FSU iGEM 2017

Human Practices

Bacteria Aimed at Removing and Terminating Intestinal Invaders

We have engaged with our community, our city, and our state to explore Celiac Disease and the ramifications of a cell-based therapy as a treatment option. We recognize that as a cell-based therapy available as a pill or pro-biotic for human ingestion, we have considered safety in three aspects through dialog in our community.

1. Development of a human-friendly chassis and full clinical trials for safety and efficacy and FDA regulatory approvals.
2. Potential shedding of the cells into the environment and their consideration in waste treatment and the environment.
3. Risk mitigation to address concerns of cell mutation. We have designed our cells with a plasmid approach to minimize the mutagenic possibilities, and future studies could further investigate safety concerns, potentially utilizing other iGEM biobricks to

Moonshot Night "Engineering Life"

We participated in a targeted discussion room at Moonshot Night, an event run by a local non-profit, Moonshot Florida. Moonshot Night is designed to be "themed gathering of the curious and creative designed to inform, inspire and nurture a community that invents the future." Combined with Moonshot's topic on Engineering Life, we were able to explore many of the possible impacts and ethics our iGEM project should consider.

Physicians & Individuals

We met with a family physician who routinely interfaces with patients with Celiac Disease. We also talked to multiple patients about how Celiac Disease affected their lives and what they would like to see in emerging solutions. These dialogues resulted in a comprehensive understanding of what each group expected and wanted from a cell-based therapy.

We feel we meet the criteria that we have made design considerations so that ultimately our device would be used in a way that is safe, responsible and good for the world. Please join us to delve deeper into Celiac Disease and the Human Practices considerations our team has made. **(SILVER MEDAL CRITERIA)**

Furthermore, we incorporated these design considerations into our final devices, envisioning an easy-to use cell-based therapy to neutralize gliadin and zonulin in the gut that could be readily available as a pill or pro-biotic. **(GOLD MEDAL CRITERIA)**

We hope you will take the time to read further below!

Introduction

Over the course of this summer, our Human Practices team has had the pleasure of delving into the problem of Celiac Disease to better formulate a potential solution. From our initial investigation to our patient research, we have been able to look into the social, physical, and financial implications of Celiac Disease not only on the patients, but also the community and the doctors that interact with them. What resulted was BARTII: Bacteria Aimed at Terminating and Removing Intestinal Invaders, a cell-based therapeutic intended to remove gliadin and zonulin from the small intestine while degrading the excess gliadin in the same extra-membranous space.

When initially researching the basics of Celiac Disease, we came across three fundamental problems with current treatment or remediation options. First, while there are a few treatment options for Celiac Disease currently being studied, including immune modification and even ringworm infections, most of the proposed treatments are invasive or they effect the individual's autoimmune responses. These options, though viable, effect the patient's life by either making them more susceptible to infection or require a significant change in the patient's lifestyle. The only consistently effective method of mediating Celiac symptoms is going Gluten Free. Though it is effective when the patient's food is prepared in isolation from outside sources, eating in public and purchasing pre-packaged food prepared in facilities that also package products with gluten increases the likelihood of cross-contamination and, therefore, the likelihood of unintended physiological reactions. This strictly limits the ability of patients to interact socially because their food and drink options must be monitored for the sake of their health. Second, the Gluten-Free diet is expensive, with some products regularly being 200% or more greater than analogous products with gluten. Third, Gluten-Free products are popular in part because of the Gluten-Free health food fad. Unfortunately, this fad has led to the patients with Celiac Disease being seen as an illegitimate condition though it has potentially deadly ramifications. Likewise, people who have neither Celiac Disease nor gluten allergies misrepresenting the necessity of the Gluten-Free diet has led to a generally, popularly accepted belief that removing gluten from one's diet is a choice not a necessity. Because of this, the chance of cross-contamination increases yet again for patients who try to eat meals they did not personally prepare. Not to mention, Celiac Disease is often left undiagnosed. Even more troubling is that the population with the fastest rate of Celiac Disease diagnosis is children. It is because of this that a great deal of emphasis was placed in determining how best to provide a solution for the children that live with this condition. Patients with Celiac Disease face many challenges in their attempts to stay healthy, both physically and socially. Our cursory analysis of the challenges patients face led us to the conclusion that the current method of treating a Celiac patient could be improved by providing a therapy that was minimally invasive, required next to no change from a traditional diet, and had a minimal cost associated with it.

