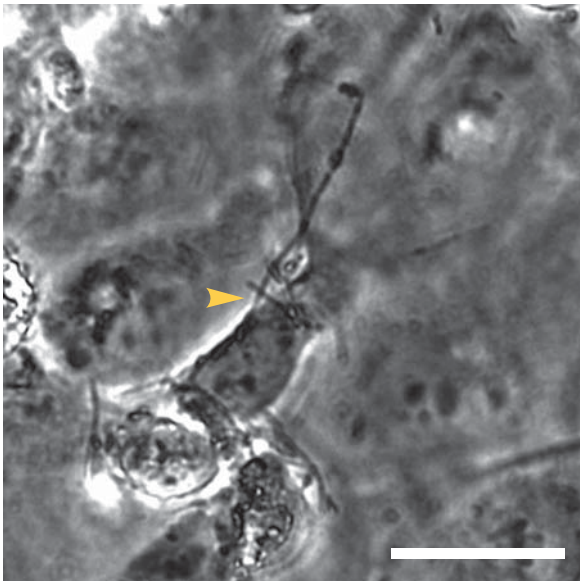


Figure 3. Airway epithelial cells grown to 100% confluence in the BioFlux plate (scale bar = 100µm).



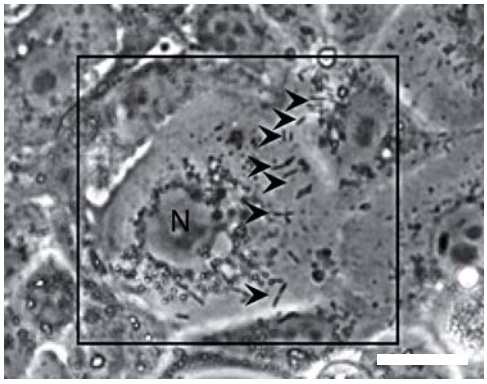


Figure 4: *P. aeruginosa* attaching under flow to airway epithelial cells (scale bars=20µm).

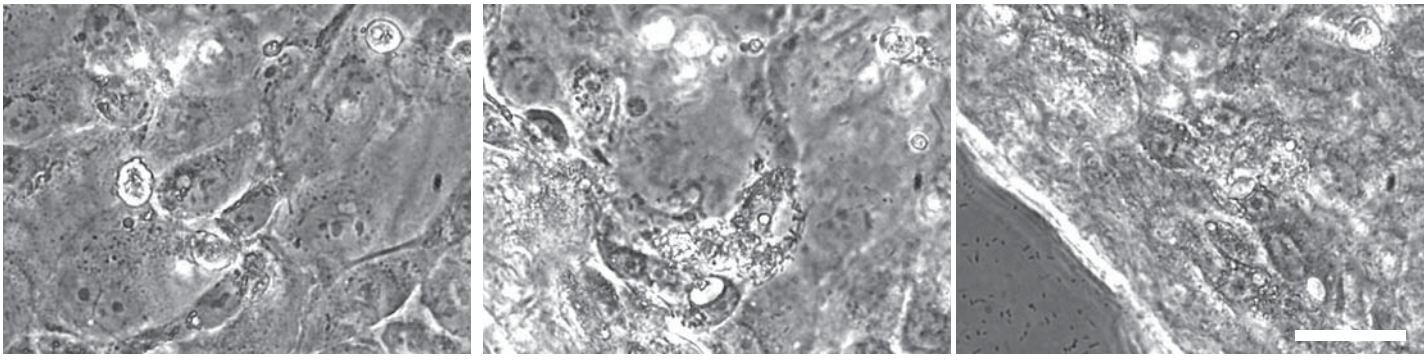


Figure 5: Cytopathic effects of *P. aeruginosa* attachment to epithelial cells. (a) start of flow (b)1.5 hours post flow (c) 3 hours post flow (scale bar= 40 µm)

Summary

Establishment of a model for the study of bacterial-host interactions was achieved in the BioFlux system to study *Pseudomonas* on lung epithelial cells. Observations were made under flow during attachment and aggregation of bacteria on cells. As a model system for CF, it is amenable to further experiments to probe both the microbiology and the cellular physiology involved in the establishment of lung infections. In a similar manner, the microfludic channels of the BioFlux plates could also be adapted to model other host-pathogen systems interactions such as: urinary tract infections with UPEC, infections in cardiac heart valves, or Chlamydial interactions with blood vessel walls. These models can be further extended to pharmacological and antiobiotic screening. The system provides the ability to run 24 assays in parallel amounting to hundreds of conditions screened per day.

Acknowledgements

Fluxion acknowledges Terry Machen, Ph.D. at University of California, Berkeley for kindly providing bacteria and host cell lines as well as advice on culture of epithelial cells and experimental design.

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