M04S APPLICATION NOTE

Host-Pathogen Interactions

INTRODUCTION

Host-pathogen interactions represent a significant area of biomedical research, encompassing the study of how virus, bacteria, and other organisms cause infections and diseases or alter normal physiology of host cells and tissues.[1] As the field of cellular systems biology advances, there is increasing interest in using *in vitro* cell culture models to study host-pathogen interactions.[2]

The proper study of host-pathogen interactions requires careful control of the cell environment to simulate physiologic conditions. An *in vitro* model that can replicate infection parameters including flow rate, exposure time, solution type, and timed introduction of therapeutic agents while sustaining long term live cell microscopy enables experiments that are difficult or impossible with standard methods. In many cases, it is crucial to precisely manipulate the exposure of pathogen to the cultured host cells—too low an exposure may prevent appropriate interactions, while too high an exposure could cause confounding effects (such as bacterial overgrowth and nutrient starvation) independent of the desired host-pathogen interaction. In addition, information on the effect of exposure times and flow conditions could prove valuable for understanding the mechanisms of disease for many infectious agents. A further challenge in host-pathogen research is the ability to sustain infections *in vitro*, since cultured host cells often de-differentiate in culture and no longer support pathogen responses found *in vivo*. For certain pathogens, the time scale of infection even under optimal conditions could take days or weeks, placing additional burdens on the cell culture method. Recent advances in microfluidic based cell culture technologies offer potential solutions to these problems.[3]

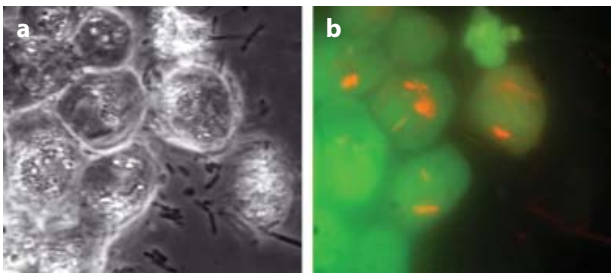

Figure 1. Human colon adenocarcinoma cells (HT-29) cultured in the M04S microfluidic chamber and infected with an invasive strain of *E. coli*. (a) Phase contrast image showing apoptotic cells, and (b) fluorescence image showing invaded bacteria (red) and cell viability (green). Images taken with a 100X objective lens.

The ONIX Microfluidic Perfusion System is well suited for host-pathogen studies by providing a stable, long term culture environment for host cells (including primary cells) with controlled pathogen exposure. The continuous perfusion format and the ability to switch media solutions enable wash-out of pathogens from the chamber and subsequent monitoring of host cell response over many days. The enclosed small volume of the culture chamber also provides practical advantages for working with infectious agents during live cell imaging. The design of the microfluidic plates enables the dynamics of infection to be tracked using live cell imaging on standard inverted microscopes.

Here, we demonstrate a host-pathogen experiment using human intestinal cells infected with engineered *E. coli* strains (see figure 1). Both an invasive and non-invasive bacterial strain was monitored for long term infection with time-lapsed imaging up to 24 hours in the CellASIC M04S microfluidic plate.

PLATE DESIGN

The M04S microfluidic plate is built on the ONIX platform developed by CellASIC (www.cellasic.com/ONIX). The plate has a SBS standard 96 well footprint to fit typical microscope stage holders. The custom well layout was designed to maximize live cell imaging capabilities. The M04S has 4 independent units (A-D), with each unit containing 8 wells (1 gravity inlet, 4 switching inlets, 1 cell inlet, 2 wastes). The four cell culture chambers are centralized under a single large imaging window (see figure 2). The chamber to chamber distance is 5.2 mm, reducing objective travel time and focus drift. The bottom surface of the plate is a #1.5 thickness (170 µm) optical glass slide to maximize quality of high resolution, high numerical aperture imaging. The plate houses all experiment solutions allowing control with an external pneumatic manifold (see figure 3). The manifold lets the user direct flow rates and select exposure

