CELL Microsystems®

APPNOTE

Biofilm Growth

Growth of environment-specific biofilms using the BioFlux system

Introduction

Biofilms are composed of one or more types of microorganisms, such as bacteria, fungi, or protists which are encapsulated in an extracellular matrix to form communities that can interact with each other. The majority of microorganisms exist in biofilms that can be found throughout nature; however, many microorganisms can become sources of infection or disease. Common examples of harmful biofilms include dental plaque, chronic wound infections, food-spoiling bacterium, and opportunistic pathogen growth on medical devices and implants. To understand how to treat and prevent biofilms, research on how they develop and grow is necessary. Because biofilms form and thrive in environments where they are periodically or continuously suffused with water or other nutrient-rich liquids, static cell culture models do not accurately reflect the true mature structure and function of biofilms in a "real world" environment.

Here we describe co-culture experimentation with Streptococcus oralis and Actinomyces naeslundii for the formation of a mutualistic biofilm in saliva, as well as the growth of a biofilm from the environmental bacterium, Pseudomonas fluorescens, a close relative of the opportunistic pathogen Pseudomonas aeruginosa. This application note demonstrates the ability of the BioFlux system to form environment-specific biofilms under shear flow conditions.

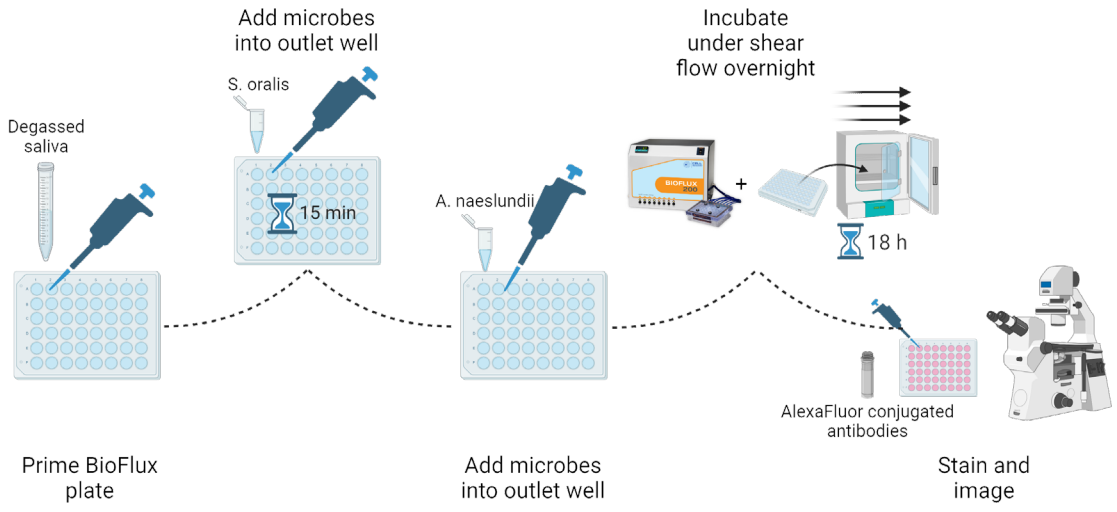
Key Highlights:

1. Image real-time growth kinetics
2. Co-culture various bacteria strains
3. Simulate in vivo conditions by culturing biofilms with saliva

Questions this App Note Answers

1. Can environment-specific biofilms be grown under shear flow conditions?
2. How can bacteria be co-cultured under shear flow conditions?
3. Does culturing under shear flow impact biofilm viability?

Co-Culture Biofilm Growth Workflow



Methods

Inoculating cultures

The channels of a 48-well BioFlux Plate were primed from the outlet ports (Figure 1) with degassed saliva or growth media until the channel was coated, as determined by visual observation under a microscope. For co-culture experiments in saliva, S. oralis was added first, followed by A. naeslundii, both at a low shear force. The cells were allowed to settle and attach to the plate for 15 minutes in between additions. A 15-minute incubation was determined to be sufficient for bacterial attachment but not long enough for the formation of isolated biofilms of individual strains. For the P. fluorescens experiment, cells from an overnight culture were added to the channel and allowed to settle and attach to the plate for 1-hour before the application of shear flow.

*Note: Depending on experimental requirements, bacterial strains can be mixed prior to plating.

Co-culture/ biofilm growth assays

In order to grow biofilms from the cultured bacteria, the entire interface coupled to the BioFlux Plate was placed in an incubator, and saliva or media was perfused overnight. For the oral biofilm co-culture experiments, after an overnight incubation, cells were live-stained in the BioFlux plate with AlexaFluor® conjugated antibodies against each species. For the P. fluorescens biofilm, phase contrast images were taken live at 1-hour intervals until 7 hours post-inoculation and again following an overnight incubation. After the overnight incubation, bacteria were stained for viability.

