Senior Design Project
May - 2020

Design and Methodology of a Sampling Device for the In Vivo Collection of Gut Metabolites

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Abstract

We designed an orally consumable device that would allow for the untargeted sampling of the gut microbiome in a non-invasive, qualitative, and semi-quantitative manner. We developed an associated clinical methodology to analyze the binding capacity of our microbeads.

Introduction

C18 Hydrophobic Reaction Principles

Our microbeads contain C18 chains that induce hydrophobic reactions with small metabolites in the gut that possess hydrophobic domains, such as butyrate.

Size Based Exclusion Method

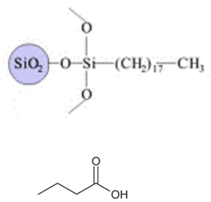
The silica coating of our microbeads contains pores of sizes of (0.1-1nm) to exclude larger molecules based on size.

Protective Enteric Coating

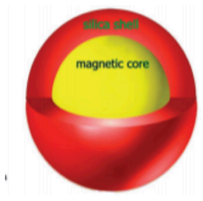
Microbeads have a 55 Coated shell to protect them from the harsh environment of the stomach and control the release time of the microparticles.

Recovery Method

Particles will have Maghemite (Fe+) Core that allows for magnetic separation from fecal contents



Butyrate



Methods I Design I Analysis

Protocol 1A	Protocol 1B	Protocol 1C	Protocol 1D
• Sample Preparation	• Prepare C18 Spin Columns	• 4-Nitrophenyl-Butyrate Binding	• 4-Nitrophenyl-Butyrate detection assay
❑ **Sample binding** Remove 150µL of each 4-nitrobutyrate-butyrate stock samples to a separate microfuge tube and add 50µL of Sample Buffer which gives a final concentration of 0.5% TFA in 5% ACN. ❑ **Sample buffer control (no C18)** Remove 150 ul of each 4-nitrobutyrate-butyrate stock samples to a separate microfuge tube and add 50uL of Sample Buffer which gives a final concentration of 0.5% TFA in 5% ACN. (These samples will not be passed over the C18 column but will serve as a positive control.) ❑ **PBS C18 binding control** Remove 150µL of each 4-nitrobutyrate-butyrate stock samples to a separate microfuge tube and add 50µL of PBS. ❑ **Control standards (no C18)** Remove 150µL of each 4-nitrobutyrate-butyrate stock samples to a separate microfuge tube and add 50µL of PBS. (These samples will not be passed over the C18 column but will serve as a positive control.)	❑ **Label the column/tube 1-8 for samples 1A1 and 17-24 for samples 1A3** Tap the column to ensure the resin is settled in the base of the column, and then remove the top and bottom caps. Place column in clean collection tube. ❑ **Add 200µL activation solution** Done to rinse the walls of the column and wet the resin. ❑ **Briefly centrifuge and discard the flow through** Repeat the Activation Solution wash once. ❑ **For columns 1-8:** Add 200µl Equilibration Solution, centrifuge as before and discard solution. Repeat Equilibration Solution wash once. ❑ **For columns 17-24:** Add 200µl of PBS centrifuge as before and discard solution. Repeat Equilibration Solution wash once.	❑ **Transfer the column to 14 clean collection tubes** Apply 150µL each sample from section 1A1 and 1A3 to the top of the resin bed of corresponding column. ❑ **Centrifuge for 1 minute and recover the flow-through**	❑ **Resuspend the Lipase** Followed by centrifugation. The supernatant was collected and used afresh as the enzyme solution. ❑ **Label 32 Eppendorf tubes 1-32** Add to each tube 850µL of PBS, 100µL of Lipase and 50µL of each of the corresponding samples (1-32) and controls. ❑ **15-minute incubation at RT** Then quick spin in microfuge remove 200 ul into the wells of a microtiter plate. Take the absorbance at the maxima of 4-nitrophenol is 405nm.

Results

Due to COVID-19 we were unable to perform our killer experiment, however the expected outcomes for our device go as follows:

- Our device will demonstrate its capability to bind to small metabolites in a qualitative and semi-quantitative manner.
- The buffer conditions will not affect the device's ability to detect metabolites fluctuation.
- The magnetic recovery of magnetic microparticles is expected to be greater than 95%.
- Our experiment will demonstrate the utility of the novel methodology we designed as an inexpensive and easily accessible alternative to traditional metabolomic approaches.

Conclusion

After the research and manufacturing phases of development, our device must pass several pre-clinical and clinical trials before FDA approval for commercial use. These trials will establish the safety and efficiency of the device in an In Vivo animal model. Once FDA approved, we will be ready for the mass production and shipment of our device to begin the collection of metabolic profiles to add to current metabolite libraries and improve global understanding of the gut microbiome. In the future, we would like to compile and submit our data to the Human Microbiome Project (HMP) to aid in the diagnosis and treatment of human gut associated disorders.

Acknowledgments

We would like to thank the University of Miami, Dr. Farbice Manns, Dr. Jorge Bohorquez, Dr. Fotios Andreopoulos, and the Department of Biomedical Engineering for their support and help during this entire design process.

References

1. Baxter, Nielson T., et al. “Dynamics of Human Gut Microbiota and Short-Chain Fatty Acids in Response to Dietary Interventions with Three Fermentable Fibers.” *MBio*, vol. 10, no. 1, 2019, doi:10.1128/mbio.02566-18.
2. Donia, M. S., and M. A. Fischbach. “Small Molecules from the Human Microbiota.” *Science*, vol. 349, no. 6246, 23 July 2015, pp. 1254766–1254766., doi:10.1126/science.1254766.