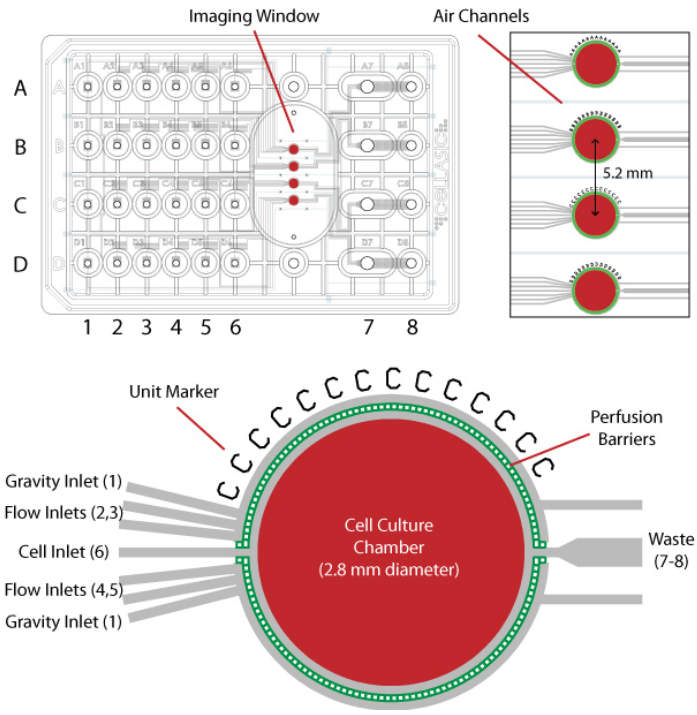

Figure 2. The M04S plate contains 4 independent flow units (A-D), each with 4 upstream solution inlets, a gravity flow inlet, a cell inlet, and 2 waste wells. The culture chamber is 2.8 mm in diameter (100 µm height) and is surrounded with a microfabricated perfusion barrier (4 µm pores). Inlet 1 is a gravity flow well, allowing long term cell culture in a standard incubator without a pressure system. Continuous flow of solutions from the inlets creates a dynamic exposure profile during live cell imaging.

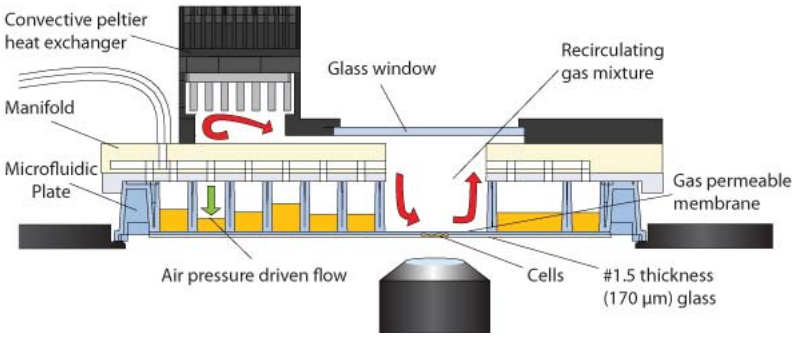

Figure 3. Side view schematic of the microfluidic plate with micro-incubation manifold on a microscope stage. The bottom surface of the microfluidic plate is a thin glass sheet, allowing high quality cell imaging. The plate is sealed to a pneumatic manifold, allowing user control of the flow profile during imaging. Additional air channels allow control of the gas environment.

solutions without perturbing the microscope stage. The programmable software interface automates flow switching times. A gas line allows control of the environment within the microchambers through a network of gas permeable air diffusion channels. Temperature is regulated through an onboard heater/chiller on the manifold.

BACTERIAL INFECTION MODEL

To demonstrate time lapsed imaging of host-pathogen interactions in the microfluidic culture system, a human colon adenocarcinoma cell line (HT-29, ATCC) was exposed to two *E. coli* strains (courtesy of Tim Lu, MIT). The strains tested included one capable of HT-29 infection and one that could not. A schematic of how the perfusion system was used to monitor invasion is depicted in figure 4.

