BIOLUMINESCENT BIOREPORTER INTEGRATED CIRCUITS (BBICS): WHOLE-CELL ENVIRONMENTAL MONITORING DEVICES

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INTRODUCTION

The bioluminescent bioreporter integrated circuit represents a new advance in the development of whole-cell biosensors. These devices consist of a genetically engineered bioreporter organism interfaced with an integrated circuit (Fig. 1). The bioreporter is engineered to luminesce when a targeted substance is encountered, while the circuit is designed to detect the luminescence, process the signal, and communicate the results. The chief advantage of this approach is that the entire sensor, including all signal-processing and communication functions, can be produced as a single-chip, low-power, rugged, inexpensive device. We envision these devices being used in a variety of distributed sensing and environmental control systems. Future work will focus on using more information processing capabilities of the cells to create highly functional sensing/computing/actuating devices.

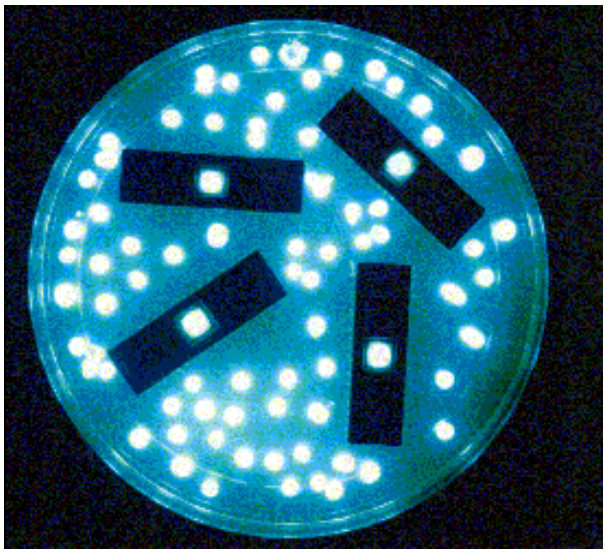

Fig. 1. This photograph shows maximally-induced bioluminescent bacteria on a CMOS microluminometer to form bioluminescent bioreporter integrated circuits. All the light for this photograph was provided by the bacteria (45 minute exposure time).

CURRENT STATUS OF RESEARCH BIOREPORTERS

We have on-going efforts to develop several bioreporters. In particular, we are developing: (1) an ammonia bioluminescent biosensor constructed using the bacteria *Nitrosomonas europaea;* (2) An estrogen bioluminescent reporter using an existing beta galactosidase bioreporter (the beta galactosidase (*lacZ*) reporter gene will be replaced with a fused *luxAB* gene cassette); and (3) bioluminescent reporters for the herbicide 24D, dinitrotoluene, and blood glucose concentration.

CELL ENCAPSULATION

Our current work has concentrated on sol-gel as the primary encapsulation medium. Utilizing sonication methods, we have been able to initiate polymerization under pH conditions conducive to cell survival. Both a toluene bioreporter (*Pseudomonas putida* TVA8) and a naphthalene bioreporter (*Pseudomonas fluorescens* HK44) have successfully been encapsulated in sol-gel and shown to produce bioluminescence when exposed to their specific inducers. However, difficulties remain in the prevention of cracking and drying within the thin sol gel matrices after polymerization.

Alternatively, a common alginate polymerization matrix is also being studied for on-chip applications. To increase mechanical stability, we are currently encasing alginate encapsulated cells within 0.1 μm low adsorption/absorption filter membranes and hollow

fiber membranes which will allow for influx of chemical analytes while inhibiting alginate degradation and cellular release into the surrounding medium. We are also attempting to lyophilize these structures to increase long term storage capabilities.

MICROLUMINOMETER

Fig. 2 shows a photograph of the complete microluminometer chip. The chip measures 1.9 mm × 1.9 mm with the photodetector occupying ~ 25% (1.2 mm^2) of the total chip area. For testing purposes the chip was mounted in a 40-pin ceramic dual inline package. Bioluminescence was determined for cultures containing different concentrations of *P. fluorescens* 5RL cells growing in LB supplemented with 10 ppm of the inducer molecule salicylate and 14.7 mg/L tetracycline. Bioluminescence was determined using the integrated circuit microluminometer and a light-tight enclosure mounted above the chip. Linear regression analysis showed that the data fit a linear model indicating that bioluminescence per cell remains constant for cell concentration ranging from 4 x 10^5 to 2 x 10^8 CFU/mL and for detector responses ranging from 0.05 to 20 pA. Using a linear model, the limit of detection (2 σ) for this experimental geometry was estimated to be 4 x 10^5 cells per mL. The results obtained with the BBIC microluminometer were compared with results collected with the Azur PMT-based luminometer at each cell concentration. The data showed that the measured bioluminescence responses were proportional for cell concentrations ranging 4 x 10^5 to 2 x 10^8 CFU/mL, indicating that the BBIC microluminometer gave consistent results to standard PMT-based detection systems.

