I. SIGNIFICANCE

Just as penicillin was a life-saving drug to combat infections at the turn of the century, food fortification was critical in preventing life-threatening disease and fetal deformities. Despite these discoveries, we are now plagued with both antibiotic-resistant 'superbugs' [1,2], and our low-fiber Western diet is further increasing mortality and disease risk [3,4]. **We hypothesize that precision dietary prebiotic fibers may prevent or reduce deadly infections and improve cancer outcomes.** Dietary fiber is critical to support the health of the bacteria in the gut, the microbiome, especially microbes which possess the ability to evict pathogens [5]. Deaths from pathogen infections accounts for approximately 700,000 death each year globally, with 23,000 U.S. deaths attributed to antibiotic resistant bacteria [1]. Among the major culprits are *C. difficile,* which is related to approximately 15,000 deaths per year in the U.S., and enterotoxic *B. fragilis* (ETBF), which is a major cause of hospital-related infections. The standard treatment for these infections is a combination of antibiotics, however, as we run out of antibiotics to combat these 'superbugs', we must look for alternatives and preventative strategies, which has ignited a new effort to develop pre- and probiotics to address this critical situation. Currently, we have very little understanding of *which types* of dietary fibers support those microbes that convey protection from infection nor the mechanisms by which specific dietary fibers are protective [6-8]. To address this issue, we have designed a multi-phase strategy to develop precision medicine prebiotics by **utilizing a new tool the Mini-Bioreactor Array system (MBRA)[9].** This new system will allow us to test dietary fibers in a controlled gut microbiome community setting. The purpose of this research is to identify the prebiotics and microbial factors conveying resistance to infection while accounting for inter-individual microbiome variability. Indeed, a recent study showed a 5-fold improved response to cancer immunotherapy with a high fiber diet, which supports our focus on cancer treatment [10].The results from our initial study (Phase I) will allow us to pursue a multi-phase research strategy (**Fig. 1**) designed to address an outstanding need in our field to identify critical microbes and metabolites that are altered as a result of exposure to dietary fibers and pathogens. *Ultimately, this evidence will expand our understanding of the fiber-microbiome relationship, and allow us to develop preventative interventions using prebiotics*. (**Initiative=Health/Data Science)**.

INNOVATION: **1)** Application of the *MBRA system* to study diet-microbiome interaction, as well as, drug-microbe, microbe-microbe, or nanomaterial-microbe interaction in a controlled environment **(Initiative=Health)**. **2)** Innovative use of microbial populations to study dietary fibers through *metabolic modeling* **(Initiative=Data Science/Health)** and their effects over time on resilience, stability, and resistance to pathogens. **3)** Development of a precision medicine prebiotic; a) **Phase I,** will set the stage for several more powerful studies **(Initiative=Health)**: b) **Phase II -** *in vitro* studies to investigate the differential response to dietary fibers between stool communities from healthy individuals and those with colon cancer, and c) **Phase III -** *in vivo* studies to determine the response to fiber-treated microbiota in healthy and cancer models.

IMPACT: The NIH released its draft Strategic Plan for Nutrition Research; this research will position Baylor to take advantage of these funding opportunities. Specifically, our research addresses 8 priorities (**see Appendix).** *Results from this pilot data will be used to develop a large (R01) multi-phase strategy (**Fig. 1**) that includes multiple study inputs and outputs poised to address questions regarding host-microbe and fiber-microbe interactions.* Thus, this study, in combination with future studies, has the potential to lead to development of precision medicine prebiotics for infection resistance and improvement of cancer treatment.

