Use of the BioFlux Apparatus to Study Environmental Biofilms.

Carmen Allen and Shauna L. Reckseidler-Zenteno.

Centre for Science, Athabasca University Athabasca, Alberta, Canada T9S 9Z9

ABSTRACT

Background
The Science Laboratory at Athabasca University has recently acquired the BioFlux 200 system from Fluxion Biosystems. This system employs the use of shear flow to model physiological and environmental conditions. The apparatus has a number of applications in the areas of cell biology, immunology, and microbiology. One of the applications of this system is that it allows for real-time analysis of biofilm growth and development under environmentally controlled conditions.
Methods
The BioFlux system contains a number of sterile and contained tubes that are capable of delivering a constant shear flow of fluids through channels embedded between the wells of 24 or 48-well plates. Each channel contains an input well and an output well which allows different types of fluids and chemicals to be added in order to study the effect of biofilm development under a variety of conditions. A suspension of bacteria is inoculated into the output well and shear flow applied in order for the bacteria to flow into the channel. Once the bacteria have been seeded into the channels, fluid is added to the input well and shear flow applied to allow the fluid to move through the channel and into the output well. Antibiotics, peptides, various concentrations of ions, and other chemicals can be added. The biofilm can then be monitored over the length of the experiment due to the fluorescence microscope and camera attached to the BioFlux apparatus. The camera included with the instrument can be programmed using software to capture images of the biofilms at desired intervals. The microscope can image in bright-field, phase contrast, and fluorescence. Images of the biofilms can be analyzed for density, fluorescence intensity, and other variables using the Montage software that is included.
Results
The BioFlux apparatus has previously been used to study biofilm development in a few species of bacteria. We are currently using this apparatus to determine the effect of a number of synthetic peptides on biofilms produced by the bacterial species *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. This project is in collaboration with the University of British Columbia.
Conclusions
The BioFlux apparatus is an effective way to study biofilms in environmentally or physiologically relevant conditions. It is advantageous over other methods for studying biofilms as it allows for constant shear flow in a controlled environment. It also allows for direct imaging of the biofilm without having to remove the biofilms from pegs or plates. Finally, it is capable of real-time imaging throughout the course of the experiment.

INTRODUCTION

The BioFlux system by Fluxion Biosciences is an instrument that can deliver constant shear flow to 24 or 48- well plates in order to mimic physiological and environmental conditions, which adds relevance to a number of studies. This system has a pump which delivers shear flow to the plates through a series of contained hoses at a controlled rate. The system is attached to a microscope and camera to enable visualization and image capture. The software that comes with the system enables one to program the timing of the experiment and the frequency of images so the experiment can be monitored in real-time. The software also enables detailed analysis of the images. This system has applications for a number of disciplines including: microbiology, immunology, vascular biology, oncology, and developmental biology.

The BioFlux system utilizes their innovative Well Plate Microfluidic™ technology to embed micron-scale fluidic channels on the bottom of a 24 and 48-well plate. A wide range of conditions can be simulated by controlling the flow across the experimental channel. The channels are embedded between the wells of the plates. Two wells are designated for each channel which allows for flow in and out of the channel.

The applications for microbiology include biofilms, which have relevance medically and environmentally. This system enables real-time analysis of biofilm development. The channels allow for compounds to be introduced into the biofilm in order to study their effect. The BioFlux is an excellent system to facilitate drug discovery as it allows for screening of a number of compounds in a controlled and relevant environment. Our laboratory, in collaboration with the University of British Columbia is investigating the efficacy of a number of synthetic peptides for anti-biofilm activity.

MATERIALS AND METHODS

Preparation of biofilms:

Bacterial cells were grown overnight in 3 ml of Luria broth (LB) at 37° C with shaking at 250 rpm. Cells were then sub-cultured (100 µl into 3 ml) in LB and grown to an optical density (OD_{600}) of 0.3. Cells were then inoculated into the output well of a 48-well microfluidic plate that was primed with LB and shear flow applied to allow the cells into the channel. The plate was then incubated at 37° C for 1 hour to allow the bacteria to adhere. Following incubation the excess fluid was removed from the output well and fresh LB added to the input well. Shear flow was applied at a rate of 2 dyne/cm^2 for 8 hours. Images of the biofilm were captured at the end point of the experiment.

