Design Brief

Harnessing synthetic microbial communities for gut health: A novel approach to addressing the impact of unhealthy diets on mental well-being*

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The rise of unhealthy dietary choices across America contributes to a concerning trend of poor mental health outcomes. Studies have shown that diets high in processed foods, low-quality carbohydrates, and saturated fats lower the diversity of beneficial dietary components in the gut, increasing the amount of unhealthy gut microbiota. This negatively impacts brain function, contributing to poor regulation of neuro-immune signaling and neurotransmitters, increasing the risk of conditions such as depression and anxiety. By restoring homeostasis within the gut microbiome and balancing pH discrepancies, it is possible to engineer and replicate synthetic microbial communities (SMCs/SynComs) to target short-chain fatty acids (SCFAs), which act as neural signaling molecules that can lead to the production of neurotransmitters, and their modulators, the GPR41 and GPR43 genes. The treatment plan includes administering a high initial dose of SynCom with a protective coating to fully establish itself in the gut and co-administering prebiotics selected to support the growth of specific bacterial strains within the SynCom. We will monitor gut microbial activity, dietary patterns, and potential health markers with capillary electrophoresis. This process involves regular analysis of the gut microbiome composition through stool analysis to track the SynCom's establishment and activity, alongside assessing overall gut health markers and patient symptoms to gauge intervention effectiveness. The next step is building a multi-stage capsule design that includes an outer enteric coating to protect the capsule from stomach acid and a pH-sensitive polymer layer that degrades only in the slightly acidic environment of the lower small intestine and upper colon, indicating potential gut dysbiosis. This targeted delivery mechanism ensures that the contents of the capsule are released in the specific gut region where they can be most effectively utilized. By engineering and delivering SynComs, individuals will be able to restore a healthy gut microbiome and modulate neurotransmitter production through the targeted release of beneficial metabolites to increase brain function and improve mental health.

Keywords: Neuroscience, gut microbiome, targeted drug delivery, microbiota modulation, synthetic microbial communities

In recent years, there has been an increasing awareness of the prevalence of mental health problems in society; mental and substance use disorders are now the leading cause of disability, and 70% of those suffering from a mental illness worldwide

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lack the necessary treatment options (Henderson et al., 2013).

Mental illness, or mental health disorders, is characterized by its ability to disrupt one's thinking, mood, and behavior for a prolonged period of time. The most notable examples include depression, different forms of anxiety disorders, eating disorders, bipolar disorders, schizophrenia, attention-deficit hyperactivity disorder (ADHD), and more. Such disorders may prevent an individual from having healthy relationships with others, functioning and performing daily activities, and maintaining happiness. Common symptoms of mental health disorders include constant sadness, inability to concentrate, detachment from reality, excessive feelings of doubt, worry, stress, anger or guilt, major changes in appetite, and suicidal thoughts (Mayo Clinic, 2022).

Mental health disorders are generally the result of problems with neurotransmission, the communication between neurons in the brain. Imbalances in neurochemicals in the synaptic gap between neurons can impact the number of neurotransmitters in the synaptic gap, thus affecting neurotransmission (National Institutes of Health, 2007). Neurotransmitters are chemical compounds released between a presynaptic neuron and a postsynaptic neuron that act as chemical messengers; examples of neurotransmitters include norepinephrine, dopamine, serotonin, glutamate, acetylcholine, GABA, and glycine. Each neurotransmitter carries out a specific function and allows for a particular response. For example, serotonin controls one's mood, wellbeing, and sexual desire, while dopamine controls satisfaction, motivation, and pleasure (National Institutes of Health, 2007).

