Application Brief

Dynamic live cell imaging of bacterial biofilm communities using the CellASIC® ONIX Microfluidic System

Research reveals connections between metabolic oscillations and membrane potential-based signaling.

The challenge of studying signaling and growth behavior in dynamic bacterial colonies.

What do wildebeest herds, metastatic tumors, and bacterial biofilms have in common? They are all complex dynamic social environments that have intricate signaling mechanisms. The challenge in studying and manipulating such dynamic systems is in the measurement and control of the immediate environment coupled with constant monitoring of changes and outcomes over extended periods of time. Even within seemingly uniform populations that compose bacterial biofilms, careful control and manipulation of their microenvironment has revealed surprising fundamental signaling mechanisms that could open up whole new approaches to anti-bacterial therapeutics.

The most attractive approach to studying microbial community dynamics would be in creating a model cell culture system with a completely controllable environment that still allows extended, continuous observation. Recent advances in live cell analysis technology have made many such experiments possible[1]. The controllability of cellular microenvironments has been significantly improved with the introduction of biologically designed microfluidic chambers coupled with computer control[2]. One of the major technical challenges for long-term cell analysis is being able to control the temperature, gas, and nutrient composition of the cell environment during the course of the experiment, without impeding optical access to the cells. This is complicated by the proximity of microscope elements (objective lens, condenser, stage), as well as the need for an unobstructed light path through the sample without hindering stage or objective movement. These challenges facing live cell analysis can be met by using microfluidics-based cell culture, which uses very small volumes of fluidics and culture chambers, to enable dynamic changes in gas and temperature conditions. Such a small thermal footprint allows the user to perform precise, quick changes of temperature or gas conditions during live cell experimentation, without interfering with optical analysis and enabling experiments that have traditionally been hard to perform. Therefore, a microfluidic platform is a promising basis on which to design an improved live cell incubation system with dynamic control.

Here we report on two recent *Nature* publications[3, 4] utilizing the CellASIC® ONIX Microfluidic cell culture platform (Figure 1) for the observation and manipulation of bacterial biofilms.

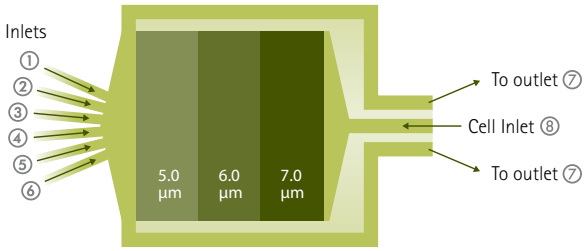

Figure 1.

CellASIC® ONIX microfluidic cell culture system. The low-profile manifold (foreground) seals to the standard footprint microfluidic plate for viewing on any inverted microscope. Pressure-driven perfusion, temperature and gas lines route through the manifold to the microfluidic plate without blocking optical paths. The CellASIC® ONIX Microfluidic Plate offers gaseous microenvironment control with rapid response through aeration channels and gas-permeable materials.

The culture chamber is 9 mm^2 in area with trap heights of 5.0, 6.0, and 7.0 µm. Nine position markers indicate unit number and relative position. The inlet/outlet functions and minimum/maximum volumes for each culture unit are listed below.

1–6 Inlet for solution switching
7 Outlets to waste wells
8 Inlet for cell loading

Creating a controllable biofilm environmental chamber

Bacterial biofilm communities consisting of millions of cells can show considerable heterogeneity in nutrient access. The UCSD researchers studying biofilm growth reproduced that environment in a continuously observable culture. They used the CellASIC® ONIX Microfluidic Platform and a modified version of the Y04D microfluidic plate (Merck Millipore). This plate provided unconventionally large chambers, allowing the formation of large cell colonies, yet not restricting media flow (Figure 2).

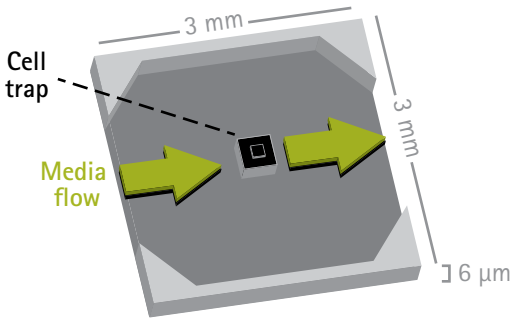

Figure 2.

Schematic of the microfluidic device used throughout the biofilm experiments. Direction of media flow is indicated by the green arrows.

Media flow in the microfluidic chamber was completely controllable and driven by a pneumatic pump from the CellASIC® ONIX Microfluidic Platform, and the pressure from the pump was kept stable during the course of the experiment. In most of the experiments, they used a pump pressure of 1 psi with only one media inlet open (there are 6 media inlets in the Y04D plate), which corresponded to a flow speed of16 µm/s in the growth chamber. Since the microfluidic chamber could remain continuously on the microscope stage, time-lapse microscopy recordings of the biofilm colony growth was recorded using phase contrast microscopy with images taken every 10 minutes and subsequently analyzed using image analysis software (Figure 3).

