CELL Microsystems®

APPNOTE

Host-Pathogen Interactions

Pseudomonas aeruginosa growth on airway epithelial cells

Introduction

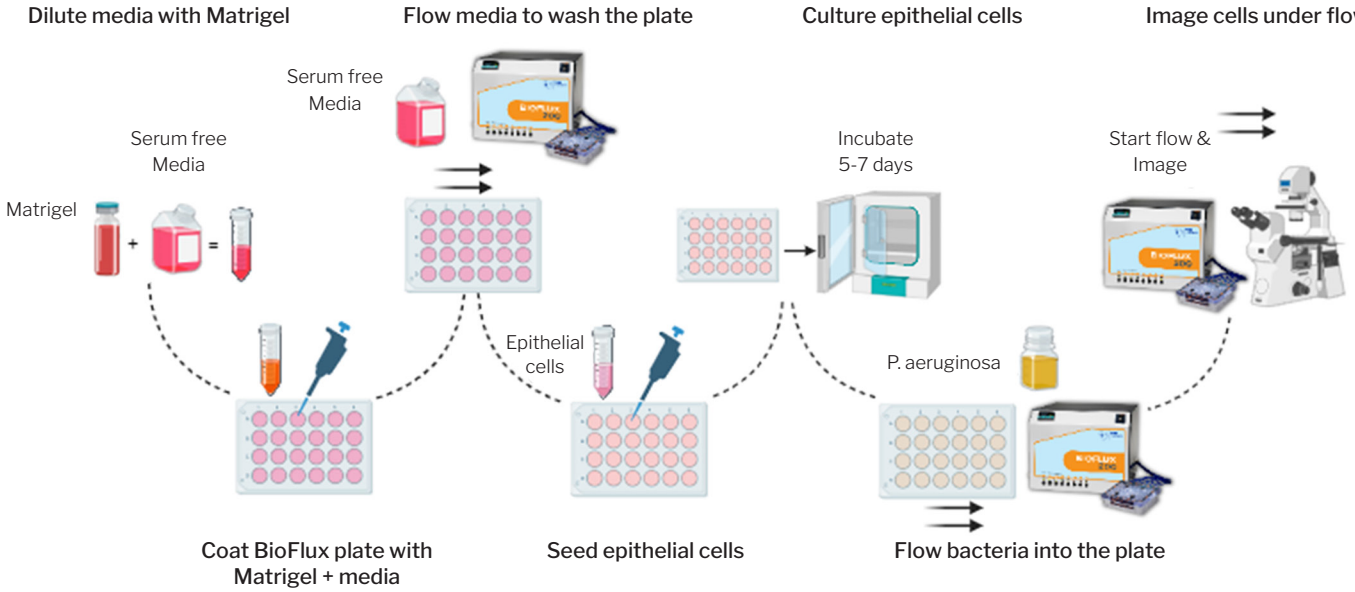
Cystic fibrosis (CF), a genetic disorder affecting cellular ion transport, is complicated by chronic lung infections of various opportunistic bacteria, including P. aeruginosa. The study of host-pathogen interactions in CF is germane to discovering how infections are established and how to prevent them. Here, we mimic in vivo conditions by developing a model of airway epithelial cells and P. aeruginosa under physiological shear flow conditions using a BioFlux system.

Shear flow conditions enabled by the BioFlux system can be used to either simulate physiological conditions (e.g., urinary tract, gastrointestinal tract, respiratory tract, bloodstream) or be used for the introduction of organisms and buffers during uninterrupted microscopic observation. Both benefits of this system can be used together to develop physiologically relevant in vitro models for the study of host-pathogen interactions.

Key Highlights:

1) Seed and grow epithelial monolayers under physiological shear flow conditions
2) Observe attachment and aggregation of bacteria to epithelial cells under shear flow
3) Track the effects of bacteria on epithelial cell structure and monolayer contraction

Host-Pathogen Interaction Workflow



Questions this App Note Answers

1. How can epithelial cells be used in a BioFlux plate?
2. What is the protocol for creating a physiologically relevant model of host-pathogen interaction?
3. How can I simulate the respiratory tract of a person with cystic fibrosis?