Mental health disorders are generally the result of imbalances in neurochemicals (National Institutes of Health, 2007). This can be linked to imbalances in the gut–brain axis, which refers to the bidirectional communication between the central nervous system (including the brain) and the gastrointestinal (GI) tract, a structure that comprises organs responsible for digestion and absorption (Mitrea et al., 2022). The gut microbiome, which consists of the microorganisms that inhabit this environment, contributes significantly to the GI tract's function and health. Studies have revealed that certain dietary patterns can significantly alter the composition and function of the gut microbiome, leading to a state of dysbiosis (Shan et al., 2019). Unfortunately, methods such as changing one's diet only stimulate slow changes over time, and the result is an ineffective treatment for the immediate onset of an imbalance in gut bacteria when in dysbiosis. Additionally, changes in diet can bring extreme cravings, fatigue, headaches, and low energy. Certain life circumstances and influences such as age, family dynamics, socioeconomic status, and religious and cultural traditions can hinder one's ability to alter their diet (*Why is it so hard*, n.d.). Our project aims to improve the state of the microbiome and mental health without having to face such barriers; by improving the state of one's microbiome, one's mental health (which may make dieting difficult) is also expected to improve (Xiong et al., 2023).

Our proposed treatment plan aims to eliminate the struggles some people face when attempting to improve mental health through adopting healthy diets, and it involves administering a synthetic microbial community (SynCom) designed to target key metabolites and signaling pathways in the gut. SynComs are small consortia of microorganisms that are simple and defined in nature; these communities are used as models for studying functional, ecological, and structural aspects of native microbiota. SynComs have been applied in various fields, including crop resiliency, marine bacteria–plankton interactions, and inflammatory bowel diseases (van Leeuwen et al., 2023).

Additionally, when gut bacteria ferment soluble fiber from food, they produce short-chain fatty acids (SCFAs) as byproducts (Dalile et al., 2019). These SCFAs not only serve as an energy source for the gut microbiota but can also enter the bloodstream and reach the brain, acting as signaling molecules. In the brain, SCFAs bind to G protein-coupled receptors (GPCRs) expressed on neurons and glial cells, which are supportive cells for neurons. This binding triggers a signaling cascade that ultimately increases the production of certain neurotransmitters (Dalile et al., 2019). For instance, the SCFA propionate can bind to

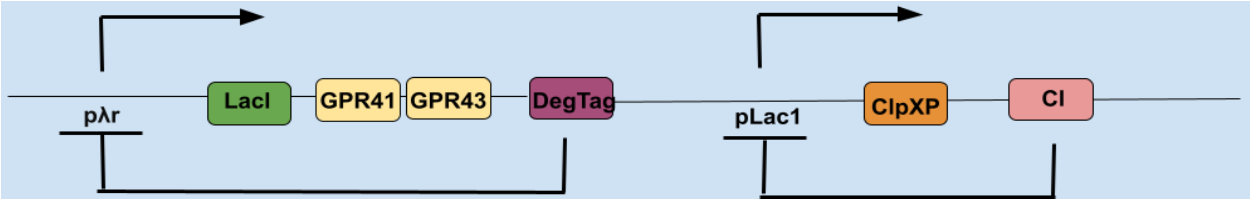

Figure 1. Inducible circuit describing parts of the pλR-LacI-GFP-LAA-pLac-ClpXP-CI construct (Lambert iGEM, 2016)

the GPR41 receptor found on both neurons and glial cells, stimulating the production of the neurotransmitter GABA in neurons and the release of acetylcholine from glial cells. Similarly, another SCFA, butyrate, can increase acetylcholine production by binding to the GPR43 receptor on neurons. Thus, the consumption of soluble fiber leads to SCFA production by gut bacteria, and these SCFAs modulate neurotransmitter levels in the brain by interacting with specific GPCRs on neural cells, which can potentially influence brain function and behavior (Dalile et al., 2019).

Specifically in this project, the SynCom will modulate SCFAs utilizing the GPR41 and GPR43 genes, aiding in the regulation of gut homeostasis (Ang & Ding, 2016). This gut balance ultimately results in an improvement in mental health. These engineered microbes will be delivered through a delayed-release capsule to ensure that their contents reach the gut microbiome unharmed from the acidic environment in the GI tract.

