MICROFLUIDIC CHEMOTAXIS SCREENING PLATFORM FOR QUANTIFICATION OF BACTERIAL VIABILITY

Anand N. P. Radhakrishnan, Department of Chemical Engineering, University College London, UK
anand.radhakrishnan@ucl.ac.uk
Asterios Gavriilidis, Department of Chemical Engineering, University College London, UK
Elaine Allan, Division of Microbial Diseases, Eastman Dental Institute, University College London, UK

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It is estimated that by 2050, over 10 million people globally will die each year as a direct result of antimicrobial resistance. This global health crisis has arisen from the use and often misuse of antibacterial drugs and poses a serious threat to all aspects of modern healthcare. The discovery of new antibacterial agents is, therefore, imperative. In this regard, novel surfaces with light activated antimicrobial agents and antimicrobial paints for use in healthcare environments are being developed and need rapid evaluation. To this end, we aim to utilise the natural phenomenon of bacterial chemotaxis (the ability of bacteria to move towards or away from environmental signals by flagella-mediated motility) to perform live-dead analysis on bacterial cells after exposure to antibacterial agents in a microfluidic device. One of the challenges of this approach is to quantify chemotactic motility independent of convective-diffusive forces, to distinguish between alive and dead cells. Operational parameters (e.g. flow rates) must be carefully chosen in order to avoid masking the chemotactic separation by lateral movement of bacteria due to diffusion in the direction of the chemotactic gradient. In this work, chemotaxis defined by Fick's Law in a T-junction microfluidic chip was modelled by computational fluid dynamics (CFD), where chemotactic velocity was given as a function of the chemoattractant concentration and concentration gradient. The microchannels of the T-junction in the microfluidic device were 500 µm wide, 100 µm deep, and with a main channel length of 25 mm (Figure 1a). A range of flow rates was tested, and the spatial concentration of live bacteria estimated. The chip was fabricated by micro-milling channels on 1 mm thick polycarbonate. *Pseudomonas aeruginosa* PAO1 (mini-Tn7-GFP) was used as the model organism (in the mid-log phase to ensure maximum motility), as it causes serious infections in immunocompromised people and is frequently multidrug resistant (MDR). A chemoattractant, L-Threonine (4mM in 10 mM HEPES buffer) was tested on live bacterial culture. Bacteria and L-Thr were injected into the chip at three flow rates: 0.1 µl/min, 1 µl/min and 5 µl/min (each inlet). Fluorescence images were taken at the T-junction and 10 mm downstream of the channel to quantify the bacterial distribution across the channel width.

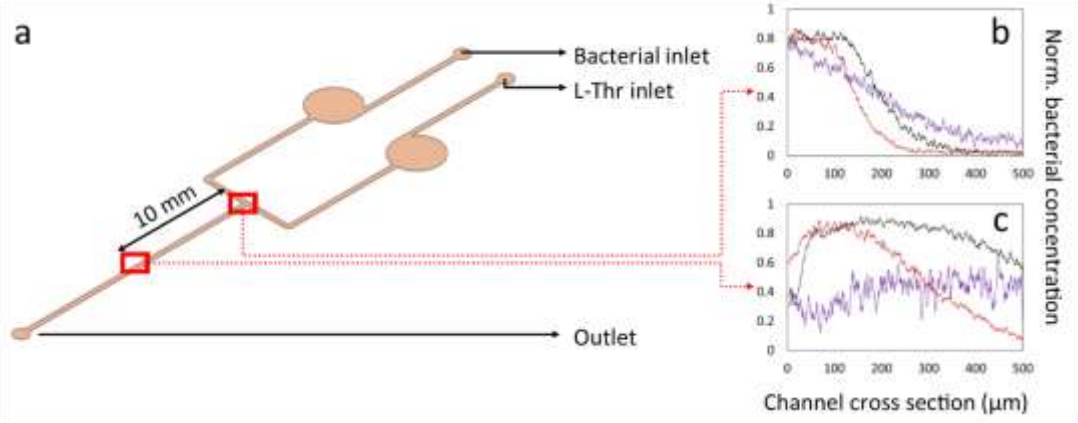

Figure 1: (a) Schematic of the microfluidic T-junction device (width = 500 µm, depth = 100 µm, length of main channel = 25 mm. Graphs show the normalised bacterial concentration from fluorescence images at the T-junction (b) and 10 mm downstream (c) for 3 different flow rates: 0.1 µl/min (purple), 1 µl/min (black), and 5 µl/min (red) each inlet. The decrease in the fluorescence signal at the left edge of the channel (10 mm downstream) is indicative of chemotactic motility.

At 10 mm downstream, results indicated that bacteria moved ~75 µm from the left-side channel wall at 1 and 5 µl/min (Figure 1c). At 0.1 µl/min flow rate, concentration of bacteria became homogenous due to diffusion, while at 5 µl/min the chemotactic focusing was the most apparent, in agreement with the CFD model. CFD-assisted testing has hence proven to be an efficient way to determine suitable operational parameters. Work is being conducted to fabricate novel designs to create chemotactic gradients for efficient live-dead bacterial separation, leading to a rapid bacterial viability analysis.