Dynamic control and quantification of bacterial population dynamics in droplets

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Quantification of bacterial population dynamics and gene expression is critical for developing a mechanistic understanding of bacterial physiology and for evaluating and optimizing performance of engineered gene circuits. Such quantification is typically carried out in bulk cultures that are not scalable and require large quantities of reagents. Droplet-based microfluidics can potentially overcome such limitations. But it also suffers from difficulty in dynamically manipulating the chemical environment or in maintaining droplet stability for long-term experiments. We developed a microfluidic system that integrates droplet injection and trapping to overcome these limitations.

***Keywords* — Quantitative biology, microfluidics, droplet injection and bacterial population dynamics.**

I. Introduction

Quantification of bacterial population dynamics and gene expression is critical for developing quantitative insights into gene regulation or bacterial physiology [1, 2]. Droplet-based microfluidics represents a promising alternative of bulk culture to overcome the limitations of large volume and high cost. However, it is also limited by manipulability of the chemical environment, and capability of long-term monitoring of population dynamics to acquire high temporal resolution.

We developed a microfluidic system that is integrated with an electrode-free injection technique for dynamic manipulation of droplets, and a trapping device to allow long-term, stable maintenance and monitoring of individual droplets. As a demonstration of our platform, we used this system to characterize both natural bacteria and engineered bacteria in response to diverse environmental cues, such as cell density and antibiotics.

II. RESULTS

A. Droplet injection and long-term data acquisition

In this work, we explored the electrode-free injection [3] to dynamically control the chemical environment of droplet. The custom device can used to monitor the population dynamics in individual droplets for at least 240 hours. To demonstrate the capability of this microfluidic system, we used it to quantify different population dynamics generated by either engineered or natural bacterial strains.

B. Programmed population control by an ePop circuit

When *ePop* cells were cultured in droplets, they generated population dynamics consistent with the circuit function. The population in each droplet started from a low density (1~5 cells per droplet), and reached its threshold density at approximately the 6th hour when the population crashed. The population then recovered at roughly the 20th hour.

C. Inoculum effect (IE) in response to antibiotics

IE refers to a population-dependent phenomenon in which bacteria at high initial densities are able to survive with intermediate antibiotic concentrations, while populations at low initial densities are eliminated. As our experiments in droplets, when streptomycin was 4μg/ml, the droplets with a high initial density survived, while those with a low initial density did not, which is the defining feature of IE.

D. Programmed altruistic death (PAD) of engineered bacteria in droplets

We then examined the dynamics of PAD of bacteria to test to capability of droplet injection technology. The survival and death of PAD cells are tunable by two chemicals, IPTG and 6-APA. When 6-APA was injected into the droplets containing PAD cells, the population without any induction of IPTG initiated growth for a short time window but stopped growing thereafter. In comparison, when the droplets were also injected with 1mM IPTG, the population in droplets grew to higher densities.

III. Conclusion

Our work demonstrated the feasibility of using liquid droplets to control and quantify dynamics of small bacterial populations, which has implications for the analysis of population dynamics of bacteria or other microbes in diverse contexts.

References

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