The experiment was prepared by culturing HT-29 cells in the M04S microfluidic plate in a standard cell culture incubator for 3 days with gravity perfusion of McCoy's 5A medium + 10% FBS. This allowed the cells to form close cell-cell contacts and enter growth phase. On the day of the experiment, *E. coli* cells were cultured in LB on a shaker to achieve log phase growth. The M04S plate was then loaded with culture medium (McCoy's 5A + 10% FBS) in well 2 and bacterial suspension in well 3. Within each plate, the invasive strain, non-invasive strain, and no bacteria control were run in parallel chambers. For live cell imaging, the M04S plate was sealed to the ONIX Microincubator Controller manifold (for perfusion, temperature, and gas control) and imaged using an Olympus IX71 microscope. Conditions were set at 37ºC and 5% CO_2 for the duration of the experiment. The cells were initially perfused with culture medium for 1 hour at a flow rate of 5 µl/hr to stabilize imaging, and then exposed to the bacteria solution for 30 minutes, followed by washout by medium flow at 5 µl/hr for the remainder of the experiment. Figure 5 shows images comparing the invasive strain (a and b) against the non-

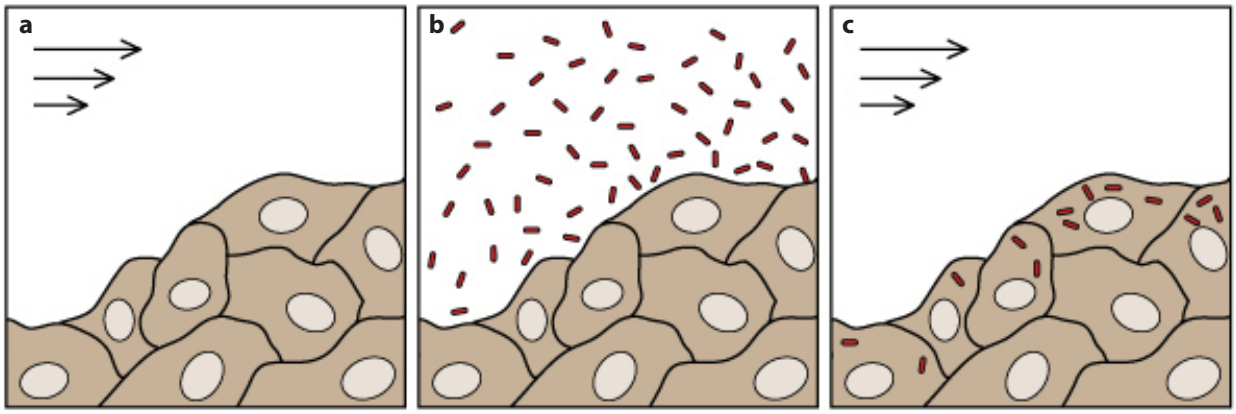

Figure 4. Schematic of host-pathogen experiment. (a) Human cells are cultured in the M04S microfluidic plate to establish a healthy, differentiated, and stable host population under continuous media perfusion. (b) Pathogen (bacteria) are introduced by flow to expose the host cells. (c) Media perfusion is re-established, allowing long term monitoring of host-pathogen interaction via live cell microscopy.

