Microfluidic Devices for Studying Chemotaxis and Calcium Signaling in Environmental Policeman *Tetrahymena pyriformis*

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Microorganisms rapidly respond to environmental changes surrounding them and coordinate their behaviors to adapt to the changes. Many of microbiology tools such as agar plate and plastic tube are too simplified to reflect the subtle changes occurring in the microecosystems. Microfluidic devices, which have controllable low Reynolds environments and reproducible topology, offer both microbiologists and physicists opportunities in mimicking many microecosystems in nature. Using microfluidic devies, we have explored undiscovered aspects of microorganisms' behaviors, such as chemotaxis [1], quorum sensing [2], mechanosensation [3], etc.

In this talk, we will present our recent applications of microfluidic devices in real-time imaging of chemical and biochemical changes in microorganisms. The first target of our interest was the fresh water ciliate *Tetrahymena pyriformis* because the microorganism is a good model for the study of eukaryotic chemotaxis. Because of its high motility and tendency to follow hydrodynamic flow, it is difficult to observe the chemotactic response and intracellular calcium signaling mediated by chemical stimuli. To solve the problems, we developed a microfluidic device equipped with pneumatically actuated valves, generating a linear gradient of chemoeffectors to quantify the chemotactic response of *Tetrahymena* [2]. The components of the device include electronically controlled pneumatic microvalves, microchannels and microchambers. The linear gradient of the chemoeffectors was established by releasing a chemical from a ciliate-free microchamber into a microchamber containing the ciliate. The ciliate showed chemotactic behaviors by either swimming toward or avoiding the gradient. The ciliates in the microfluidic device were sensitive enough to be attracted to 10 pmol glycine-proline, which indicates a 10^5 increase in the ciliate's known sensitivity. However, we still did not know why the ciliates were highly sensitive to the attractant. To answer the question, we suppressed its motility by adding a polyethylene glycol (PEG)-based thermo-polymer (10 Pa at 25 ^{o}C) to the central chamber in order to maintain the highly viscous conditions [5]. Once the viscous condition was achieved, directional chemical gradients were formed inside the center chamber via the release of N-methyl-D-aspartate (NMDA), a known chemoattractant, from the surrounding chemical reservoirs into the center chamber. As a result, intracellular Ca^{2+} in the ciliate increased up to three-fold, and its distribution was skewed in the direction of NMDA stimulation. However, the Ca^{2+} in ciliates pretreated with phospholipase C (PLC) or phosphatidylinositol-3-kinase (PI3K) blockers did not increase even after stimulation. Additionally, the PI3K blocker induced the secretion of granules, the size of

which was dependent on the concentration of the blocker. The results indicate that both PLC and PI3K perform pivotal roles in controlling the levels of intracellular Ca^{2+} in *T. pyriformis* during chemotaxis.

Collectively, these results demonstrate that microfluidic devices are highly useful for studying behaviors and chemical signaling in many microorganisms.

References

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