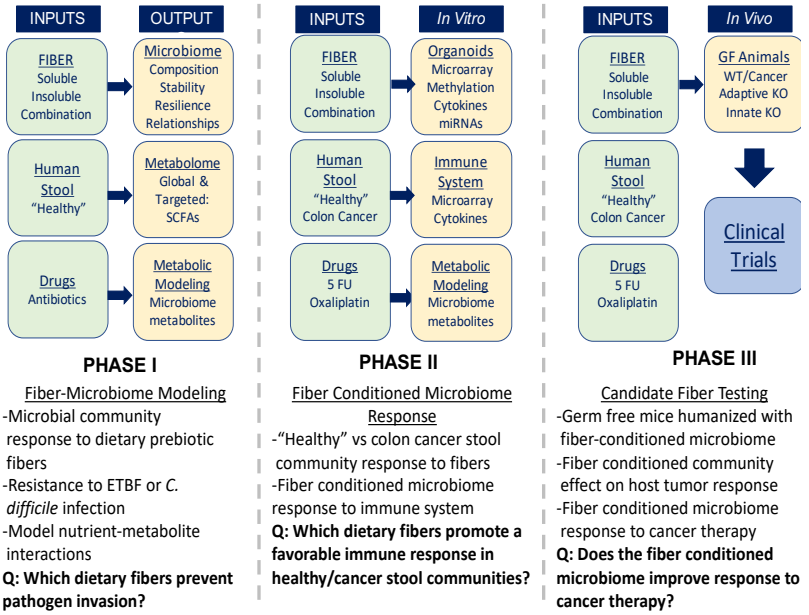


Fig. 1. Three Phase Strategy for Developing Precision Medicine Prebiotics. Phase I is described in this proposal.

II. RESEARCH QUESTIONS ADDRESSED

This proposal seeks to illuminate the dietary fiber-microbiome relationship using a **powerful new tool - fecal mini-bioreactor arrays (MBRAs)**[9]. First used to study *Clostridium difficile* infection, the MBRA allows for continuous cultivation of complex fecal microbial communities to study infection [9]. Using the MBRA system, developed by **Co-PI Dr. Robert Britton**, we seek to develop a prebiotic that is capable of preventing or reducing infection, focusing initially on the effects of two types of fermentable fiber, inulin and resistant starch in Phase I of this research strategy (**Fig. 2**).

Studies indicate that fermentable dietary fibers (prebiotics) are paramount in maintaining a healthy microbiome that is resistant to disease, including infection and cancer [8,11]. Little is known; however, about *which types* of dietary fiber are responsible for these protective effects nor their mechanisms [12]. With the global prebiotics market size anticipated to reach $7.91 billion by 2025, we are facing a critical need to move beyond our current 'one size fits all' application of dietary fiber to that of a tool for precision medicine [13]. The bacteria in the gut, collectively termed the microbiome, is significantly affected by our diet [14]. Our understanding of how this dietary fiber-microbiome relationship impacts response to infection however remains rudimentary, in particular with respect to the effect on the microbiome. Dietary fiber is one of the main sources of carbohydrates the microbiome uses for fuel in the production of short chain fatty acids (SCFAs), which supports gut barrier function [8,15]. Without fiber, the gut bacteria feed off the mucus lining the gut, leaving the host susceptible to infection and disease, including cancer [11,13,15] .

Our long-term goal is to develop prebiotics for precision medicine using the MBRA system in our multi-phase strategy (**Fig. 1**). The goal of this proposal (**Fig. 2**) is to operationalize the MBRA system by testing two types of dietary fibers in healthy stool communities, and their ability to resist pathogen colonization**.** We are choosing to focus on two of the most common dietary fibers, insulin and resistant starch, because they have consistently demonstrated the ability to favorably modify the gut microbiome to produce SCFAs [7,16]. Additionally, by establishing this new tool, **we will be able to train graduate and undergraduate students in using the MBRA** system (**see Appendix)**, which will give them critical skills to enhance learning and the ability to be competitive for fellowships or jobs in the STEM fields. To meet these goals, we have developed the following specific aims:

