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.

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 \textit{Nitrosomonas europaea}; (2) An estrogen bioluminescent reporter using an existing beta galactosidase bioreporter (the beta galactosidase (\textit{lacZ}) reporter gene will be replaced with a fused \textit{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 (\textit{Pseudomonas putida} TVA8) and a naphthalene bioreporter (\textit{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 \textmu m low adsorption/absorption filter membranes and hollow

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).
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.

MICROLMINOMETER

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 mm²) 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 10⁵ to 2 x 10⁸ CFU/mL and for detector responses ranging from 0.05 to 20 pA. Using a linear model, the limit of detection (2σ) for this experimental geometry was estimated to be 4 x 10⁵ 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 10⁵ to 2 x 10⁸ CFU/mL, indicating that the BBIC microluminometer gave consistent results to standard PMT-based detection systems.

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.