Cell Based Biosensors

Living cells as active sensing elements

Overview

Living cells, in a manner analogous to the canaries once used to detect toxic gases in coalmines, respond to unknown toxins, gases, etc. in a physiological relevant manner. In this work, molecular biology and genomic-scale technologies are being used to create sensor arrays that utilize living cells as active sensing elements. We are generating cell lines that uniquely fluoresce in response to a variety of target agents. In addition these cells will be patterned /printed onto a variety of substrates including MEMS fluidic architectures, insects, or micro-robots for remote sensing.

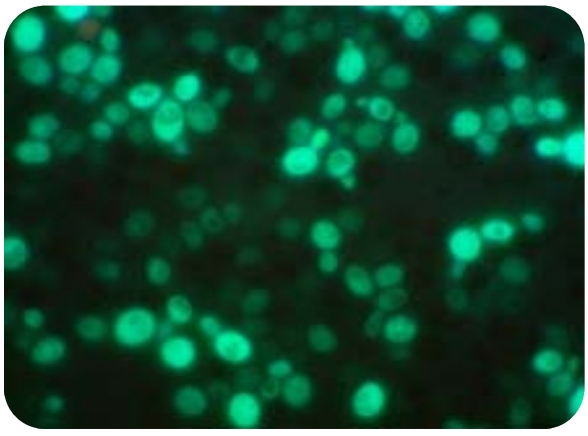

Figure 1. Genetically engineered cells fluoresce (green fluorescent protein, GFP) in response to the binding subunit of cholera toxin.

Current designs of whole-cell biosensors have not taken advantage of the complexities and dynamics of the cell to make "flexible" sensors. A cell will respond to numerous stimuli (chemical, biological, nuclear) and therefore biosensors incorporating whole cells should allow detection of multiple analytes. The use of microarray analysis to identify promoters permits an unbiased screen – leading to maximal robustness of sensor design. Although current whole-cell biosensors have yet to expand upon this asset, our sensor will potentially detect many stimuli, simultaneously. In addition, by fusing genetics with molecular biology, we are able to impart to the cell-based sensor a dynamic range of capabilities including amplification and resetting of the sensor an important component for continuous monitoring of weapons, etc.

Approach

Microarray analysis, utilizing the complete Saccharomyces cerevisiae (yeast), genome, was used to identify sub-sets of promoter genes that are regulated in response to external stimuli such as cholera toxin. Yeast cells were grown tostationary phase. In this phase, yeast can survive for months or years in various environmental settings. Groups of these cells were exposed to our target agents; cholera toxin (the binding subunit) and a supernatant from media containing anthrax

simulants Bacillus subtilis and Bacillus thuringensis. After exposure, the cells were lysed and microarray analysis was used to determine changes in gene expression in the cells in response to the target agent.

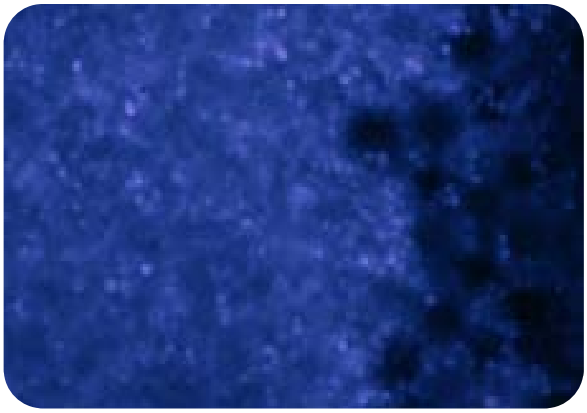

Figure 2. Response of cells (blue fluorescence) to environmental stimulus in a MEMES fluidic channel.

Essentially DNA microarray technology allows us to know the entire compliment of genes that is induced in yeast as a result of a particular stimulus. These expressions are characteristic to a specific stimulus—a fingerprint. VxInsight analysis (a visualization software package developed at Sandia by George Davidson) was used to quickly identify genes whose induction was specific for one or more agent stimuli versus those whose regulation was more general. We then selected three gene promoters that were specifically triggered by an exposure to cholera toxin. The selected promoters were fused into DNA plasmids containing a gene encoding for GFP, CFP, or RFP (red fluorescent protein) and introduced into yeast cells to create the multiple cell lines, each encoded with a unique genetic response pathway. Upon exposure to the targeted compound (cholera toxin) promoter genes are activated, initiating expression of a fluorescent protein.

For additional information or questions, please email us at BioNano@sandia.gov.

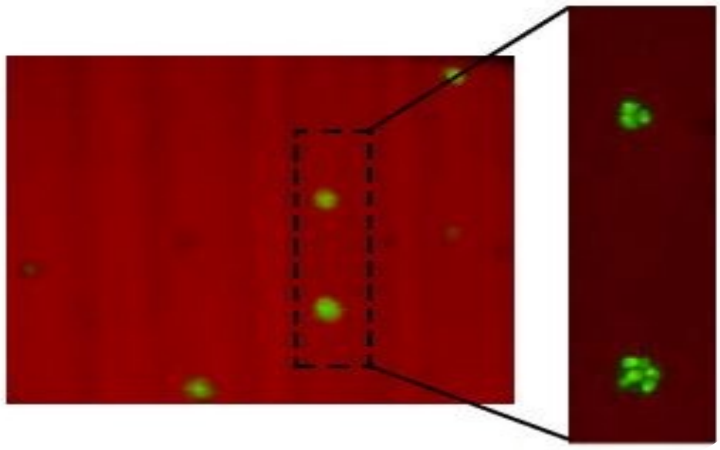

Figure 3. Optically patterned array of cells in biocompatible sol gels expressing GFP.

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