A.1. Specific Aim 1: Quantify changes in model stool community composition and fatty acid metabolism after exposure to inulin or resistant starch. The gut microbiome is reliant upon dietary fiber for nutrients, which in turn supplies energy for the intestinal epithelial cells. Data show that lack of dietary fiber results in dysbiosis, changes in metabolites (e.g. SCFAs), and increased disease risk [15,17]. However, human dietary fiber intervention studies with inulin or resistant starch each show different effects on the microbiome and SCFA production [18-21]. *Thus, we hypothesize that the gut microbiome composition and SCFA production will be differentially altered in response to inulin or resistant starch, which will be dependent upon the donor microbial stool community supplied.*

A.2. Specific Aim 2: Measure the ability of fiber-conditioned stool communities to resist pathogen colonization. The ability of microbial communities to resist pathogen colonization involves mechanisms that are, in part, reliant upon fermentation of prebiotic fibers to produce SCFAs. Specifically, commensal *Bacteroides spp.* confer colonization resistance again *Salmonella* infection through the production of the SCFA propionate [22]. Further, the combinations of prebiotic fibers with the probiotic *Lactobacillus reuteri* 1063 prevents colonization of the pathogenic strain of *E. coli* by limiting mucus adhesion [23]. *Thus, we hypothesize that inulin and resistant starch will confer differential colonization resistance to the pathogens, enterotoxic B. fragilis (ETBF), and C. difficile.*

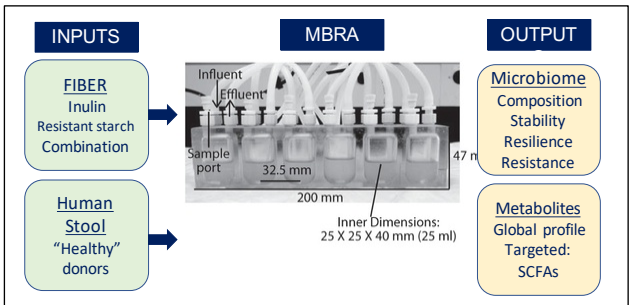


Fig. 2. Study Design Schema for Phase I.

Our research plan is supported by a highly-experienced team of scientists at Baylor University, Baylor College of Medicine, and Mayo Clinic, with expertise in microbiology, metabolic modeling, and metabolite analysis [24-28]. Completion of this research will provide a tool (MBRA) to **enhance the current space available** and allow recruitment of students and faculty of high research caliber. This research will also provide the foundation for an externally funded research program dedicated to elucidating the dietary fiber-microbiome relationship. *These findings will impact our field by not only answering outstanding questions that remain with regards to microbiome resilience to infection, but also lead to novel prebiotic interventions to prevent or reduce infection.* ***Our research will benefit Baylor by providing cutting-edge tools, collaboration, student training, and a sustainable research focus that can be used to make Baylor a leader in prebiotics and infectious disease research.***

A.3. Research Approach

Approach: To initiate our study, we (**Dr. Greathouse** and a doctoral student) will begin by training with **Dr. Britton** to setup and run the MBRA system at Baylor College of Medicine. This training will allow us to fully operationalize the MBRA system in the **laboratory of Dr. Greathouse** and begin experiments **(Fig. 2)**. Further, this doctoral student will be co-mentored by both Dr. Greathouse and Dr. Britton through the Biomedical Science (BMS) program at Baylor University. (**see Appendix – Training Plan)**

