PhD Thesis Defense
Department of Microbiology and Cell Biology
Montana State University

Katrina N. Lyon

1:00 p.m., Monday, November 18, 2024
Norm Asbjornson Hall 165
"Human organoid modeling of gastric mucus layer physiology"

ABSTRACT

The stomach has two major secretory modes of self-defense: acid that aids in digestion and kills harmful microorganisms, and a sticky mucus layer across which this gradient exists. *Helicobacter pylori*–a pathogen responsible for gastric inflammation, ulcers, and cancer–attempts to overcome these two defenses in its quest to colonize and infect the stomach lining. Human gastric organoids–3D constructs derived from patient tissue–have been useful tools for mimicking such disease *in vitro*. Despite progress, there remains a gap in our understanding of how the gastric pH gradient is maintained. In addition, how closely organoid models can mimic this microenvironment has not been fully investigated. In this research, we first developed a novel technique for the measurement of pH inside our organoids using microelectrodes. We demonstrated reliable measurement of organoid pH, as well as evidence of a pH gradient. The pH was alkaline however, indicating a lack of acid secretion in our model. To investigate the luminal microenvironment further, we used particle tracking microrheology and demonstrated that the organoids contain heterogeneously distributed, viscoelastic mucus. As organoids are topologically closed, we next explored a method for culturing the cells as a monolayer at the air-liquid interface, thus enabling access to the mucus. We showed that milliliter quantities of clean and sterile "bioengineered gastric mucus" (BGM) can be harvested from these cultures over multiple weeks. We found that BGM shared many protective properties with native mucus, such as molecular composition, internal architecture, and rheological behavior. Proteomic analysis of BGM compared to native human mucus confirmed the impurity of native mucus that render it quite flawed for functional studies, further emphasizing the need for a physiologically relevant *in vitro* model. Overall, this research has contributed to a more complete biophysical understanding of mucus and acid production by human gastric organoids. These advanced insights into the use of organoids in gastric physiology studies may inspire translation of such models toward personalized treatment approaches for gastric disease.