A novel approach for antemortem small intestinal microbiota sampling

Caroline Bruer[1], Bret Ulery[2], Aaron Ericsson[1], Samantha Huddleston[2], Drona Madugula[2], Farnoushsadat Rezaei[2], Craig Franklin[1], James Amos-Landgraf[1,3]

[1]Department of Veterinary Pathobiology, University of Missouri, Columbia MO, [2]Department of Chemical Engineering, University of Missouri, Columbia MO,
[3]Mutant Mouse Resource and Research Center, University of Missouri, Columbia MO

Veterinary Research Scholars Program, University of Missouri

College of Engineering, University of Missouri

Veterinary Pathobiology, University of Missouri

MMRRC Mutant Mouse Resource & Research Centers supported by NIH

Background

- Pronounced regional differences between the upper and lower gastrointestinal tract (GIT) in both humans and mice reinforce the importance of analyzing the complete gut microbiota when sampling
- No commercial system currently exists to survey upper GIT contents without requiring high invasive procedures or post-mortem sampling
- Anatomical similarities between human and murine gastrointestinal tract systems (Figure 1)
- Development of a device for capturing small intestinal microbiota has promising market potential and could revolutionize research on the human gut microbiome, enabling longitudinal studies and advancing diagnostic and therapeutic applications in gastrointestinal medicine

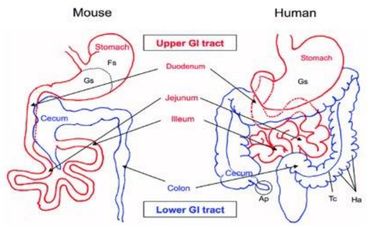


Fig. 1. Comparison of human and murine gastrointestinal tracts, distinguishing between the upper and low GIT.

Objectives

- This study is part of an ongoing project to develop an environmentally sensitive in situ sampling nanoparticle to allow for capture, recovery, and subsequent analysis of upper GIT microbiota
- Department of Engineering created a novel pH sensitive molecule
  - Positively charged molecule associates with bacteria in lower pH environments
  - Charge collapse prevents bacterial association at higher pH environments

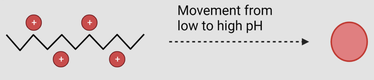


Fig. 2. Charge collapse as the sampling molecule moves from a low to a high pH

- The principal objective of this study was to visualize association between bacteria and the novel sampling molecule using fluorescence microscopy

Hypothesis

- Bacteria will associate with the novel compound under dynamic pH conditions similar to the transition from the upper GIT to lower GIT

Molecule Visualization

- The sampling molecule was tagged with a TAMRA fluorophore using click chemistry and visualized by fluorescent

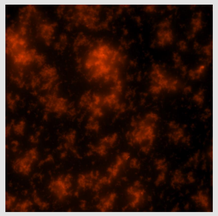


Fig. 3. Molecule visualized at conditions similar to the upper GIT.

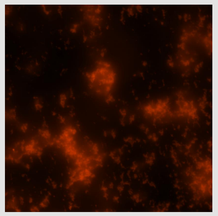


Fig. 4. Molecule visualized at conditions similar to the lower GIT.

Methodology

Experiment 1: *L. lactis* Association
- GFP fluorescence in *Lactococcus lactis* is induced with Nisin
- Fluorescent bacteria is combined with the sampling molecule a solution with a pH of 5.5 and a solution with a pH of 7.5

Experiment 2: Gram-positive Association
- Gram-positive bacteria and the sampling molecule are combined at a pH of 5.5
- The pH is raised to 7.5 and WGA conjugate is added to visualize the cell membranes of the bacteria

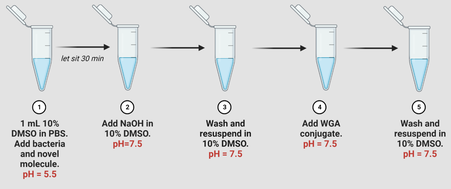


Fig. 5. Experimental procedure for testing association between Gram-positive bacteria and the sampling molecule

