Nanochannel-based microfluidic device for quantitative measurement of bacterial chemotaxis

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The organization and behavior of plant-microbe communities is dictated by a complex network of physical and chemical interactions between organisms within the rhizosphere. These interactions produce spatially and temporally localized micro-environments that change as organisms grow, consume, and produce new materials, altering their surroundings. Previous studies show that some bacterial species reproducibly colonize specific regions of the plant root, suggesting that the bacteria are sensing and responding to physical and chemical cues from the host plant. In order to better understand the effects of hundreds of different chemical signals released from the plant on the bacteria, it is necessary to develop high-throughput platforms to quantitatively evaluate the effect of these chemical signals.

We have designed and fabricated a nanochannel-based microfluidic device for quantitative measurement of bacterial chemotaxis. The device consists of a central imaging chamber with nanochannels on one side and microchannels on the other side. The geometry of the nanochannels as well as the geometry of the central chamber control the chemical gradient in the device (Figure 1). The bacteria travel through the microchannels in response to the chemoattractant gradient, but cannot pass through the nanochannels. The number of cells in the central chamber is quantified using image analysis to assess the bacterial response to the target chemoattractant. The device is made of PDMS with a mixing ratio of 5:1 (base to crosslinker) and cured in a 70 degree Celsius oven for two days. The mold is fabricated using e-beam lithography and photolithography.

Our preliminary results show that the number of bacterial cells (*Pantoea* spp.) trapped in the central chambers increases with glutamate concentration from 0 to 100 mM, and decreases at 1 M (Figure 2), which may suggest that this strain detects an optimal range of attractant. The number of cells in the chamber is a direct result of the strength of the bacterial response to that chemoattractant, and thus can be used to quantitatively compare which chemicals act as chemoattractants and, ultimately, assess their influence on the rhizosphere community composition and organization. We are currently using this platform for high-throughput screening of chemical candidates secreted from plants to study microbe-plant interactions and understand colonization of microbes in plant roots.

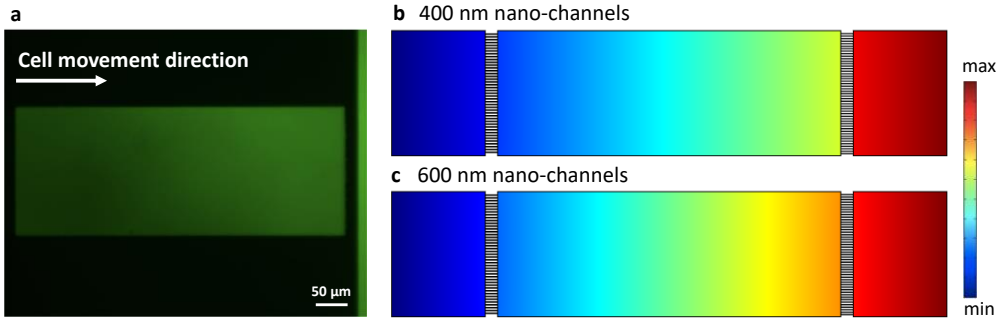

Figure 1: Geometry and Chemical Gradient of the Microfluidic Device: (a) fluorescent image of the microfluidic chamber when fluorescein in glutamate solution is introduced from the left side of the chamber. COMSOL simulation of the chemical gradient inside the microfluidic device with nanochannels of (b) 400 nm and (c) 600 nm.

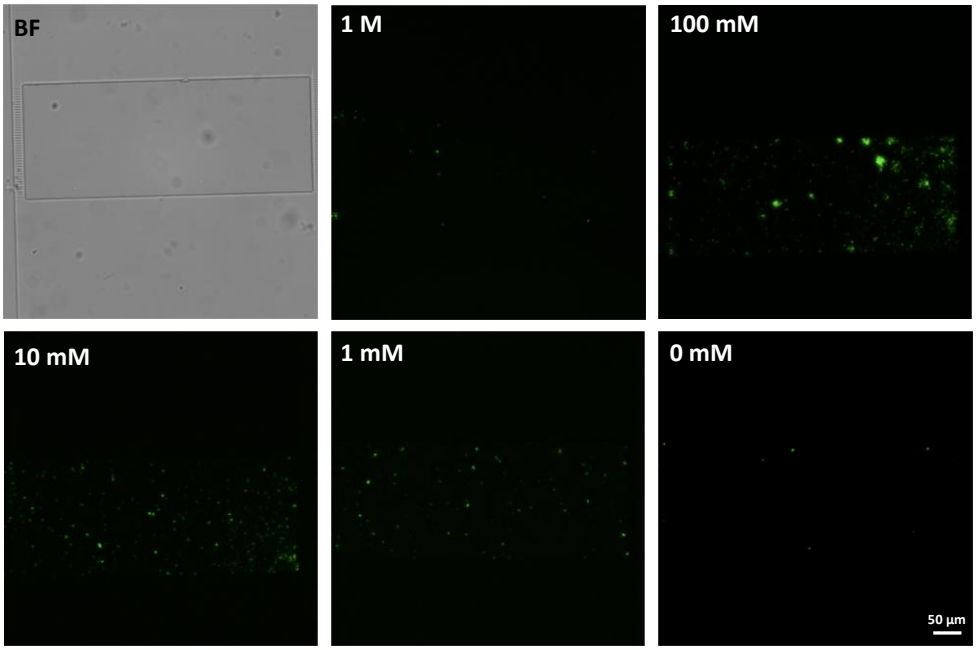

Figure 2: Bright Field (BF) and Fluorescent Images of Central Chambers with Different Glutamate Concentrations (1 M, 100 mM, 10 mM, 1 mM and 0 mM).