Yeast-based Biosensor for Detection of Interleukins in Human Sweat

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Today, chronic inflammatory diseases (CID) are determined to be the leading cause of deaths worldwide. People suffering from CIDs need to monitor their disease progression regularly. Current inflammation detection methods are invasive and require frequent hospital visits. We aim to utilize the advances in synthetic biology to design a sweat collecting skin patch as a non-invasive, use-at-home biosensor for CIDs. The patch will contain genetically modified yeast capable of responding to interleukins (IL) in sweat. The yeast will be cloned with a modified human IL-receptor and upon ligand-receptor binding, a signal will be translated into the yeast pheromone pathway via a modified G-alpha mechanism. The pheromone pathway will induce the transcription of a visible color pigment. We envision our design as a model platform for future development of non-invasive monitoring devices for CIDs as well

Index Terms- iGEM, chronic inflammatory diseases, engineered yeast, interleukins, biosensor, G-alpha, colour change

I. INTRODUCTION

Chronic inflammatory diseases (CIDs) are determined to be the leading cause of deaths worldwide [1]. Estimations attribute over 50% of premature deaths to CIDs and this number is predicted to increase according to WHO [2,3]. They are life-long, debilitating illnesses, where patients can suffer from pain, fatigue, swelling and fever. Some examples of CIDs are Rheumatoid Arthritis, Crohn's Disease, and Inflammatory Bowel Disease.

Treatment of CIDs focus on the alleviation of symptoms. However, due to the complication of the disease profiles, treatments do not always work and usually there is a long searching process for the right treatment. Monitoring tools to follow the diseases and assess treatment are essential but current tests are time consuming and not always available or accessible, causing a reduction in the quality of life.

Diagnosis of CIDs is very complicated due to the difference in biomarker profiles for each individual patient. However, in all CIDs, the level of inflammation is high, which is commonly treated with various medications. Similar biomarkers, such as interleukins (ILs), are found in many illnesses. It is only the level of these interleukins that vary among the different CIDs. Making a diagnostic tool from their levels require extensive research and pricesness. However, for all CIDs, the monitoring of these *general* inflammatory markers is of interest for following disease progression and treatment efficacy. Some testing methods exist, such as specialized blood tests. However, home-safe non-invasive test are still new to the field of inflammation monitoring. Research regarding the existence of biomarkers in sweat demonstrate the utility for using sweat biosensors [4]. Inflammatory biomarkers are present in sweat and correlates to the levels found the bloodstream [5]. Thus, sweat is a promising tool for non-invasive testing. Here we will explore the utility of GMOs as a sweat-based biosensor tool, by engineering yeast cells to respond with a color signal to interleukins in human sweat.

II. RESEARCH ELABORATIONS

In order to detect the inflammatory biomarkers in sweat, the endogenous human interleukin receptors are cloned into yeast as the source of specialized detection [6]. Our research provides the necessary information for succesfull integration of human IL receptors into genetically modified yeast cells and outlines the functionality of the organism as a biosensor tool.

E. Research Design

Interleukin receptor modification

Most interleukin receptors fall into the category of receptor tyrosine kinases, and as such, many interleukins require the binding of both a primary and an accessory receptor for signal relay. We will test this dimerization of the two receptors by using the tried and tested yeast two-hybrid method, using split-ubiquitin to see how our extracellular domains interact. To this end, we will fuse the extracellular domain of a human interleukin receptor and its accessory protein to endogenous yeast transmembrane proteins, and then again to a one part each of a split TEV protease intracellularly.

Hijacking the pheromone pathway

The amplification and transduction of the signal to our receptors will happen through modification of the regular pheromone pathway in yeast. Normally, it is the beta/gamma complex that relays the receptor signal. We will inhibit the beta/gamma complex in the resting cell by using a modified G alpha protein, which will have TEV recognition sites inserted into its sequence. Upon reception of interleukins, our extracellular receptors will bind to their ligand, and the intracellularly fused split TEV-protease will reconstitute. This reactivated TEV protease can then recognize the cleavage sites in G alpha and cut this inhibition away, thus, rendering the remaining beta/gamma

complex able to serve as a starting point for the pheromone pathway.

Signal transduction via color expression

Triggering the pheromone pathway will result in the activation of a transcription factor, which will promote transcription of a gene encoding a pigment (betanin). Through this, our biosensor will produce a clear, visible color when the inflammation levels are high.