To address **Aim 1,** we will collect donor stool from three healthy individuals according the methods previously described [9]. Three replicate reactors will be used for each of the three donor stool samples (n=9 reactors). Reactors will be inoculated with 25% fecal supernatants by adding them to pre-reduced growth medium for 16hrs, and then fresh medium will be continuously flowed in through the MBRA system. To determine changes from baseline in community composition we will extract 1mL from each reactor on day 0 (inoculation), and daily thereafter for 20 days and store at -80 degrees. To compare the effects of inulin vs. resistant starch, 2.0 g/L of inulin or resistant starch (dose derived from previous studies) will be added to each donor sample reactor with fresh medium flowed in; provided for 20 days [9]. Samples will be collected in the same manner as at baseline. DNA will be extracted from each sample using bead beating and the Qiagen DNeasy Tissue Kit, and will be quantified with QuantIT. Samples (n=594) will be sequenced on the Illumina MiSeq (**Dr. Petrosino, BCM**) using 16S rRNA sequencing (V4 region) and bioinformatically processed as previously described [9,29,30]. For analysis of metabolites, SCFAs, a separate 1mL sample will be collected at all time points previously indicated. Samples from each donor will be pooled together by time point and sent to **Dr. Lavado (ENV)** for SCFA metabolite analysis (pooled, n=36). Data from the microbial and metabolite profiling will integrated by summing microbe-microbe metabolic interactions to construct an interaction landscape (**Dr. Chia**) from response to dietary prebiotics.

To address **Aim 2,** we will perform invasion studies using *C. difficile* (CD2015 from **Dr. Britton**) or ETBF (**Dr. Greathouse**), both of which are responsible for life-threaten infectious diarrhea, chronic disease, or colon cancer. Prior to inoculation, we will test samples for *C. difficile* or ETBF presence. Using an experimental design similar to the one described previously [9], we will inoculate three reactors with either CD2015 or ETBF at a dilution of 1:100 from a culture grown to an OD_{600} of 1.5 to measure CFU/mL in pure culture by plating and by qPCR. We will test the ability of the pooled communities to resist pathogen invasion by pretreating with i) antibiotics, ii) inulin + antibiotics, iii) resistant starch + antibiotics, or iv) control media. To measure resistance to invasion, we will take 1 mL samples at 15 min and 3 hours post-inoculation, and once daily for 15 days, and measure CFU/mL by plating and by qPCR. To determine community structure changes, we will also collect 1mL samples for 16S rRNA sequencing (n=405). Using these data, microbial and metabolic, we will use the interaction landscape to identify which prebiotic fibers can support the microbes capable of evicting or preventing *C. difficile* or ETBF engraftment (**Dr. Chia**).

Specific Aim	Year 1 Benchmark	Year 2 Benchmark	Year 3 Benchmark
Aim 1	Trained/Initialize MBRA Model communities growing	Samples collected from inulin/starch exposure	Community/metabolite analysis finalized
Aim 2	CD2015/ETBF growing in MBRA reactors	Samples collected from pathogen exposure	Community/invasion analysis finalized
Manuscripts/Grants		Grant funding identified/manuscript outlined	Grant/manuscript submitted

I. CLEAR LINK TO INITIATIVES AND PILLARS

Pillar #1. Commitment to an unambiguously Christian educational environment. This proposal will support the first pillar through two mechanisms, a) opportunity for students to practice communicating their science with a Christian worldview to children and young scientists through mechanisms such as “Present your PhD”, b) demonstration of scientists working through their Christian faith to make important scientific breakthroughs, and c) Christian mentorship of students from Baylor research faculty during this study.

Pillar #3. Excellence in Research & Scholarship. To achieve the University’s goal of R1 status, establishing this MBRA system and research area will be paramount in allowing us to compete for external funding with R1 Universities and Medical Centers. This area of research is highly critical both scientifically and monetarily.

