Yo Suzuki, Ph.D. Updates

Update on 4-11-18 *(6 Month Progress Report)*

Executive Summary

The purpose of this project is to test to see if skin bacteria that respond to glucose levels and produce insulin can be engineered and introduced into laboratory animals to reduce their blood glucose levels, as a precursor to establishing engineered skin bacteria as painless substitutes for glucose sensors and insulin pumps. During the first six months, we made rapid progress and established genetic tools for engineering skin bacteria. This accomplishment sets the stage for the second phase of the project where expression modules for insulin genes and regulatory mechanisms for sensing glucose will be developed.

Productivity

The work performed is summarized under the aims proposed for the project.

Aim 1. Establish genetic engineering tools in selected deep skin bacteria.

Completed.

We collected six Gram-positive and three Gram-negative strains of bacteria isolated from skin samples. We determined the minimal inhibitory concentrations for nine antibiotics commonly used for genetic engineering. This test revealed that there are many strain-antibiotic combinations that can be used in our study. The Gram-positive strain Staphylococcus epidermidis ATCC12228 was the only strain tested among the nine strains for the capacity to re-enter the skin (unpublished result, R. Gallo). Therefore, we focused our resources on this strain. Gram-negative bacteria have been noted to be more proficient at proper folding of secreted proteins derived from other organisms, although there are examples where Gram-positive cells were used for making and secreting insulin. We decided to keep the Gram-negative strains as backup strains and continue to acquire genetic engineering tools for them. This marks the attainment of an initially proposed milestone (selection of strains).

We previously developed a genetic engineering approach where synthetic DNA fragments loaded with an enzyme called transposase in vitro is introduced into bacteria. Transposase facilitates the integration of DNA fragments into the genome. Because this approach is effective in a wide variety of organisms, we tested it in the S. epidermidis strain with limited tools. To cost-effectively perform this procedure, we purified transposase and confirmed the activity of the purified enzyme. We then used the enzyme and succeeded in introducing a DNA fragment only containing a puromycin resistance gene into the S. epidermidis strain. The positive result was obtained when the DNA sample was pretreated with the lysate of the organism. The idea was that methylases in the lysate generated the methylation pattern on DNA found in the native organism so that the incoming DNA was accepted as its own DNA.

Aim 2. Implement a biocontainment measure.

In progress.

Before we engineer the capability to express and secrete insulin in the S. epidermidis strain, a mechanism for biocontainment needs to be implemented, as an organism capable of expressing human insulin would be a hazard to laboratory workers, should they become infected. We are attempting to use CRISPR genome editing to knock out the thyA gene needed to make thymidine, an ingredient for DNA, to make our bacterial cells dependent on thymidine supplied from outside and keep the cells within designated culture tubes.

Aim 3. Express SCIs in deep skin bacteria.

In progress.

Recent studies have resulted in single-chain insulin analogs (SCIs) that match native insulin in potency (Hua et al., 2008, J. Biol. Chem. 283:14703-14716). In beta cells, proinsulin is folded and cleaved to produce biologically active insulin, which consists of two peptides that are linked by disulfide bonds. Because native bacteria cannot cleave proinsulin, SCIs are essential for the strategy of cell-intrinsic and self-sufficient production of active insulin within bacteria. We are currently designing expression constructs incorporating the published SCI-57 design (Hua et al., 2008).

Aim 4. Evaluate the capacity of bacterially produced SCIs to stimulate glucose uptake in adipocytes.

Not started.

We plan to test whether the bacterially produced SCIs are biologically active by treating mouse adipocytes with the bacteria in a glucose uptake assay. We expect this work to be started as soon as the SCI-producing strains are made. We have culture cell expertise needed to prepare the adipocytes.

Aim 5. Evaluate the ability of SCIs to reduce blood glucose in mice after application of the engineered bacteria to the mouse skin.

Not started.

We plan to determine if the SCI-producing bacteria can colonize the skin of a mouse to result in a reduction of blood glucose levels in the mouse. We will prepare for this work when the SCI-producing bacteria are made.

Aim 6. Implement a glucose-mediated regulation of SCI production.

Not started.

For effective blood glucose control, it is critical that SCI production be adjusted based on glucose concentration. We initially proposed to use a glucose sensor in Gram-negative bacteria, but we decided to focus on a Gram-positive strain. We will start researching mechanisms available in Gram-positive bacteria to enable a smooth transition to this phase of research.

Synergistic Activity

The Diabetes Research Connection grant enables the critical first step toward a microbial treatment for diabetes that circumvents the struggle of injected insulin therapy. The preliminary data obtained in this project were incorporated into a grant preproposal submitted by J. Glass (JCVI) to the Larry L. Hillblom Foundation. We have identified a suitable NIH grant mechanism (PAR-18-434) to further develop our approach. We also attended the 2017 Chemical and Biological Defense Science and Technology Conference in Long Beach, California sponsored by Defense Threat Reduction Agency. Skin biology is an important aspect of their portfolio, and we received useful feedback on our research from experts.

Update on 2-06-18

Last time I told you that I purified an enzyme called transposase to be used for facilitating a process called transformation, to put engineered DNA constructs into cells from outside. It turned out that the transformation step was still not easy for skin bacteria, but by communicating with scientists from Australia and multiple universities in the US working on related bacteria of the Staphylococcus species, I got better at the process. I started getting colonies, or dots in a Petri dish each originating from a single transformed cell. Tests confirmed that these colonies had the DNA material I introduced into the cells. With this process being established, the next step for me will be to knock out a gene (thyA gene) needed to make an ingredient for DNA, to make our bacterial cells dependent on the ingredient (thymidine) supplied from outside, so that we can keep the cells at designated sites like a culture flask (as opposed to my skin). Also, the time is right for making a DNA construct for expressing insulin to be introduced into the skin bacteria. Designing DNA constructs is what I love the most. I am grateful that your support enables us to proceed to this phase of the project.

Update on 11-22-17

Toward my aim 1 goal of establishing genetic engineering tools for skin bacteria, I wanted to test the approach of introducing synthetic DNA fragments loaded with transposase in vitro. Transposase facilitates the integration of DNA fragments into the genome. I believe that this is a widely applicable approach that is suitable for our project with multiple target organisms to engineer. For optimizing this process, I would need quite a bit of transposase, but this is an expensive reagent if you buy it. Just 10 µl of it costs $500, and you can use up all 10 µl in one experiment. Therefore, I decided to make my own preparation of transposase. I received a DNA construct containing a transposase gene that can be expressed in E. coli from a research group in

Sweden. I introduced this construct into an E. coli strain suitable for recombinant protein production. I induced transposon production, lysed the cells, and purified the protein from the lysate using an affinity chromatography column. I confirmed the activity of the purified transposase using a standard assay in our laboratory. I succeeded in this process and obtained an amount of transposase worth $93,000. Encouraged by this result, I am now tackling establishing transformation protocols for skin bacteria.