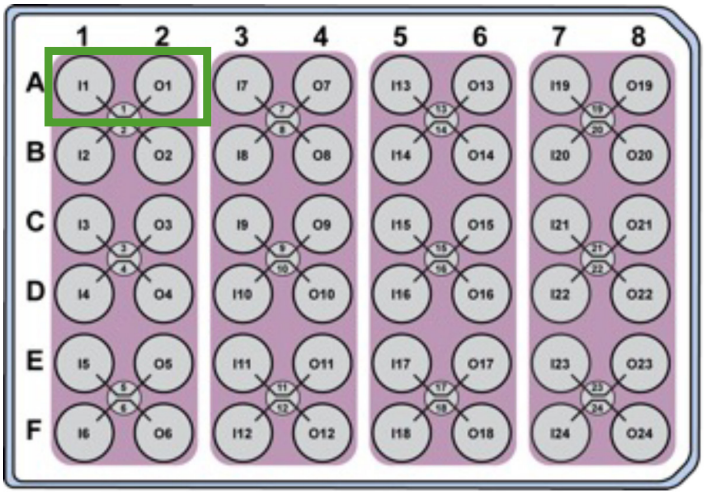


Figure 1. 48-well BioFlux plate. The green square highlights an inlet (A1) and outlet (A2) well with a viewing channel connecting the two wells. Each pair of inlet and outlet wells represent independent assays.

Results

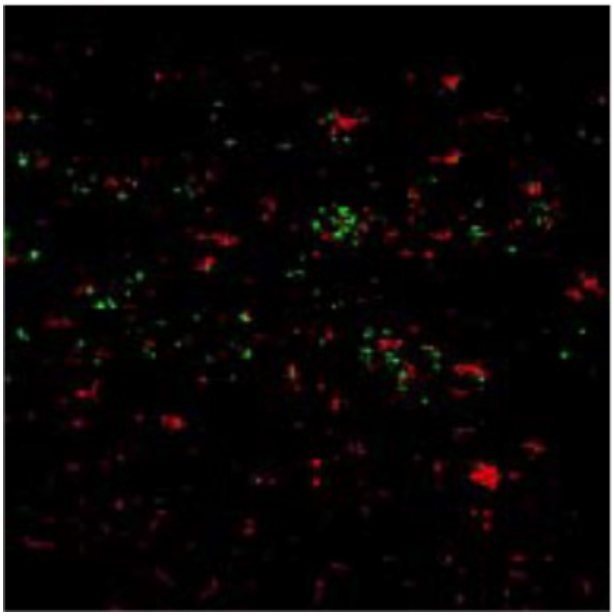
Co-culture of Streptococcus oralis and Actinomyces naeslundii.

Using saliva as the growth medium, the bacterial strains of S. oralis and A. naeslundii were successfully co-cultured within the same BioFlux channel. Both strains were found to be highly viable, as evidenced by viability staining (data not shown). In addition, AlexaFluor staining revealed successful coaggregation of the bacterial strains (Figure 2).

Pseudomonas fluorescens growth.

P. fluorescens was successfully cultured within the BioFlux plate and grew to form a biofilm within 6.5 hours of shear flow (Figure 3). Importantly, shear flow conditions did not impact viability of the P. fluorescens, and the bacterium maintained normal morphology (Figure 4) rather than adopting changes in membrane organization that are typically observed when grown in static conditions.

A



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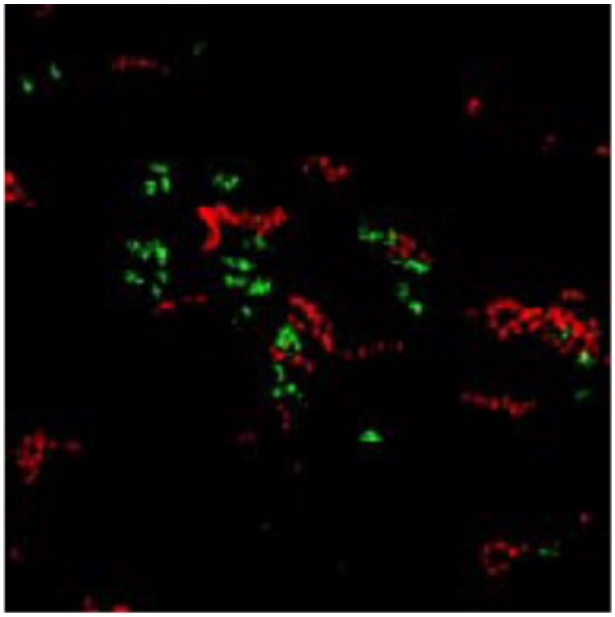