Image acquisition:
Images were obtained using an inverted Nikon microscope in phase contrast and analyzed using the Montage software package that accompanies the BioFlux system. The Nikon microscope is also capable of fluorescence and bright field microscopy. Images were obtained using a 20 power objective lens as this enables viewing of the entire channel and the overall biofilm that has developed.

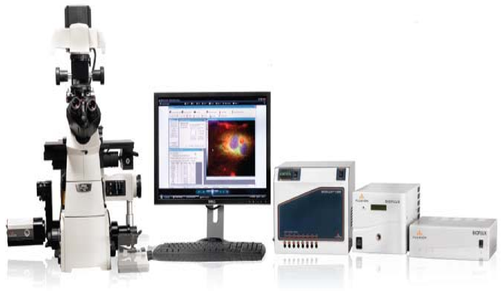
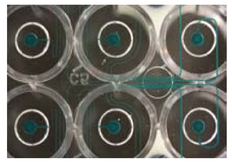


Figure 1. The BioFlux system by Fluxion Biosciences. The system contains a pump that delivers shear flow through a series of hoses which is delivered to channels embedded on the bottom of 24 and 48-well plates. The pump is integrated to an inverted microscope as well as a camera. Software accompanies the system that allows programming for conditions and image acquisition as well as data analysis.



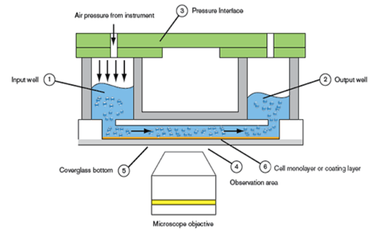


Figure 2. The BioFlux Microfluidic™ well plate by Fluxion Biosciences. Hoses from the pump deliver shear flow through channels embedded underneath well plates in a 24 or 48-well format. Each channel has an input and an output well so fluid can be delivered through the channels. The bottom of the plate sits over the objective lens of an inverted microscope connected to a camera so images can be captured over time in order to analyze biofilm development.

Figure 3. Biofilm development in the bacterium *Pseudomonas aeruginosa* following 8 hours of incubation. Bacteria were seeded into the channels at an optical density of 0.3 and shear flow applied at a rate of 2 dyne/cm^2 for 8 hours using Luria broth. This image is at 200X magnification.

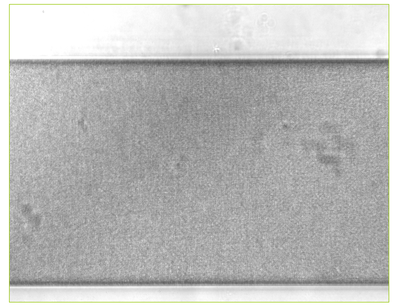


Figure 4. Biofilm development in the bacterium *Klebsiella pneumoniae* following 8 hours of incubation. Bacteria were seeded into the channels at an optical density of 0.3 and shear flow applied at a rate of 2 dyne/cm^2 for 8 hours using Luria broth. This image was generated at 200X magnification.

DISCUSSION

The Science Laboratory at Athabasca University has recently acquired the BioFlux 200 system from Fluxion Biosystems. This system employs the use of shear flow to model physiological and environmental conditions. Our laboratory is currently interested in the application of biofilm growth and development. The BioFlux system contains a number of tubes that are capable of delivering a constant shear flow. Each channel contains an input well and an output well which allows different types of fluids and chemicals to be added in order to study the effect of biofilm development under a variety of conditions.

We have recently been using the BioFlux apparatus to study biofilm development in a few species of bacteria. We are currently using this apparatus to determine the effect of a number of synthetic peptides on biofilms produced by the bacterial species *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. This project is in collaboration with the University of British Columbia. We are interested in studies involving environmental biofilms as well, and are interested in future collaboration in this area.

The BioFlux apparatus is an effective way to study biofilms in environmentally or physiologically relevant conditions. It is advantageous over other methods for studying biofilms as it allows for constant shear flow in a controlled environment. It also allows for direct imaging of the biofilm without having to remove the biofilms from pegs or plates. Finally, it is capable of real-time imaging throughout the course of the experiment.

Athabasca University
Canada's Open University®