Systems level

Inspired by the Lambert iGEM 2016 project, our construct is a similar switch system that involves using a negative feedback mechanism to regulate the synthesis of SCFAs. To do this with *Escherichia coli*., we used the pLac-ClpXP-CI, a transcriptional regulatory system that can work in cooperation with other transcriptional regulatory systems, such as p-λ-r=LacI, to switch from one metabolic pathway to another, controlling SCFA production. (Lambert iGEM, 2016).

Before induction, the LacI gene produces lac inhibitor molecules that will bind to the pR11 (pLac1) promoter. This prevents the pLac promoter from driving the expression of ClpXP and the Cl, which are responsible for the feedback mechanism, aiding in the degradation process by unfolding and breaking down proteins. However, upon isopropyl β-D-1-thiogalactopyranoside (IPTG) induction, IPTG molecules will deactivate LacI repressor molecules, allowing the expression of ClpXP and the CI. The ClpXP degrades SsrA-tagged GFP and chromoproteins GPR41 and GPR43 (Lambert iGEM, 2016). The CI will block the p-λ-r promoter, which prevents the further synthesis of LacI repressor molecules and effectively functions as a genetic toggle switch. They would combine GPR41 and GPR43 with the p-λ-r part, then combine it with the rest of the inducible system (Lambert iGEM, 2016).

Device level

In our project, our device is based on two key composite constructs: a genetically engineered microbial consortium and a multi-layered capsule delivery system. The engineered microbes house an inducible circuit composed of pLamdaR-LacI and pLac-ClpXP-CI. This circuit strictly regulates the expression of proteins associated with GPR41 and GPR43 genes, which are modulators of SCFAs, to minimize the harmful effects of dysbiosis on gut–brain communication. This system will be delivered to the gut microbiota via a Eudragit L (Evonik Industries AG, Essen, Germany) (methacrylic acid-co-methyl methacrylate) capsule, which prevents circuit saturation by allowing for a gradual release within the intestines (PharmaCentral, 2022). This method allows the beneficial components of the capsule to withstand the highly acidic

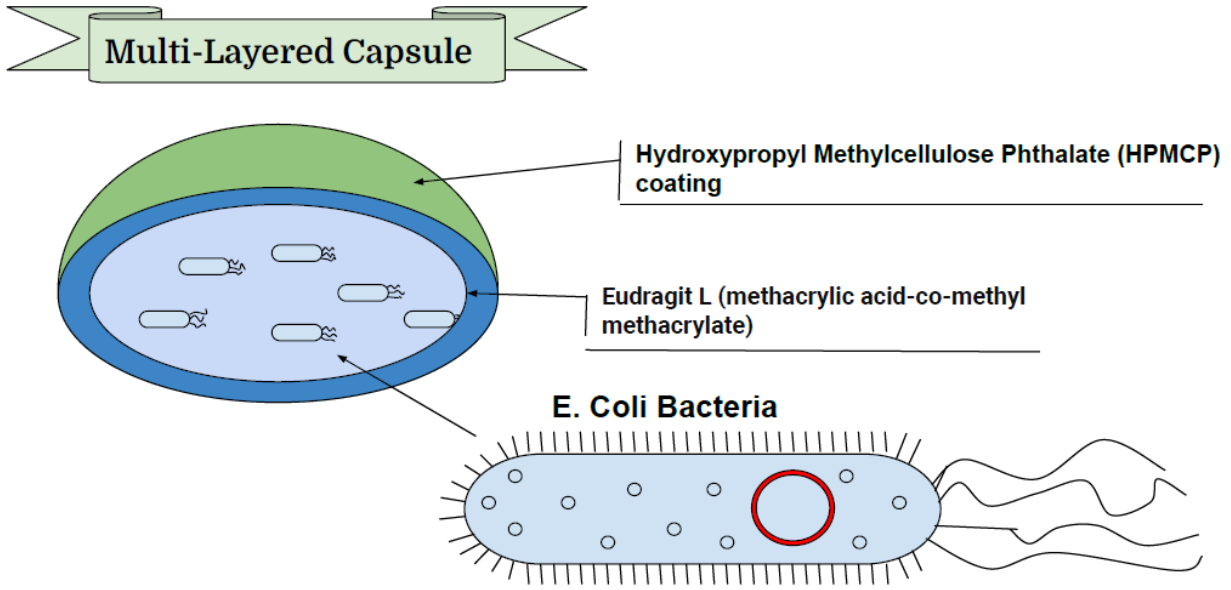

Figure 2. Multi-layered capsule design with integrated hydroxypropyl methylcellulose phthalate (HPMCP) and Eudragit L (methacrylic acid-co-methyl methacrylate)