III. METHODS AND MATERIALS

In order to accomplish the development of a biosensor design with the capacity to relay a significant signal from the interleukin concentration in sweat, we have designed two methods of signal propagation. Here, we use computer simulations on MATLAB and Rosetta to compare the applicability of the different designs.

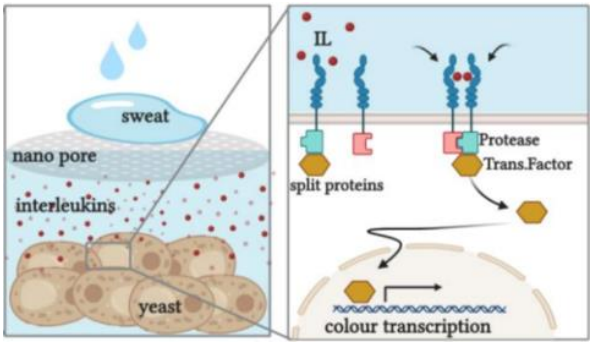

Figure 4: Illustration of split-protein design

In the first design, the receptor-ligand binding causes a split-ubiquition or TEV protease to release a transcription factor (*Figure 1*). This will be accomplished by cloning the yeast with the receptors and making a split ubiquitin assay. Modeling techniques based on ODE (ordinary differential equations) showed that these two designs are very similar in terms of dynamic ranges. However, from our ODE studies, we found that these designs do not have the necessary sensitivity to respond to physiological concentrations of interleukines. In other words, in order for the biosensor to be functional, a different design is needed.

Our second design incorporates the signal amplification from the endogeneous pheromone pathway. The cascade that is part of this pathway leads to a phenomenon known as hypersensitivity, i.e. very high levels of response even with very small initial concentrations.

The simplicity of the split-ubiquitin design makes it extremely useful for initial assays analyzing the functionality of our cloned interleukin receptor. However, after the success of this assay, the pheromone pathway design will be necessary for sufficient signal transduction.

IV. RESULTS AND DISCUSSION

The presence of inflammatory biomarkers in sweat makes it a promising tool for monitoring of chronic inflammatory diseases. *Figure 2* shows the dynamic range of this design, and the correspondence with physiological concentrations of interleukins can be seen (i.e. nanomolar concentrations).

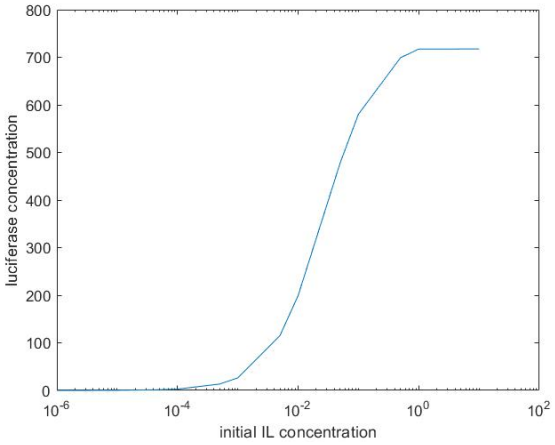

Figure 5 : Dynamic range of the first two designs (split ubiquitin and split TEV protease).

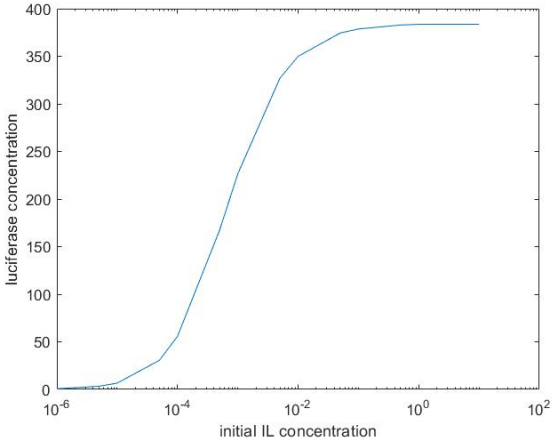

Figure 6 : Dynamic range of the design based on the pheromone pathway.

V. CONCLUSION

Our modified yeast can not only be used for a sweat biosensor but is a valuable tool for research in diagnostics and management of diseases related to inflammation, both to biomedical researchers and future iGEM teams. As a cheap, sustainable source of biomarker detection, it can provide the basis for research into CIDs across the world and potentially aid in the development of new medical discoveries within the field. All this is enabled by engineering signaling pathways that provide significant signal amplification, and based on our simulations, the pheromone pathway seems to be a good candidate for achieving functional biological implementation.

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