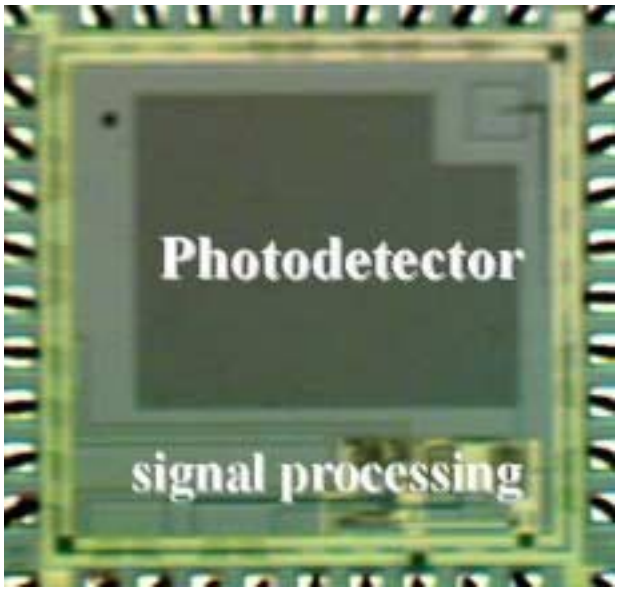

Fig. 2. The microluminometer integrated circuit.

FUTURE WORK

The focus of our future work will be to enhance the capabilities of whole cell bio-microelectronic devices and systems by engineering more functionality (logic gates, interconnectivity, group behavior, etc) into the cells.

â€œWholeâ€”Cell Bioluminescent Bioreporters For The. Detection Of Biogenic Amines In Food. â€ Presented At The.Â Bioreporters are genetically engineered organisms chemicals in the environment via production of visible light. designed to detect speci?c compounds by incorporating a. gene responsive to a selected external compound, for. Detection of pathogenic organisms, as opposed to chemi. Bioluminescent bioreporter integrated circuits: potentially small, rugged and inexpensive whole-cell biosensors for remote environmental monitoring. J Appl Microbiol. 2004;96(1):33-46. doi: 10.1046/j.1365-2672.2003.02114.x.Â Affiliation. 1 Center for Environmental Biotechnology, University of Tennessee, Knoxville, TN 37996, USA. By interfacing bacteria to integrated circuits, NASA-supported researchers have created a device that can sense almost anything. Like a canary in a mine, a microbe can often sense environmental dangers before a human can. It's easy to see a canary's reaction. But how can you can you tell what a microbe's feeling? How can you coax a microbe to communicate? One way is to interface it to a silicon chip.Â The researchers have already used these devices, known as BBICs, or Bioluminescent Bioreporter Integrated Circuits, to track pollution on earth. Now, with the support of NASA's Office of Biological and Physical Research, they're designing a version for spaceships. Right: Glowing colonies of microbes. Whole-Cell Environmental Monitoring Devices: Bioluminescent Bioreporter Integrated Circuits. August 2000. SAE Technical Papers 762:197-205. DOI: 10.1021/bk-2000-0762.ch014.Â Bioluminescent bioreporter integrated circuits (BBICs) are novel biosensor devices that utilize light emitting microorganisms as biological sensors for chemical contaminants. The microorganisms are coupled to a full-custom integrated circuit containing photodetector units and low-noise signal processing circuitry for measuring and communicating chemically induced cellular light responses. Two prototype BBICs have been designed and tested using naphthalene and toluene sensitive bioluminescent bioreporters. The bioluminescent bioreporter integrated circuit (BBIC) can be developed in which a bioreporter is engineered to luminescence when target substrate (arsenite) is encountered, while the circuit detects the luminescence and after processing the signal, the results are communicated by remote sensing.Â Cell- Based Sensing Systems or Bioreporters Whole cells as the sensing element offers some unique advantages compared with isolated proteins, whole cells are often less susceptile to changes in environmental conditions such as pH and temperature and the presence of other solutes.Â The light is absorbed by a photodiode array on the device resulting in the generation of photocurrent, which is proportional to the intensity of the light.