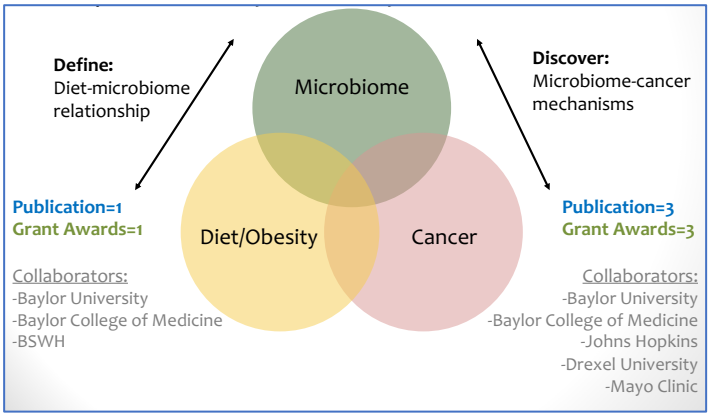
Several funding agencies, private, state, and federal, have issued multiple requests for research proposals focused on the elucidating the diet-microbiome relationship; indicating this is a highly sought after and fundable project. Also, multiple high-impact research articles regularly report a lack of understanding of the mechanisms governing the resistance to infection and disease conferred by the diet-microbiome relationship, which is addressed this proposal.

Collaboration between faculty/schools/Universities

Collaboration between faculty and schools will be a key aspect of this research: 1) **Dr. Greathouse + Dr. Lavado** (Nutrition/ENV) – will work together to identify microbial metabolites using state-of-the-art equipment in the Lavado Laboratory and provide training for undergraduate and graduate students; 2) **Dr. Kearney** (Biology) – will be able to use MBRA system to develop and test his antimicrobial peptide engineered bacteria [31], 3) **Dr. Bruce** (ENV) – will be able to test multiple drug combinations using the MBRA. This research will also take advantage of established and new collaborations between hospitals and Universities by integrating the expertise of multiple investigators: 1) **Dr. Robert Britton** (Baylor College of Medicine) – will provide training and expertise in MBRA system setup and establishment at BU, study design, and microbiome analysis.; 2) **Dr. Nick Chia** (Mayo Clinic) – with whom I have an established publication record [26], will provide metabolic modeling and network analysis [32,33] from data generated from this study, and future studies using this system **(Initiative – Data Science/Health)**, and will be actively engaged in postdoctoral training (**see Appendix)**.

II. PROJECT SITUATED WITHIN A BROADER RESEARCH AGENDA

The main focus of my research is to understand and identify biomarkers and molecular mechanisms that can be used to define the relationship between obesity, the microbiome and diet. Using big data and machine learning techniques, we seek to elucidate the relationship between diet, the microbiome and cancer. Our goal is to **1)** delineate the dietary factors that modify the microbiome and its function, **2)** develop microbial predictors that improve stratification of patients for obesity or cancer treatment (**2 publications/1 grant awarded/2 grants pending funding**), and **3)** identify key functional pathways and mechanisms of the microbiota-host communication (**2 grants awarded/2 grants pending)**. Currently, my lab is working together with several collaborators inside and outside of Baylor University. One of our projects involves the study of the prebiotic Prebiotin as part of an RCT in medical residents to prevent excess weight gain. We are analyzing microbial (stool), stress, activity, and dietary data collected from physicians taking Prebiotin or placebo X12 weeks (**1 URC grant awarded)**. We are using both microbiome data and serum to identify key features that we can use to understand how prebiotics alter the microbiome and mediate weight gain and stress. A separate project involves assessing the microbiome-host relationship by asking whether sRNA, contained in outer membrane vesicles (OMVs) shed by bacteria, can activate the Toll-like receptors (TLRs) on epithelial or immune cells, and thus trigger inflammation. This project is profiling the sRNAs (RNA-seq) contained in colon-cancer associated bacteria (*B. fragilis*) and those associated with the TLRs to identify mechanisms of immune evasion and activation (**3 abstracts published/2 grants awarded/2 grants pending funding).** This research proposal is linked to my broader agenda through my aforementioned goals. These goals will be achieved by testing the effects of two fermentable dietary fibers in a controlled polymicrobial community, which will allow us to identify the dietary and microbial factors that are key in protecting from pathogen invasion and infection, as well as, discovering novel mechanisms through which the microbiome may mediate the response to cancer therapy. Further, the proposed work augments ongoing studies in which we are investigating the mechanisms of host-microbiome communication between known colon cancer promoting pathogens (e.g. *B. fragilis*) and colon epithelial cells. In the future, we are planning to study how different dietary fibers differentially impact the microbiome in individuals with and without colon cancer, which will address an outstanding need in colon cancer prevention and treatment.