Focus Group Research

Beyond just the initial research, we concluded that to find a solution that was readily acceptable for both patients and practitioners, we would have to create dialogues with the parties involved. This resulted in conversations with three patients, a Family Practice doctor, and community members in the form of an event called Moonshot Night. These dialogues resulted in a comprehensive understanding of what each group expected and wanted from a cell-based therapy.

Having conversations with Celiac patients helped inform our potential solutions by giving us insight into the needs of those who deal with Celiac Disease on a daily basis. These patients, though from a wide range of ages, deal with the same social, physical, and financial burdens incurred because of Celiac Disease. For this reason and the nature of the condition, many of their responses during our dialogues were similar. They all wanted a therapy that mitigated symptoms with minimal side-effects, the ability to discontinue the Gluten-Free diet if they so choose, and an option that was less expensive than the Gluten-Free diet. Beyond this, they had few requirements. As patients with a life-altering condition, they were willing to risk potential side effects if the usefulness of the therapy outweighed the risks, they were open to all forms of drug delivery, and they were content with a solution that was able to work for a range of time periods.

The effects of Celiac Disease greatly impact the patient's everyday life in such a way that they are acquiescent of most solutions so long as they work and prevent the threat of cross-contamination or gluten from dictating their interactions with food and their communities. Another point of coincidence was that the patients were willing to maintain the Gluten-Free diet if they were able to eat in public, because, as they all made a point to say, the debilitating pain and nausea caused by trace amounts of gluten was enough to keep them from eating foods prepared by others. These perspectives led us to envision our cell-based therapeutic as a probiotic-like solution that is not only effective, but minimizes the magnitude of a behavioral change as Celiac patients already require strict observance over everything they consume. [see Patient Transcriptions]

We had the opportunity of speaking with a Family Practice doctor in Tallahassee to gain a better understanding of Celiac Disease, its diagnosis, and what a treatment option would look like from a doctor's perspective. Dr. Goforth has been practicing medicine for the last 34 years and has dealt with the diagnosis process of Celiac patients fairly regularly over that time period. His experience allowed him to clarify the process of diagnosis and treatment using the Gluten-Free diet, as well as, a few short comings that have arisen as the diet continues to gain popularity in Western culture.

When speaking on the topic of a cell-based therapy for Celiac Disease, he was able to formulate a model that reduced cost, increased availability to the general public, and was easy to use.

"You always want something that is the least expensive, that you can take orally and don't have to inject…I think if you come up with therapies that are easy to use; primary care physicians will be able to take care of all of that… I think the longer it lasts the better. The problem with long-acting agents is that they are extremely expensive like $2000 for the dose you get annually and one could argue that its a better deal than taking a pill daily or weekly but a lot of insurance companies just won't pay for it because it is so expensive… You always want something that is the least expensive, that you can take orally and don't have to inject… that tones down the immune response…[and has] very few side effects."

Dr. Goforth presented a unique perspective because, initially, a long-term solution made sense; all one would have to do was input a population of cells able to mediate symptoms and be kept at relatively stable levels within the small intestine for a prolonged period of time. His input allowed us to develop a model that would likely decrease overall cost and would be less likely to affect the homeostasis in the jejunum.

When we spoke with the attendees of Moonshot Night, we were able to view our cell from a new and different set of perspectives. While the patients and doctors were not necessarily concerned with the regulations and controls placed on a cell-based product, the public was. They were concerned that a therapy that was purely cell-based could mutate easily and present a health risk for those to used it. Similarly, they were wary of a product that was intended to stay in the body for any prolonged period of time. For this reason, they were much more amenable to probiotic-like pill or consumable solution that stayed in the system for a period of time, then was completely removed. We asked the question of what kinds of treatments they would be willing to undergo if they had Celiac Disease, and, on the whole, they were able to accept everything from a topical solution to a treatment

with Chemotherapy-like side effects. However, in discussion, most were able to more readily accept a pill presented like a probiotic in that it contained lyophilized cells because there are popular analogues available over the counter or in everyday foods.