Figure 2. Bacterial coaggregation. Representative images of coaggregation of S. oralis (red) and A. naeslundii (green) during overnight growth in microfluidic channels. Overview of the microscopic field (A) and 4X magnification (B). Micrograph courtesy of Albert Ding and Robert J. Palmer NIDCR/NIH

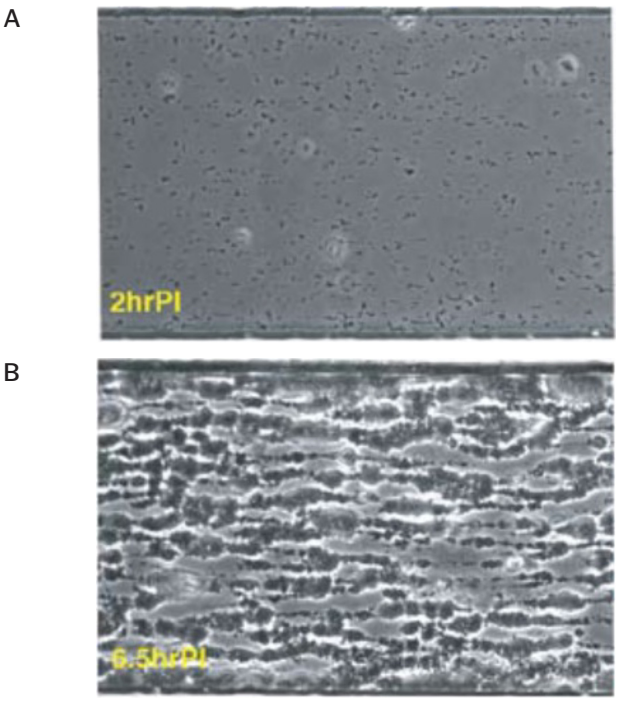

Figure 3. P. Fluorescens biofilm growth. 10X magnification of P. fluorescens biofilm growth under shear flow after 2 hours (A) and 6.5 hours (B).

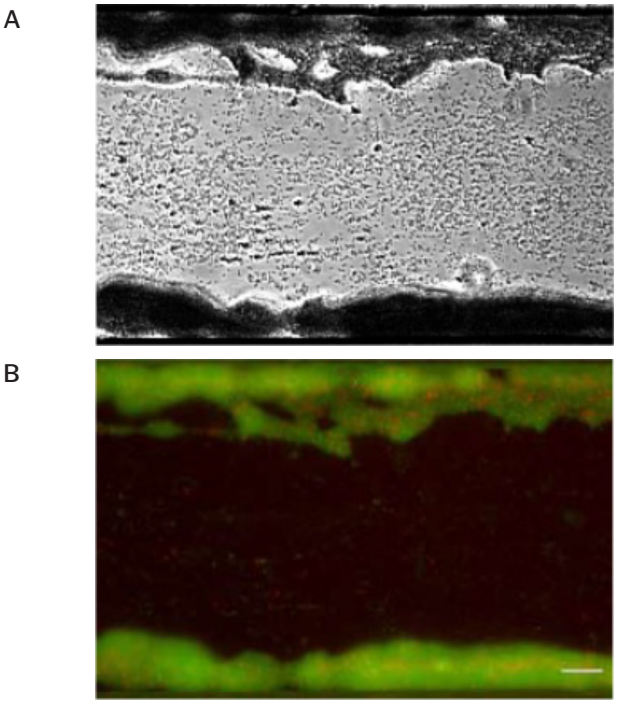

Figure 4. P. fluorescens biofilm morphology and viability. 10X magnification of P. fluorescens biofilm 21 hours post-inoculation. Phase-contrast (A) and viability stain (B).

Conclusion

Using the BioFlux microfluidic system, we have demonstrated an efficient, user-friendly workflow for growing and experimenting with bacterial biofilms. These experiments show that the BioFlux system can be used to generate the mechanical stress and nutrient exchange necessary to rapidly grow mature biofilms in an environment that more closely mirrors their natural conditions, providing excellent models to study the prevention and treatment of harmful biofilms. These in vivo-like culture and testing conditions are critical for developing effective treatments and preventions for biofilms in “real-world” environments.

For more information on BioFlux Technology, visit cellmicrosystems.com

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