III. REQUESTED FUNDING LEVEL

PERSONNEL

K. Leigh Greathouse, PhD, MPH, MS, RD, *Principal Investigator (9.6 calendar months for years 1-3).*

As PI, Dr. Greathouse will be responsible for maintaining the overall scientific and procedural integrity of the project and executing the scientific agenda described in this proposal. She will supervise and/or directly perform IRB applications, training, data collection, and data analysis. She will coordinate meetings and communications amongst her collaborators and students regarding study progress and will have ultimate responsibility for monitoring of research progress and safety. She will be responsible for budgeting resources and for communications regarding the progress of the study with the Baylor University IRB. Dr. Greathouse will request class release in lieu of salary support.

Robert A. Britton, PhD, *Co-PI (1.2 calendar months for years 1-2).* Dr. Britton, Professor at Baylor College of Medicine, will oversee training on the MBRA system, but will not require salary coverage, only $5048 to cover supplies and services for one week of bioreactor runs along with training.

Ramon Lavado, PhD, *Co-PI (2 calendar months for years 2-3).* Dr. Lavado will conduct SCFA analysis in his laboratory and oversee student training in SCFA measurement and analysis. He will not require any salary coverage for this project.

Nicholas Chia, PhD, *Co-PI (1.2 calendar months for years 2-3).* Dr. Chia, Mayo Clinic Co-Director of Microbiome Research, will oversee postdoctoral training and conduct the metabolic modeling of data obtained from the MBRAs. He will require 0.1FTE at $13,852.80/year for this work for years 2-3.

RESEARCH BUDGET

We are requesting a total of $237,560 in Other Personnel, Materials and Supplies, Equipment in years 1-3 as follows:

OTHER PERSONNEL

Postdoctoral assistant. We are requesting 50% coverage for postdoctoral assistant (0.5 FTE) $28,312.50/year for years 2-3 to assist with metabolic modeling to share with Dr. Chia (other 50% covered by Dr. Chia).

Graduate research assistant. We are requesting as part of the research budget, funding to cover one graduate student, who will be working under the guidance of Dr. Greathouse and Dr. Lavado to assist with collecting, storing, and processing biospecimens for metabolite analysis for the duration of the study ($24,000/year).

MATERIALS AND SUPPLIES

Research supplies. We are requesting a total of $ 41,098 to cover consumables, labware, bacterial reagents, DNA extraction, and qPCR reagents (**Dr. Greathouse**). For metabolite SCFA analysis, we are requesting $20,000 (**Dr. Lavado**).

Contract services. For the 16S rRNA sequencing of the microbiome we are requesting: $25,000 for Year 2 and $25,000 for Year 3. (BCM, **Dr. Petrosino**)

EQUIPMENT

To initiate the setup and installation of the Mini-bioreactor array (MBRA) system we are requesting $89,208. This cost includes an anaerobic chamber and necessary components (oxygen sensors, heaters, H2S scrubbers, and pumps). The cost is based on the established system developed by Dr. Robert Britton (**see Appendix**). If this proposal is funded, it has been agreed (Drs. Meohnke and Chambliss) that space within the Molecular Bioscience Center in the Baylor Sciences Building would be utilized making this available to a larger user base.

TRAVEL AND PUBLICATION FEES

To support dissemination of findings from this study we are requesting $4,500 in Year 3 for travel to conferences ($1,500) and fees associated with publication ($3000).

Budget Items	YEAR 1	YEAR 2	YEAR 3	Total
TOTAL BUDGET	**$146,855**	**$87,312**	**$91,814**	**$366,184**

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