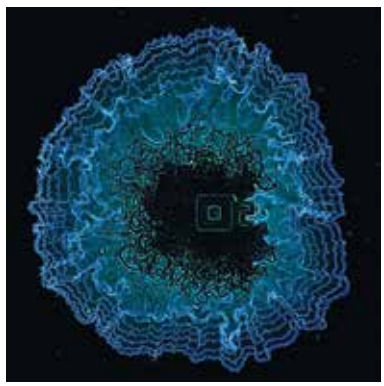

Figure 3.

Composite image of a growing bacterial biofilm community. In the center is a microscopy image of the actual biofilm. This image is combined with the surrounding contour lines that show the edge of the biofilm over time. Courtesy of Gürol Süel Lab, UCSD.

Biofilm growth oscillations and electrical signaling.

Jintao Liu and colleagues in Gürol Süel's lab used the microfluidic culture platform to investigate an internal conflict within bacterial biofilms: cells at biofilm periphery protect cells in the interior from external attack but also starve them through nutrient consumption (Figure 4)[3]. The researchers discovered that biofilms resolved this conflict through long-range metabolic co-dependence between biofilm periphery and interior. This co-dependence leads to periodic halting in biofilm growth, therefore preventing interior cells from starving to death.

In a further study, Arthur Prindle and colleagues of the Süel lab demonstrated that bacterial potassium channels enable active electrical communication and surprising coordination with the oscillatory dynamics of the biofilm communities[4]. This potassium based ion channel mediated oscillatory signaling allows cells to rapidly communicate their metabolic state to enhance the previously revealed long-range metabolic codependence in biofilms.

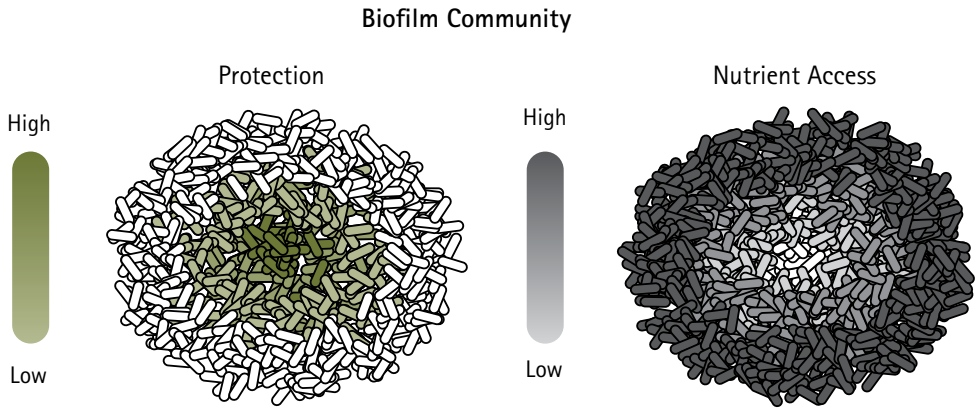

Figure 4.
Biofilms must reconcile opposing demands for protection from external challenges (gradient indicated in green) and access to nutrients (gradient indicated in grey).

Conclusions

The CellASIC® ONIX microfluidic system is specifically designed to control cell culture parameters and create a stable, manipulatable environment and can be used to conduct long term study of bacterial biofilm dynamics. This design has been demonstrated for numerous bacterial species for long-term culture, fluorescence quantification, and nutrient manipulation. Further, the ease of use, flexibility, and accessibility of this advanced technology platform should prove beneficial to a wide range of bacterial cell biology applications.

References

1. Sung MH, McNally JG. Live cell imaging and systems biology. Wiley Interdiscip Rev Syst Biol Med. 2011 MarApr;3(2):167-82.
2. Lee P, Gaige T, Hung P. Microfluidic systems for live cell imaging. Methods Cell Biol. 2011;102:77-103.
3. Liu J, Prindle A, Humphries J, Gabalda-Sagarra M, Asally M, Lee DD, Ly S, Gacia-Ojalvo J, Süel GM. Metabolic co-dependence gives rise to collective oscillations within biofilms. Nature 2015 July 523:550-554.
4. Prindle A, Liu J, Asally M, Ly S, Garcia-Ojalvo J, Süel GM. Ion channels enable electrical communication in bacterial communities. Nature. 2015 Nov 5;527(7576):59-63.

To place an order or receive technical assistance

In Europe, please call Customer Service:

France: 0825 045 645
Germany: 069 86798021
Italy: 848 845 645
Spain: 901 516 645 Option 1
Switzerland: 0848 645 645
United Kingdom: 0870 900 4645

For other countries across Europe, please call: +44 (0) 115 943 0840

Or visit: **www.merckmillipore.com/offices**

For Technical Service visit: **www.merckmillipore.com/techservice**

MERCK MILLIPORE

www.merckmillipore.com/offices

Lit No. AB7755EU00 BS GEN-15-12127 01/2016