MONTANA STATE UNIVERSITY
NORM ASBJORNSON College of ENGINEERING

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PhD Defense
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1:00-2:00 PM
Roberts 307

"Bacterial cultivation in microscale drops and capsules to resolve single-cell growth physiology"

Abstract:
Single-cell heterogeneity contributes to the complex population dynamics of infectious microbial communities. Improving our understanding of single-cell physiology and heterogeneity may aid in mitigating microbial infections; however, assaying large populations of single cells can be challenging. Despite recent developments in single-cell assaying, tracking the physiology of large numbers of individual cells and their lineages over time is difficult to achieve using current technologies. Here, I apply drop-based microfluidics to develop microscale tools for improving high-throughput single-cell microbial growth assays. Drop-based microfluidics is a technology that generates and manipulates microscale drops. In this work, I create water-in-oil drops and hydrogel-shelled microcapsules using drop-based microfluidics to study the growth of P. aeruginosa bacteria, a key pathogen implicated in chronic lung infections and wounds. The growth of single bacterial cells inside drop microcompartments is observed via time-lapse confocal microscopy. Bacteria were cultured in water-in-oil drops and prepared for long-term storage in a novel microfluidic device environment, which we call a DropSOAC (Drop Stabilization on a Chip) chamber.

The DropSOAC method prevents drop destabilization by saturating microfluidic devices with equilibrated water and oil, maintaining phase equilibrium in the drop emulsion. Using DropSOAC, the single-cell growth of starved P. aeruginosa wildtype and hibernation promotion factor mutants were characterized, revealing significant growth heterogeneity in the mutant strain. Finally, we present a method for generating hydrogel-shelled microcapsules that enables the culturing of single cells in microscale environments where nutrients and waste can diffuse in and out of the microculture environment. A 3-D microfluidic device and capsule generation protocol are designed, resulting in an optimized approach for capsule production using phase-separating polymer systems and rapid hydrogel crosslinking. The growth of hundreds of individual P. aeruginosa cells is observed over time with the hydrogel-shelled microcapsules. Due to the permeability of the microcapsules, antibiotics can be introduced at various times during growth to investigate single and biofilm P. aeruginosa physiology. Overall, this work introduces novel approaches for high-throughput, single-cell microbial

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growth characterization that enables a deeper understanding of the role of heterogeneity in bacterial populations.