Methods

Culturing Airway Epithelial Cells

Matrigel (BD Biosciences, Franklin Lakes, NJ) was diluted in serum-free media 1/50 (v/v) and used to coat microfluidic channels of a BioFlux plate 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, 10 mM Hepes, and 2 mM glutamine. Flow was stopped and cells were allowed to attach for 5-8 hours. Feeding was accomplished by initiating gravity flow from the inlet well. Confluent monolayers were achieved after 5-7 days of culturing at 37°C with 5% CO_2 (Figure 1).

Note: High flow rates should be avoided during culture, as airway epithelial cells are sensitive to high flow rates.

Host-pathogen Interactions

Overnight cultures of Pseudomonas 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. For attachment, bacteria were incubated for 1 hour at 37°C under gravity flow. Phase contrast, time-lapse images were captured at 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 was initiated at 0.8 dyn/cm^2 and images were captured at 1 frame per minute for 3 hours.

Confluent airway epithelial cells

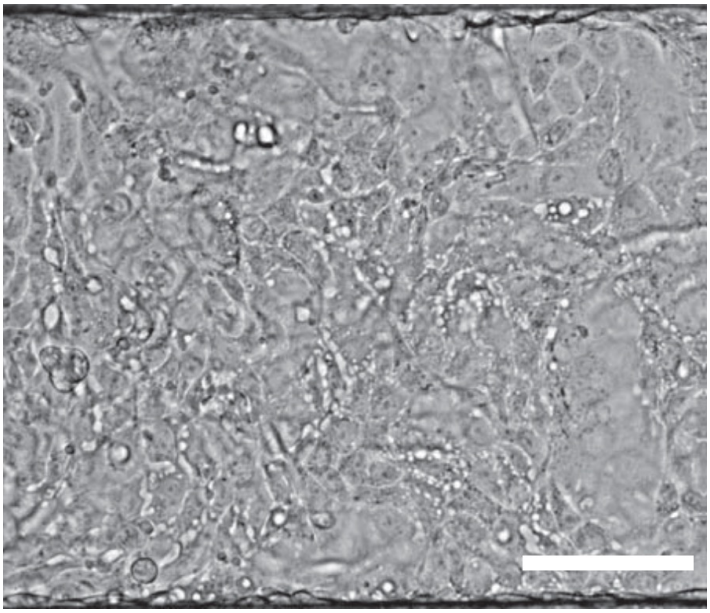


Figure 1. Airway epithelial cells grown to 100% confluence in the BioFlux plate (scale bar = 100 µm).

Results

Cells were observed for attachment, aggregation, and morphological effects within the epithelial cell-bacterial interaction model. Bacteria adhered to cells in the monolayer under low shear flow conditions (Figure 2A). Aggregation of bacteria was also observed (Figure 2B). Morphological effects and the contraction of the monolayer were observed later in the data collection period (Figure 3).

P. aeruginosa attachment under flow

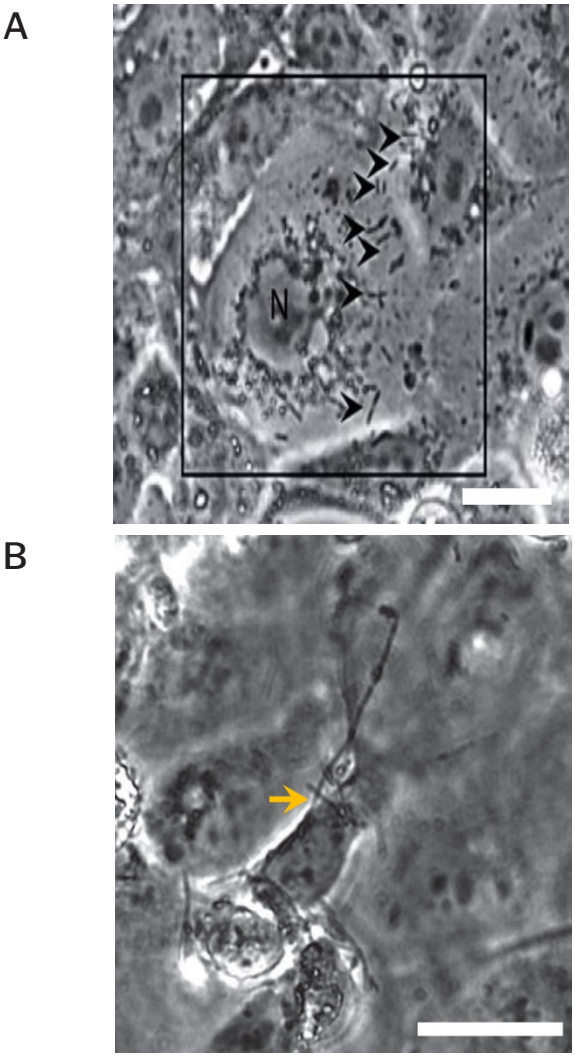


Figure 2. (A) P. aeruginosa attaching to airway epithelial cells under flow. (B) Aggregation of bacterial cells (yellow arrow) (scale bars = 20 µm).