environment in the stomach, helping to maintain viability and efficacy. Ultimately, through gradual release, beneficial microorganisms can reach their intended destinations in the digestive system before they are released in the body (Ortiz, 2024). Additionally, the outermost part of our capsule will be composed of a hydroxypropyl methylcellulose phthalate (HPMCP) layer—a white, odorless powder that serves as a cellulose derivative for enteric coating (*Hydroxypropyl*, n.d.).

Parts level

In our project, we plan to create two new parts utilizing the GPR41 and GPR43 genes. The GPR41 and GPR43 encode two mammalian GPCRs found in human adipocytes, colon epithelial cells, and peripheral blood mononuclear cells. These receptors are activated by SCFAs, which are generated through the fermentation of dietary fiber by gut bacteria in the digestive tract. These genes will be assembled into an inducible genetic circuit in which ClpXP will degrade proteins with degradation tags for recycling (Ang & Ding, 2016).

One composite construct, pLamdaR-LacI (part BBa_K1911000), enforces the inducible system (Lambert iGEM, 2016). The p-λ-r=LacI system is a transcriptional regulatory mechanism that can collaborate with other systems, such as pLac=CI, to transition between metabolic pathways (Andersen et al., 1998). For instance, when introduced into *E. coli* cells and triggered with IPTG, IPTG binds to the LacI repressor, enabling the system to switch pathways. There, the synthesis of competitive inhibitor-producing repressor molecules further inhibits the synthesis of LacI molecules and the GPR41 and GPR43 genes (Lambert iGEM, 2016).

Our second composite construct, pLac-ClpXP-CI, is a transcriptional regulatory system that can collaborate with other systems, such as p-λ-r=LacI (BBa_K19110000), to facilitate metabolic pathway switching (Lambert iGEM, 2016). For instance, when inserted into *E. coli* cells with the switch system pλr-LacI-eGFP-LAA-pLac-ClpX-ClpP-CI (BBa_K1911004), LacI repressor molecules initially inhibit the pLac promoter, preventing ClpXP and CI expression. Upon IPTG induction, LacI repression is lifted, allowing ClpXP and CI expression (Lambert iGEM, 2016). CI blocks the p-λ-r promoter, inhibiting LacI repressor synthesis and acting as a genetic toggle switch (Ahlawat & Morrison, 2009).

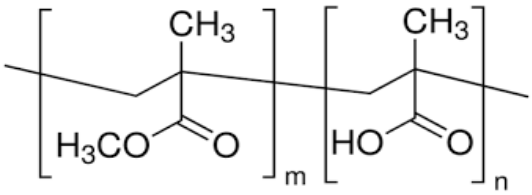

Figure 3. Chemical structure of Eudragit L (methacrylic acid-co-methyl methacrylate) (PharmaCentral, Materials and Knowledge Platform, 2022)

Safety

When working with microbial communities, it is crucial to prioritize safety throughout the entire lifecycle of the proposed system—from construction and testing to employment and deployment. During the construction and testing phases, biosafety protocols must be implemented to ensure our product is utilized effectively. This includes appropriate containment measures. All work with microorganisms should be conducted in a properly designed and maintained biosafety cabinet or other suitable containment equipment to prevent the accidental release of microbes. It is also required for all personnel to wear appropriate personal protective equipment (PPE), such as lab coats, gloves, and goggles, to minimize the risk of exposure to potentially harmful microorganisms.