The community members we spoke to were able to give us constructive feedback by raising questions that had previously been evaluated on a surface level. The Moonshot attendees were concerned with the both the ethical and practical components of a cell-based therapy. They asked the question of safety, how the therapy could affect the environment if the cells were being genetically modified, why a cell-based therapy rather than an enzyme alone, and who would be in charge of making sure the cell-based therapeutic was regulated in such a way that the patients benefited without undue cost. It was these questions along with the feedback given by both patients and doctors that led to our ideal formulation of our cell-based therapeutic and the beginning of BARTII.

Market Research

- Retail sales in 2006 of gluten-free and free-from foods had combined retail sales of 900 million U.S. dollars.
- U.S. retail sales of these categories are projected to grow to 23.9 billion U.S. dollars by 2020.
- Growing sales may be contributed to positive consumer attitudes towards gluten-free diets.
- In 2016, a survey found that 22 percent of U.S. consumers find gluten-free diets very healthy.

The expansion of this market is driven by the general consumer being more aware of gluten and its side effects, more doctors are aware of Celiac Disease and patient's loved ones are adopting Gluten Free diets to aide their spouses. What we are seeing is entire households becoming gluten free when one child or adult has CD or GI.

Bakery products are the largest segment of gluten free foods. The availability of corn, sorghum, and quinoa have made these popular substitutes in the US and Canadian markets for gluten free alternatives.

Another factor driving the market is the general demand for healthy food products. As nutritional science evolves, the association of bread, carbs, wheat, pasta, etc. as un-healthy has become a prevalent belief in both the media and social circles, viewing alternatives or elimination as healthier. This has led to the rise of gluten-conscious consumers who are neither gluten intolerant nor possess a gluten allergy for the sake of a healthy lifestyle.

A challenge for the Gluten Free market is making products that are affordable for the everyday consumer. Costs in production of replacing wheat with alternatives leads to some products being 200% more than the alternative. This is likely where a Celiac-related therapeutic would experience both positive and negative feedback; the first from Celiac patients or others suffering from gluten-related maladies and the second from the Gluten-Free industry.

Security

Another component of our Moonshot Night dialogue was understanding the concerns the community held about a cell-based therapeutic; BARTII in particular. One of the these concerns that was discussed at length was "what or who is controlling the drug/therapy?". What arose was a two-pronged assessment of necessary safety controls to make the drug more amenable to the public. The first being, the therapy must be safe for the patient so certain quality control measures must be taken, as with any biomedical product or pharmaceutical, to ensure that there are no errant cells. In the future, these control mechanisms would take the form of cellular coding intended to apoptose any malfunctioning cells, as well as, production controls to ensure no genetic shift or significant variation between chassis. The second measure intended to ensure security would be identifying exactly who had control over dispensing and regulating the therapy.

Public Policy / Law and Regulation

When evaluating who has control over BARTII, we considered the legislation in place for new/novel pharmaceuticals, the required pre-market approvals that would be necessary, and the regulating bodies that would participate in ensuring that any product brought to market would be safe and suitable for consumption.

- Section 351 of the Public Health Service Act
  - "Defines a biological product as a virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, … applicable to the prevention, treatment, or cure of a disease or condition of human beings"
  - Also, products containing cells or microorganisms
  - Regulated by the Center for Drug Evaluation and Research and Center for Biologics Evaluation and Research
  - As a drug, they are also regulated under the FDC act (Food, Drug, and Cosmetic Act)
  - Once lab and animal testing prove that the product is safe for humans, then they can undergo human clinical trials under the guise of an "investigational new drug application"
    - A biologics license application "is required for biological products subject to licensure under the PHS Act"

The PHS Act requires that the product, process and manufacturing meet standards of safety, purity and potency. With safety being, "relative freedom from harmful effects, direct or indirect, when a product is prudently administered…". As touched on before, the safety of the

  - Purity: "relative freedom from extraneous matter in the finished product, whether or not harmful to the recipient of deleterious to the product…"
  - Potency: "The specific ability or capacity of the product, as indicated by appropriate laboratory tests, to yield a given result"
- The PHS Act helps dictate the necessary standards for developing/manufacturing biologics
  - "Because, in many cases, there is limited ability to identify the identity of the clinically active component(s) of a complex biological product, such products are often defined by their manufacturing processes. Changes in the manufacturing process, equipment or facilities could

result in changes in the biological product itself and sometimes require additional clinical studies to demonstrate the product's safety, identity, purity and potency."

