Bacterial Confinement in Micro-Environments

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1. Introduction

Understanding how bacteria respond to antibiotics is important in light of a global antibiotic crisis. Research in this field generally uses bulk growth media, but it is common for bacterial infections to occur in biological niches of small volume, such as eukaryotic cell interiors and biofilms[1][2].

We aim to investigate how confining bacteria within a micro-environment of similar volume to a human cell affects their growth inhibition by ribosome targeting antibiotics such as kanamycin and streptomycin.

2. Methods

Experimental

Bacteria are confined within stabilised microfluidic droplets of aqueous growth media. Droplets are produced within a microfluidic device using a 'Flow Focus' geometry: three channels flow parallel to one another, the outer channels carrying a continuous phase whilst the centre channel carries a dispersed phase. Each phase is channelled into one another and forced to pass through a constriction, at this point droplets are generated. Droplet generation can occur upstream, downstream or within the confines of the constriction (figure 1). This method of bacterial confinement allows the production and subsequent study of 1000+ individual microenvironments near simultaneously.

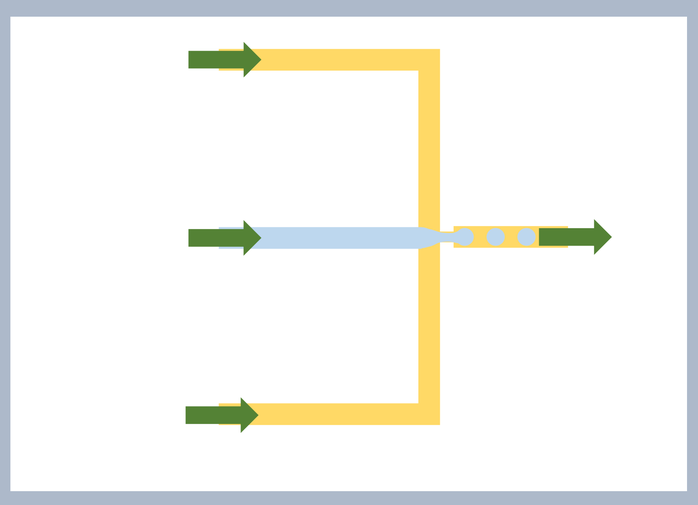

Figure (1): Diagram of Flow Focusing droplet generating geometry, green arrows indicating flow direction. The continuous phase (yellow) encapsulates an aqueous phase (blue) as they are both forced through a constriction. This geometry generates a monodisperse emulsion of aqueous droplets.

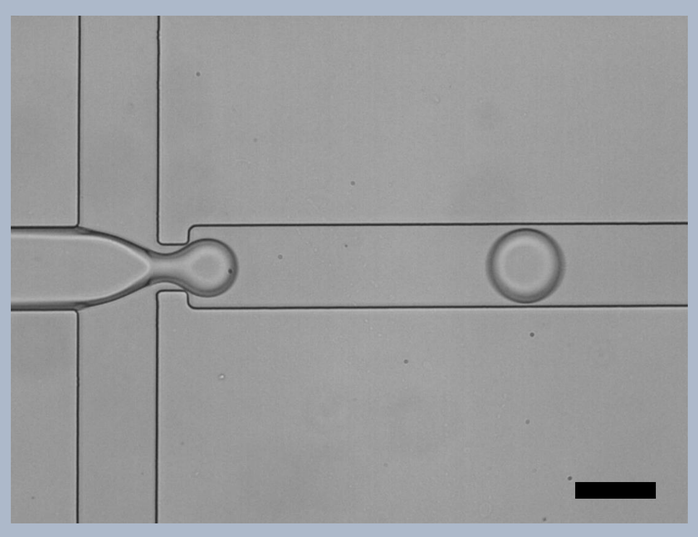

Figure (2): Bright-field microscopy image of microfluidic droplet generation using a flow-focus geometry. Droplets were generated at a flow rate of 200µL/hr, which produced droplets at a rate of 41Hz. Scale bar = 100µm.

Microfluidic devices are fabricated using polydimethylsiloxane (PDMS) based soft lithography. This method utilises equipment widely available in an academic research environment and produces devices with sufficient resolution for common microfluidic research[3].