The bacteria can also be ensured for safe consumption through two key mechanisms. The first is controlled release, in which we carefully plan and execute the release or deployment of the microbial communities (van Leeuwen et al., 2023). This ensures that the scale and location are appropriate and the potential for uncontrolled spread is minimized. Additionally, it is key that we employ monitoring and surveillance strategies. This will help track the behavior and impact of the microbial communities on the environment, allowing for early detection of any adverse effects.

There will also be safety concerns when working with patient stool specimens. Working with stool specimens in the process of analyzing SynComs' effectiveness and activity can sometimes cause the ingestion of eggs or cysts, skin penetration by infective larvae, and infection by nonparasitic agents found in stool and biological fluids. However, these risks can be minimized by adopting universal precautions as well as standard BSL-2 microbiological laboratory practices. Some examples include wearing protective safety glasses when processing specimens, decontaminating work surfaces daily, and covering abrasions on skin with adhesive dressings (Centers for Disease Control and Prevention, 2016).

Discussions

Our design outlines a promising approach to tackling the connection between poor diet and mental health through gut microbiome restoration. Some of the strengths of the plan include addressing the root cause because the plan focuses on restoring gut health and potentially addressing mental health issues stemming from unhealthy diets. With the creation of SynComs and their potential for targeted therapy, the proposed technology has the potential to introduce beneficial bacterial strains directly into the gut, maintaining internal homeostasis. In addition, the multi-stage capsule design for SMC implantation with a pH-sensitive layer ensures the implant reaches the optimal location in the gut for establishment (Yuan et al., 2023). Lastly, the plan and execution of regularly analyzing stool and monitoring gut health markers allows for adjustments to the plan as needed, allowing for a more targeted design, more efficient testing, and accurate results.

Some weaknesses need to be addressed before continuing the project. Specifically for the SMCs, the proposal mentions targeting SCFAs and the GPR41 and GPR43 genes. However, the gut microbiome is a complex ecosystem, and the challenge arises in determining the specific effects of SMCs on the specific pathways in the gut and whether the capsule will be able to reach the targeted genes of choice (Yuan et al., 2023). In addition, technical hurdles include the delivery and establishment of SynComs. The high initial dose with a protective coating to establish SynComs in the gut sounds promising, but ensuring consistent

colonization and avoiding rejection by the existing microbiome requires further development. Also, developing a multi-stage capsule design with specific pH-sensitive layers is complex and requires extensive testing to guarantee targeted release and functionality within the desired gut region. In addition, administering SynComs can impact the microbes already in the gut. For example, SynComes may compete with native gut microbes for nutrients and space, leading to shifts in the composition of the existing microbiome. Also, some SynComs members may produce antimicrobial substances or engage in other direct interactions with existing gut microbes, which may affect their survival and activity. SynComs can alter the gut environment by producing other metabolites or changing pH levels, which can, in turn, affect the growth and behavior of other microbes. Lastly, SynComs can interact with the gut immune system, influencing immune responses and indirectly affecting other gut microbes.

Currently, only one vague design has been proposed for the initial dose capsule, meaning there needs to be other methods of testing the initial design. In the context of actual drug administration and prescription, the proposed treatment plan may involve expensive technologies and specialized personnel, potentially limiting accessibility to a broader population. These higher expenses are not ideal for economically disadvantaged individuals suffering through mental health struggles. However, as further tests and leaps in treatment efficiency occur, we also hope to see a decrease in prices for these treatments, making them less experimental and more widespread. While the cost of transplanting SynComs to the gut is not established since this is still a developing field, some potential cost factors include the complexity of SynComs and how widespread of a therapy it is at the time of use. Also, there are different delivery methods. In this case, we are trying to administer a capsule, which will cost relatively less than a surgical or implant procedure. Given that SynComs are more defined and easier to produce the cost could be lowered. However, since this is still an emerging field, costs will be higher initially due to research and development. Lastly, since SMCs are a developing technology, further research must be conducted to establish long-term safety and efficacy, the scalability of the technology, and obtaining regulatory approval.