- FDA
  - "Any stem-cell based product that contains cells or tissues that are highly processed, are used for other than their normal function, are combined with non-tissue components, or are used for metabollic purposes… would able be subject to the Public Health Safety Act, Section 251, which regulates the licensing of biologic products and requires the submission of an investigational new drug application to the FDA before studies involving humans are initiated"
  - Before the investigational new drug application, you must show that:
    - Cells don't have risk of contamination or damage
      - (Really in the case where there is donor tissue, but still relevant)
    - The cell will work in vivo
      - "The FDA will probably require proof-of-concept experiments in animal models when little is known about the product, indication, or route of administration"
      - "Transplanting stem-cell–based products into animals and analyzing whether the cells travel from the site of transplantation and where they functionally integrate will be required to address this concern about site-specific integration.13 Biologic distribution studies also will be important. Tagging a stem-cell-based product with markers such as green fluorescent protein or unique surface antigens that can be seen with the use of antibodies may be an effective way to monitor the journey of cells after transplantation. It will also be important to study the longevity of the cells in the stem-cell–based product to determine the likely duration of the therapeutic effect."
    - The cells are safe, pure and potent
  - "Products containing genetically modified cells to be transplanted into patients are considered to be biologic products requiring pre-marketing approval, and they are subject to the regulations discussed here."

Environmental Impact

While the environmental impact is important to the success of a project using naturally occurring biological systems, our project used E.coli for its ease of use and it's comprehensive characterization. It is intended that later iterations of BARTII would be housed in a different chassis. This chassis and any coded modifications would have to be evaluated before a formal analysis of environmental impact could be compiled.

Design of BARTII

From the information gained through patient, doctor and community research, as well as, that obtained through a rigorous evaluation of available data on Celiac Disease and the Gluten-Free diet we were able to propose a design for BARTII. Cells that removed and degraded gliadin and removed zonulin in the extra-membranous space would prevent interactions with the autoimmune responses that characterize the irritation associated with Celiac Disease. Also, it would reduce the possibility of potentially harmful responses, minimizing risks to the patient. E.coli is prevalent in the intestines of >99% of the worlds' population and is well-characterized. For this reason and its ease of use in replication of plasmids, as well as the minimized safety concerns, it was chosen as the chassis BARTII would be developed in. The cell-based therapy that arises from this choice of test-chassis is based on the understanding that its effects will be temporary and ingested orally like a probiotic. In this case, the probiotic can be lyophilized and enterically coated for transmission to the small intestine. Ideally, a version of BARTII that could be delivered in the form of a yogurt or beverage for patients who are unable to swallow pills would allow children and elderly patients access to the therapy. This pill form of treatment would be readily available as it has analogues already approved for other conditions by the FDA. Likewise, a pill with a shortened viability in the small intestine would reduce the treatment cost making the product available to a wider range of consumers. Ultimately, BARTII is a solution that meets the needs of the patients while considering the input given by the public and specialists.

Future of BARTII

Our current design encapsulates everything we have learned from our Human Practices research this summer, but there is room for improvement. As mentioned before, BARTII was designed as an e-coli cell because it is well-characterized, and it is easily modified. Though E.coli does exist in the small intestine, it does not do so for a prolonged period of time, nor is it immune to genetic shift. For this reason, future modifications of BARTII would likely be in another cell (i.e. lactobacillus or b. subtilius). For the sake of testing, all of the parts are independent, but, ideally, BARTII will become a single cell that performs all three functions. Ideally, BARTII will become a comprehensive cell-based therapeutic alternative to the Gluten-Free diet.