Simulation

To complement our experimental work we have developed a statistical computational model that aims to emulate the experimental system. This model can be used to make qualitative predictions about the survival rates of bacteria in micro-environments of different sizes.

3. Results

Experimental

We have designed and fabricated a microfluidic device that produces a large array of monodisperse droplets within which we can image bacterial growth using a microscope. Droplets size can be easily controlled with droplet diameter ranging from 50-400µm.

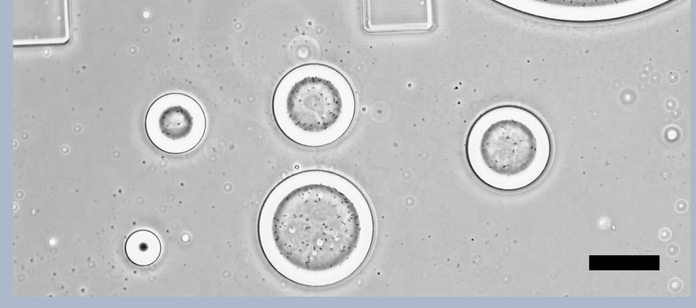

Figure (3): Bright-field microscopy image of monodisperse micro-droplets confined within a microfluidic reservoir. Scale bar = 200µm.

Figure (4): Bright-field microscopy image of bacteria confined in microfluidic droplets. Scale bar = 100µm.

Simulation

Based on simple computer simulations we hypothesise that when bacteria are loaded into small droplets, at a fixed antibiotic concentration, variation in the number of bacteria loaded into each droplet will result in a greater percentage of bacteria surviving, when compared to an equivalent experiment being conducted in the macroscopic bulk. If this can be verified experimentally, it could fundamentally change how we conduct antibiotic research.

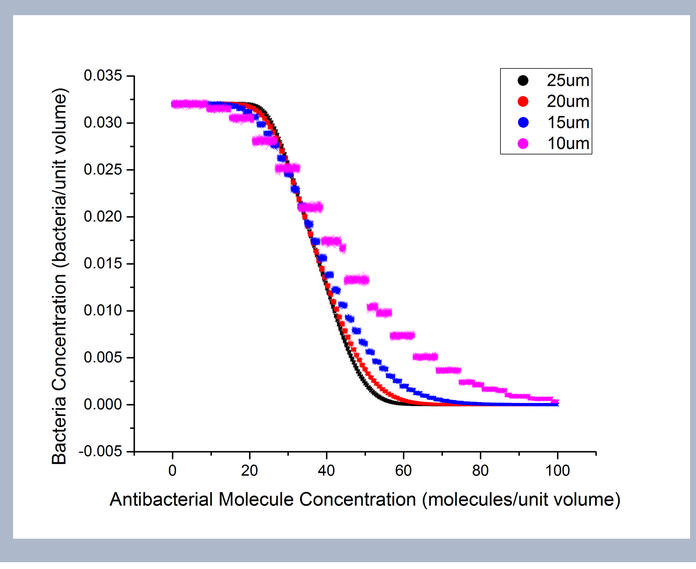

Figure (5): Average number of surviving bacteria per unit volume, after 5 generations, versus starting number of antibiotic molecules per μm^3, initial bacteria concentration was $1x10^{-3}$ bacteria per unit volume.

4. Moving Forward

We plan to test the simulation predictions experimentally; measuring growth curves of bacterial colonies trapped in microfluidic droplets. Beyond this, we hope to increase the complexity of the computational model such that it can make testable, quantitative predictions.

References

[1] J. W. Costerton, P. S. Stewart, E. P. Greenberg, Science, 21 May 1999, Vol. 284, Issue 5418, pp. 1318-1322.
[2] P. S. Stewart, J. W. Costerton, The Lancet, Volume 358, Issue 9276, 14 July 2001, Pages 135–138.
[3] G. M. Whitesides, E. Ostuni, S. Takayama, X. Jiang, D. E. Ingber, Annual Review of Biomedical Engineering, vol. 3, no. 1, pp. 335-373, 2001.

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