FLUXION

APPLICATION NOTE

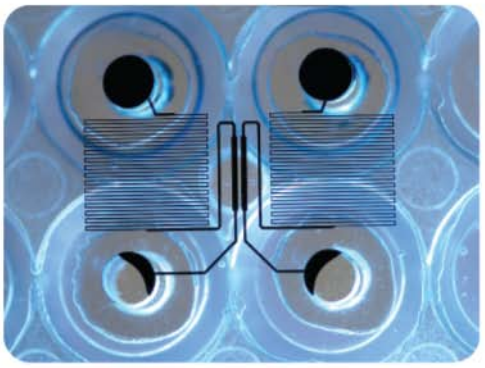
Biofilm Growth

Growth of oral and environmental biofilms using the BioFlux System

Introduction

All bateria occupy natural niches in which they proliferate. Organisms that can be isolated from the enviroment present a concern as opportunistic pathogens which grow as biofilms in wounds. Likewise, the bacteria that make up the flora of the oral cavity have complex relationships within biofilms that ultimately contribute to pathogenesis in the oral cavity as well as in other organs of the body. In order to study such relationships among bacteria, it is necessary to inoculate and culture the organism under shear flow.

The BioFlux™ System is a microfluidic platform designed to run automated shear flow protocols for cell biology and microbiology experiments in high throughput (Figure 1). This application note reports co-culture experimentation with *S. oralis* and *A. naeslundii* in BioFlux Plates for formation of a mutualistic biofilm in saliva, as well as, growth of a biofilm from an enviromnental bacterium, *P. fluorescens*, a close relative of the opportunistic pathogen *P. aeruginosa*.



Methods

A 48-well BioFlux Plate was used, which contains 24 individual flow cell experiments (Figure 2).

Inoculating cultures

The channels were primed from the outlet ports (waste wells) with degassed saliva or growth media until the channel was completely coated. For co-culture experiments in saliva, *Streptococcus oralis* was added first followed by *Actinomyces naeslundii* , both at a low shear force. The cells were allowed to settle for 15 minutes in between additions. For *P. fluorescens*, cells from an overnight culture were added to the channel and allowed to settle for 1 hour before application of shear (Figure 3).

Co-culture/ biofilm growth assays

In order to grow biofilms from the inoculated bacteria, the entire interface coupled to the BioFlux Plate was placed in an incubator; saliva or media was perfused overnight. For the oral biofilm experiments, cells were stained live after the overnight incubation with AlexaFluor® conjugated antibodies against each species (Figure 4). For the *P. fluorescens* biofilm, phase contrast images were taken live at 1 hour intervals up until 7 hours post inoculation (Figure 5) and then following an overnight incubation. After the overnight incubation, bacteria were stained for viability (Figure 6).

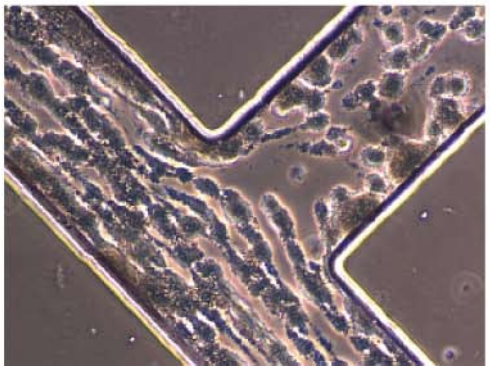


Figure 3: Detail of BioFlux channel shown with *P. fluorescens* biofilm (10x magnification).

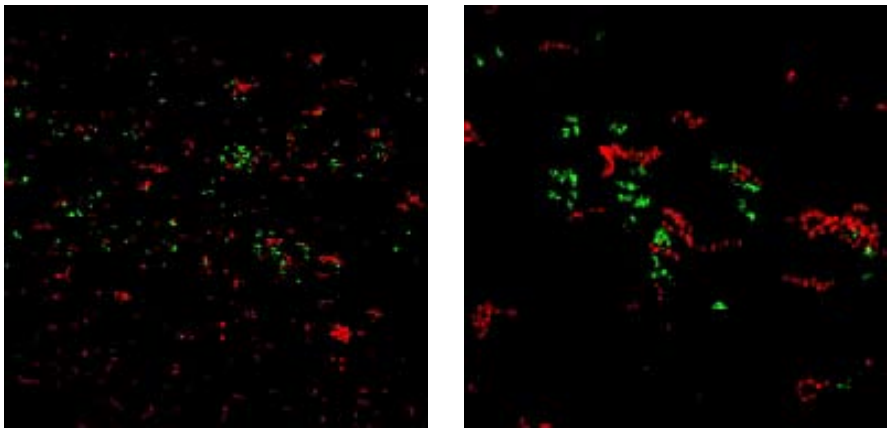


Figure 4: Example of coaggregation from overnight growth in a BioFlux channel between *Streptococcus oralis* (red), the first strain inoculated into the channel and *Actinomyces naeslundii* (green), the second strain inoculated into the channel. Overview of the microscopic field (left); 4X zoom (right).

Micrograph courtesy of Albert Ding and Robert J. Palmer, NIDCR/NIH

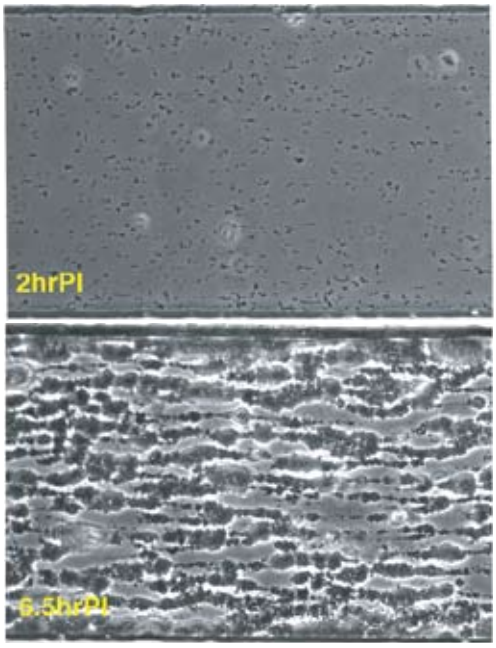


Figure 5: *P. fluorescens* biofilm, growth during 4.5 hours under shear (10X magnification).

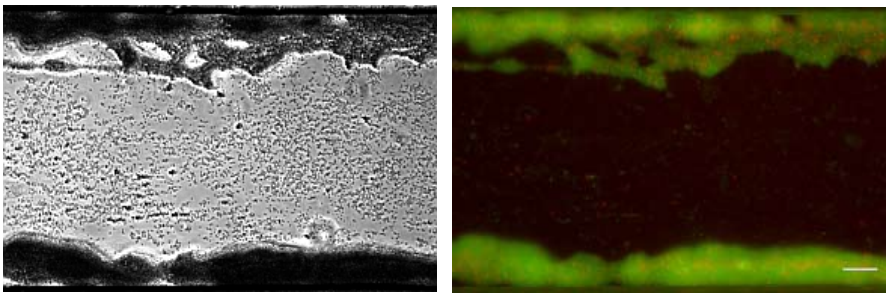


Figure 6: *P. fluorescens* biofilm 21hours post-inoculation. Phase-contrast (left) and viability stain (right) (10X magnification).

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