Final Report

Needles Be Gone for T1D

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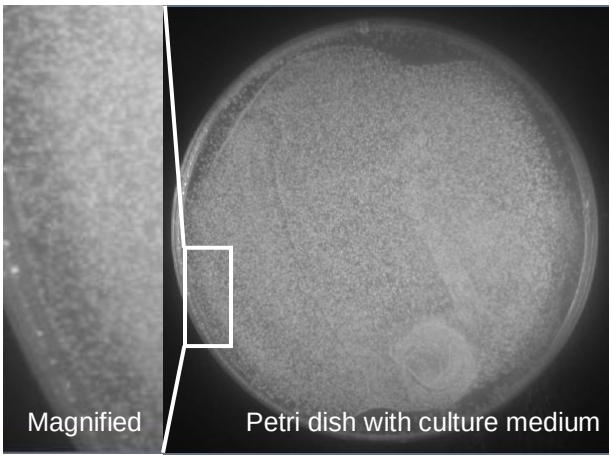
The ultimate goal of this project is to develop a better treatment for diabetes than sub-cutaneous injection of insulin to manage patient blood glucose levels. Our concept is that the bacteria that naturally reside in the deepest levels of human skin as part of our microbiome could be repurposed to serve as surrogate beta cells that would sense blood glucose levels and secrete an insulin analog in response to hyperglycemia. Our DRC project was intended to show proof of principle that skin bacteria could respond to blood glucose levels and produce insulin and thus serve as painless substitutes for glucose sensors and insulin pumps to cope with type 1 diabetes. We achieved our goal to obtain preliminary results or assessments of feasibility for all proposed aims as described below. Our primary accomplishment is the generation of insulin-secreting skin bacteria (aim 3).

Aim 1. Establish genetic engineering tools in deep-skin bacteria.
We selected *Staphylococcus epidermidis* ATCC12228 as our platform strain and became proficient at engineering this strain. This bacterium has been tested to enter the deep layers of skin when supplied from outside in the laboratory.



Strain	Culture medium	Number of colonies
RP62a (reference)	BHI	146
ATCC 12228	TSB	1,836
ATCC 12228	**BHI**	**15,040**

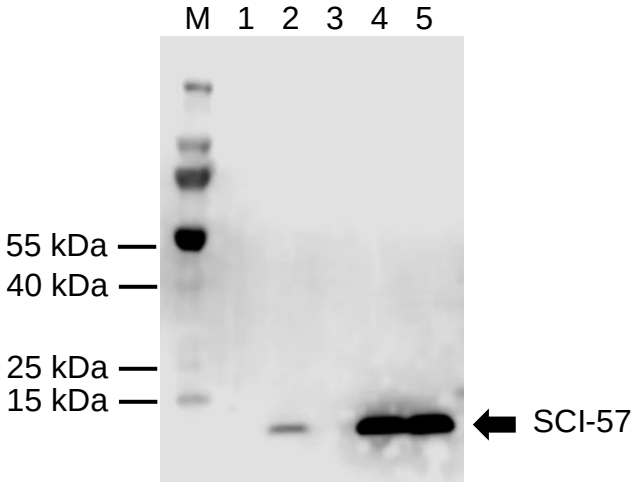
In this example, a DNA construct (not carrying the insulin gene yet) was introduced to the indicated strains of *S. epidermidis* using a technique called electroporation. Before this step, the DNA was propagated in a special strain of *E. coli* and modified so that it would be recognized by *Staphylococcus* species as its own. Only *S. epidermidis* cells that take in the DNA would grow as colonies in the presence of a certain antibiotic due to the resistance gene on the DNA. Colonies are dots in a Petri dish each representing roughly one million cells derived from a single parental cell that initially takes in the DNA and lands at a site in the Petri dish. We were excited to have reproduced the work of others with the reference strain RP62a and to show that the efficiency of DNA introduction was 100 times higher with our strain.

Aim 2. Contain bacteria within designated sites.

Because skin bacteria that are making large amounts of insulin constitutively might possibly be toxic to experimentalists, we made them safe before we started work. Initially we wished to use genetic engineering and make the bacteria dependent on a nutrient supplied from outside, but our first attempt at this indicated that we needed to develop new methods to achieve the goal in *S. epidermidis*. Therefore, we took a simpler approach of installing the insulin analog gene in *S. epidermidis* on a small circular DNA molecule called a plasmid that would only be retained by the bacteria so long as we expose the cell to an antibiotic (as in aim 1) that would kill the cell if the plasmid was not present in the cell. This way if a researcher was infected with the bacterium, the plasmid with the insulin analog gene would quickly be lost and the scientist would not risk hypoglycemia caused by the *S. epidermidis* bacteria.

Aim 3. Express insulin in deep-skin bacteria.

We made DNA constructs for expressing and secreting a single-chain insulin analog (SCI-57) that has been shown to match native insulin in potency. We then introduced these DNA molecules into skin bacteria. Using a technique to separate proteins within samples as bands on a gel, make a copy of the bands on a special membrane, and stain a specific protein on the membrane by virtue of an antibody specific to that protein, we detected the production and secretion of insulin analog SCI-57 from skin bacteria.



Small amounts (10 µl) of the medium surrounding skin bacterial cells were analyzed. M: size marker, 1: negative control cells with a construct not containing the insulin gene, 2-5: colonies from experiments with SCI-57 expression constructs. The bacterial cells forming the colony analyzed in lane 3, where there was no sign of the SCI-57 protein, probably did not have the plasmid with the SCI-57 gene.

Aim 4. Test to see if bacterially produced SCI stimulates glucose uptake in adipocytes.

We were unable to complete this work within the timeframe of the project, but we procured some of the needed materials. As soon as the adipocytes are ready, we will be able to conduct the experiment. We will update the DRC on the result.

Aim 5. Test to see if the engineered skin bacteria can reduce blood glucose in mice.
The next test would be to use a mouse and determine if the SCI-producing bacteria can colonize the skin and reduce blood glucose levels. We will need more time to make our bacteria suitable for this test. The DNA molecules we build for making SCI-57 probably will need to be stably introduced into skin bacteria by incorporating the insulin analog genes into the bacterial chromosome rather than having it on a small DNA plasmid as it is currently. If we make this change, we will need to revisit aim 2 to make the cells stably carrying the insulin constructs safe. We will update the DRC on our future experiments.

Aim 6. Implement a glucose-mediated regulation of SCI production.
For effective blood glucose control, it is critical that SCI production be adjusted based on glucose concentration. We initially proposed to use an established biological sensor for glucose in Gram-negative bacteria like *E. coli*, but the organism we selected is not of this type. We did some literature research, but it was not obvious which mechanisms would work well. At this point, we reached out to an expert of genetic circuits, Drew Endy at Stanford University. Work is underway in his laboratory to test several candidate sensors.

The generous grant from the DRC enabled the critical first step toward a microbial treatment for diabetes that circumvents the struggle of injected insulin therapy. We reached a milestone to demonstrate deep-skin bacteria producing and secreting insulin. Moreover, the knowledge we gained and the materials we generated with the DRC support will strengthen our next efforts, which are now clearly defined because we experienced aspects of the work already. Securing funding for the subsequent work was also an important goal. Regarding this, we are happy to report that our skin bacteria work is part of a project headed by our team member John Glass to be funded by the Larry L. Hillblom Foundation. Therefore, our future efforts will become a reality.