Next steps

Some next steps of execution include creating the plasmid itself and then cloning it using polymerase chain reaction (PCR), which allows for the DNA to be placed in the backbone vector with minimal limitations. PCR-based cloning makes a copy of a piece of DNA at the same time as adding restriction sites to the ends of that piece of DNA so it can easily be cloned into a plasmid of interest. Basic PCR primers for molecular cloning include the leader sequence for the restriction enzyme, the restriction site, and the hybridization sequence, which is where three primers bind to the sequence to start cloning. To select the restriction site, we will use a DNA analysis tool to identify the restriction sites in the sequence and use specific enzymes that will cut at the desired location on the plasmid. First, we will run PCR to amplify the inserted DNA using taq-polymerase and isolate the PCR product [*Plasmid Cloning by PCR (with Protocols)*, n.d.]. The digested DNA on an agarose gel will isolate the insert and conducting a gel purification will isolate the DNA. Afterward, DNA ligation will fuse the insert to the recipient plasmid with negative controls in parallel, showing how much background of uncut self-ligation recipient plasmid backbone there is compared to the ligation results to the control bacteria colonies. Lastly, we will proceed with transformation into the cells of our bacteria colonies, and we will isolate the finished plasmid and check if the transformation occurred accurately [*Plasmid Cloning by PCR (with Protocols)*, n.d.].

Dissolution testing is a crucial technique that can be employed alongside a microbial viability assay to comprehensively evaluate the solubility, degradation rate, and efficacy of the capsule formulation containing bacteria. The dissolution test determines the rate and extent of drug release from the dosage form under controlled conditions,

providing insights into the impact of the HPMCP coating thickness on the release kinetics (*Dissolution and Drug Release Tests*, n.d.). Concurrently, conducting a microbial viability assay would allow for an assessment of the effects of varying HPMCP coating amounts on the viability of the encapsulated bacteria (Stiefel et al., 2015). This combined approach would involve preparing capsules with different HPMCP coating levels and subjecting them to conditions that mimic the GI tract.

The dissolution test would reveal the release profile of the active ingredients, while the microbial viability assay, performed at various time points, would quantify the number of viable bacteria released from the capsules under different coating conditions (Stiefel et al., 2015). By correlating the dissolution data with the microbial viability results, we can optimize the formulation to achieve the desired solubility, degradation rate, and protection for the encapsulated bacteria, ensuring their viability and efficacy upon delivery to the target site. Some future improvements of this design include adding initial animal and cell tests to gather enough data to start clinical trials. The clinical trial design will be like a randomized controlled trial with a random control group to assess the efficacy of the intervention compared to a standard treatment approach. The new design should also incorporate the long-term effects of such an intervention. For example, overall administration patterns should be considered, such as whether the first initial dose is enough or if the patient has to take the capsule multiple other times in a certain period. Also, the gut microbiome is unique to each individual. While SynComs offer a standardized approach, the proposal should focus on personalizing the treatment based on individual gut analysis, allowing for a more targeted approach. The proposal also needs to be readdressed by looking into potential safety concerns and side effects associated with SynComs and high-dose prebiotics. Lastly, other issues, such as the costs of manufacturing SynComs in a widespread manner, should be considered in a revised proposal for the initial administration of the capsule, allowing for a well-rounded approach to addressing mental health from a gut perspective.

Author contributions

E.K., K.S., Y.X.L, and S.V. contributed to writing the Abstract. E.K., K.S., and Y.X.L contributed to the Keywords, Background, Systems Level, Device Level, Parts Level, Discussion, and Safety of the article. E.K. recorded the video. W.H.T.'s contributions included writing the Author Contributions, Acknowledgments, and References. K.S. and W.H.T. contributed to the original images. Y.X.L is the original author on gut and mental health and, along with K.S. and E.K., developed the plan to design GPR41- and GPR43-targeting SynComs. V.K., E.K., Y.X.L., K.P., S.R., A.S., K.S., W.H.T., S.V., and V.V. all contributed to the development of the SynComs for the gut health proposal through their research on GPR41 and GPR43 genes, SynComs, the capsule-release system, SCFAs, and homeostatic conditions in the gut, all of which made this design possible.

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