Morphological effects of P. aeruginosa

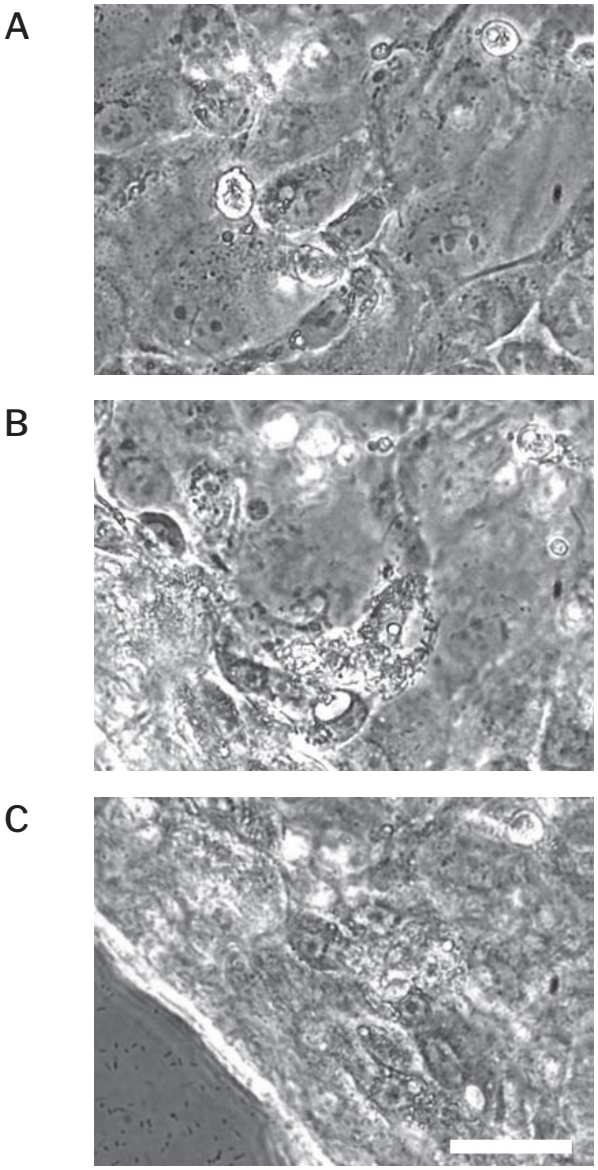


Figure 3. Morphological effects of P. aeruginosa attachment to epithelial cells. (A) Start of flow, (B) 1.5 hours post-flow, (C) 3 hours post-flow (scale bar = 40 µm).

Discussion

The establishment of a physiologically relevant model for the study of bacterial-host interactions within airways was achieved using a BioFlux system. Morphological effects and monolayer contraction were observed later in the data collection period. This may be due to the size of the inoculum or due to type II secretion, underlining the importance of controlling the number of bacteria introduced under the described conditions, as well as for experiments of a longer duration. Using this model, observations of both attachment and aggregation of bacteria to cells were made under physiological flow conditions. The model established here is useful in the study of cystic fibrosis and is amenable to experiments that probe both the microbiology and cellular physiology of lung infections. Similarly, the microfluidic channels of a BioFlux system can also be adapted to model other host-pathogen interactions, such as urinary tract infections, cardiac heart valve infections, and more. These models can be further extended to pharmacological and antibiotic screening, as a BioFlux provides the ability to run 24 assays per plate in parallel. Taken together, these data demonstrate an animal-free, physiologically relevant model of host-pathogen interaction that can be leveraged to reduce the time and cost of drug discovery and development investigations.

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For more information on BioFlux Technology, visit cellmicrosystems.com

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