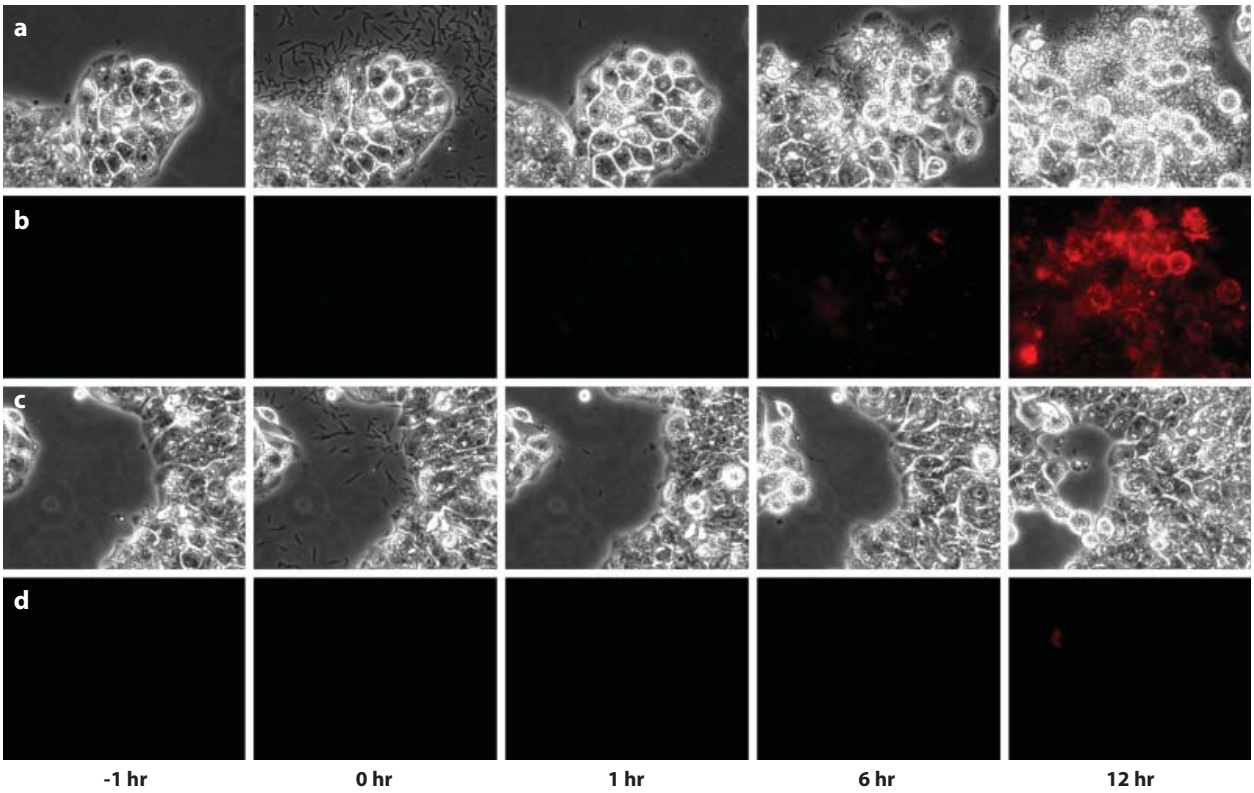

Figure 5. Time lapsed imaging of bacterial invasion into human HT-29 cells. (a) Phase contrast and (b) fluorescence images of an invasive strain of *E. coli*. (c) Phase contrast and (d) fluorescence images of a non-invasive *E. coli* strain. Both strains are designed to express mCherry following invasion into HT-29 cells.

invasive strain (c and d). During bacterial exposure, there is a high abundance of both strains visible in the solution (t = 0). The subsequent wash-out removes the majority of the bacteria, allowing the HT-29 cells to continue healthy culture. Notice in the non-invasive condition, the HT-29 cells are able to continue growth and exhibit a healthy morphology. In the invasive strain case, the HT-29 cells show clear signs of stress by 6 hours, with widespread cell death occurring by 12 hours. The fluorescence channels show mCherry expression by the bacterial strains triggered by invasion, verifying the cell death as a result of invasion instead of nutrient competition.

