352d A "Pumpless" Microfluidic Linear-Gradient Generator for the Analysis of Bacterial Chemotaxis

Jinpian Diao, Peng Zhou, Lincoln Young, Sue H. Kim, Mingming Wu, Michael L. Shuler, and Matthew P. DeLisa

Chemotaxis is the process by which cells move up or down a chemical gradient in response to an attractant or repellant and is a major factor in a bacterium's response to environmental changes. Traditional approaches for monitoring chemotaxis include capillary assay, swarm plate assay and the stopped-flow diffusion chamber assay. More recently, a microfluidic gradient generator connected to syringe pumps was developed to study bacterial chemotaxis. Along these lines, we have developed a "pumpless" microfluidic gradient generator which can provide a steady (>= 4 h) and liner (R2>0.99) chemical gradient. A simple, rapid (< 60 minutes) and quantitative bacterial chemotaxis assay was successfully developed based on this gradient generator.

Specifically, Escherichia coli RP437 cells expressing green fluorescent protein (GFP) were loaded into the middle channel of the 3-channel device. Fluid was delivered by hydrostatic force to constantly refresh the chemoeffector source and sink. RP437 cells were observed to move up l-aspartate gradients (10-4M/mm) and down glycerol gradients (1%/mm) whereas non-motile and non-chemotactic mutants of RP437 showed no bias of the bacteria's distribution. Additionally, the degree of chemotaxis was measured by two coefficients, chemotactic partition coefficient (CPC) and chemotactic migration coefficient (CMC) which were found to be consistent with predicted values for E. coli RP437. In conclusion, we demonstrated a three-channel microfluidic gradient generator that capitalized on hydrostatic force for fluid delivery and molecular diffusion for gradient generation thereby enabling highly parallel assays of bacterial chemotaxis.