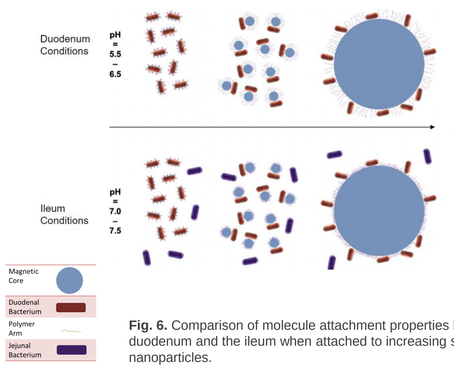
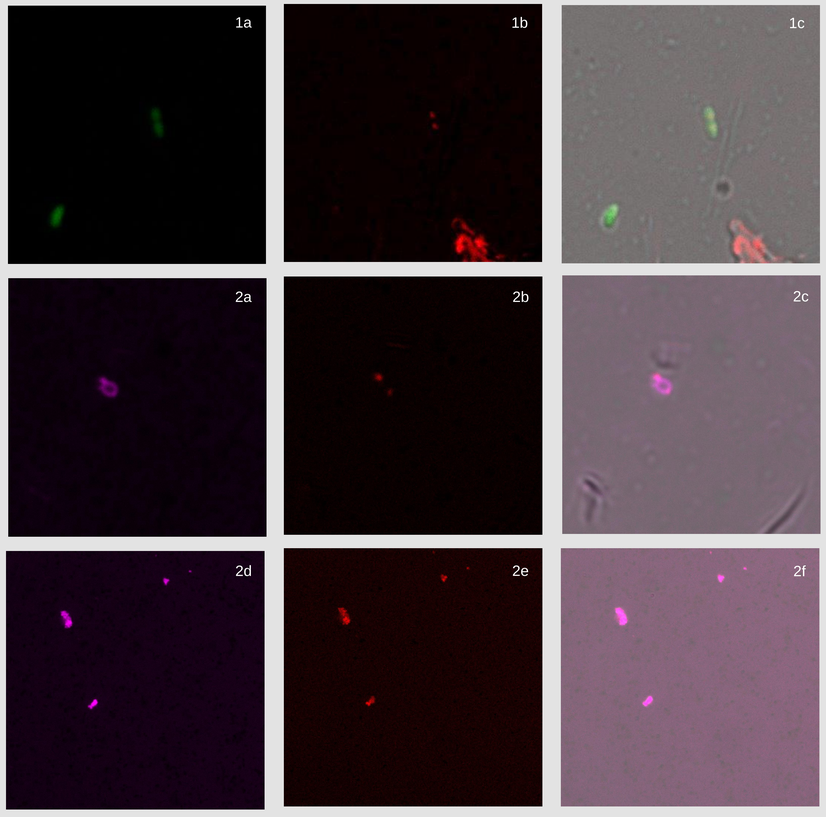


Fig. 6. Comparison of molecule attachment properties between the duodenum and the ileum when attached to increasing sized nanoparticles.

Bacterial Association

Experiment 1: *L. lactis* Association
- **Img. 1a.** *L.lactis* visualized under the GFP channel in solution with a pH of 7.5.
- **Img. 1b.** Sampling molecule visualized under the TAMRA channel in a solution with a pH of 7.5.
- **Img. 1c.** *L.lactis* and sampling molecule images overlayed to show association in a solution with a pH of 7.5.

Experiment 2: Gram-positive Association
- **Img. 2a**. Bacterial cell from the Zymbiotics Community Standard microbial sample visualized using WGA conjugate.
- **Img. 2b.** Sampling molecule visualized under the TAMRA channel.
- **Img. 2c.** Bacteria and sampling molecule images overlayed to show association.
- **Img. 2d.** *Bacillus licheniformis* cells visualized using WGA conjugate.
- **Img. 2e.** Sampling molecule visualized under the TAMRA channel.
- **Img. 2f.** *B. licheniformis* and sampling molecule images overlayed to show association.



Conclusions

- GFP fluorescent induced *L. lactis* can be used to visualize the association between the bacteria and the fluorescent tagged sampling molecule
- Wheat Germ Agglutinin (WGA) conjugate can be used to visualize Gram+ cell membranes and association with the fluorescent tagged sampling molecule
- Preliminary studies suggest positive association between the sampling molecule and bacterial cells

Acknowledgements

Special thanks to Mutant Mouse Resource and Research Center (NIH U42 OD010918) and Dr. Alexander Jurkevich of the University of Missouri's Advanced Light Microscopy Core. Additional thanks to the University of Missouri College of Veterinary Medicine Office of Research for student support.

Future Directions

- Explore additional variables
  - Microbial specificity
  - Enzyme degradation of molecule
- Attachment of novel molecule to metallic nanoparticle
  - 16sRNA sequencing to determine accuracy
  - Electron microscopy visualization
- Mouse model
  - Gastric gavage and subsequent fecal recovery and analysis