Figure 6 shows higher magnification images of the HT-29 cells exposed to the invasive and non-invasive strain. Here, the cells were stained with the green fluorescent Calcein AM viability marker, and the bacteria were red fluorescent. In the invasive case, there is clear co-localization of the bacteria with the human cells, whereas in the non-invasive case, the bacteria are effectively excluded from the HT-29 colony. When tracked over time, the invasive bacteria triggered apoptosis and eventually cell rupture, promoting bacterial growth and continued invasion. By 15 hours after

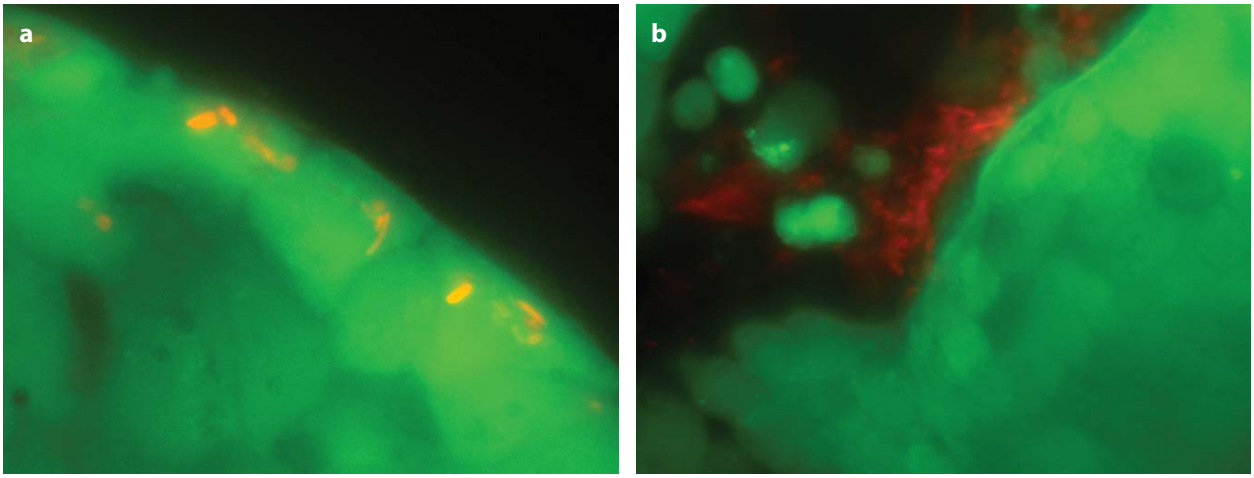

Figure 6. Images of (a) an invasive strain and (b) a non-invasive strain of *E. coli* after exposure to human HT-29 cells, washout, and perfusion culture. Bacteria express mCherry, and HT-29 cells stained with Calcein AM. Panel (a) taken with a 100X objective lens, and panel (b) taken with a 60X objective lens.

initial exposure, almost all of the HT-29 cells in the invasive chamber have died, while the majority of the HT-29 cells in the non-invasive chamber remained viable.

The continuous perfusion culture format was highly beneficial in promoting long term host-pathogen response. Without the presence of the wash-out flow, the bacteria quickly overcrowded the culture and led to cell death even in the absence of invasion. In addition, the low chamber height (100 microns) improved the ability to focus on bacterial cells in suspension. While not investigated in detail in this study, the flow environment also appeared to play a role in how the pathogen accesses host cells. For example, cells at the periphery of a colony were much more susceptible to invasion, as well as areas that served as "harbors" and prevented pathogen washout. It was also observed that the flow rate could play a role in the effectiveness of cell invasion.

SUMMARY

The ONIX microfluidic culture system provides a number of important features for live cell imaging of host-pathogen interactions. The ability to precisely control the cell culture environment on the microscope stage for long periods of time (hours to days) while maintaining a steady state perfusion of medium gives the researcher unprecedented access for tracking host-pathogen dynamics. The M04S microfluidic plate is well suited for the culture of a large variety of adherent host cells, and the programmable solution switching capability allows controlled exposure of pathogens, including bacteria, virus, and other particles. The ease-of-use and flexibility of this experiment platform has potential applications in a large number of live cell studies.

REFERENCES

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3. Lee P, Gaige T, Hung P. Microfluidic systems for live cell imaging. Methods Cell Biol. 2011;102:77-103.

E. coli strains courtesy of Professor Tim Lu of the Massachusetts Institute of Technology.

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