ABSTRACT

We present a novel microbial whole-cell sensing system for biodefense and environmental pollution monitoring. Real-time water toxicity monitoring is achieved using genetically engineered E. coli bioluminescent cells which emit light when exposed to toxic materials. The illumination intensity and its time dependence are a function of the toxicants dose and type. The bacteria are immobilized in a disposable PDMS biochip that provides live cell maintenance and micro fluidics channels for sample injection. Several bacteria strains have been developed, generating a unique response signature for different toxins.

KEYWORDS: Whole-Cell Biosensors, Bioluminescence, Environmental Monitoring, Genetically Engineered Bacteria

INTRODUCTION

Environmental pollution monitoring and maintaining the quality and security of drinking water have always been an important factor in assuring public welfare and health. Consequently, much effort is being devoted to developing fast and portable water toxicity detection systems. A promising biosensing scheme is based on bioluminescence whole-cell biosensors [1], [2]. By using live cells we are able to detect, by very simple means, very complex series of reactions that can exist only in an intact, functioning cell. Only a sensor of this type can report on the “well being” of a system, on the toxicity of a sample, the genotoxicity of a chemical or the bioavailability of a pollutant. We present a novel whole-cell biosensor which is unique in several perspectives: (a) its optical detector (b) its degree of integration, and (c) its mode of operation that takes care of reducing false positive and false negative incidents.

DESIGN

The whole-cell biosensor consists of bioluminescent E. coli cells which emit light as a response to toxins exposure. The illumination intensity and its time dependence are a function of the toxin dose and type. The E. coli cells were genetically engineered, carrying a promoter-reporter gene fusion [2]. The promoter is toxin specific and activates the reporter gene. Several bacteria strains with different promoters have been developed, generating a unique response signature for each class of toxins [3]. Light detection is achieved by a Single Photon Avalanche photodiode (SPAD) working in the Geiger mode. The bacteria are immobilized in a disposable PDMS biochip that provides live cell maintenance and micro fluidics channels for sample injection. The PDMS biochip layout is shown in Fig. 1.
The biochip consists of the following four parts: sample test, positive test, negative test and constitutive test. The sample test is the water source test. In the positive test, a diluted toxin is injected to the bacteria periodically in order to check its positive response. The negative test is a control test in which the response to pure water is tested. The sample, positive, and negative tests consists of four strains with a repetition of three bacteria wells for each strain. The constitutive test consists of one strain which works in "Normally On" mode which normally emits light and stops emitting when exposed to a toxin. In our measurements we use Nalidixic Acid (NA) as a model toxin.

A schematic cross section of the system is shown in Fig. 2. The PDMS layer is sandwiched between a glass slide and a PMMA base. The bacteria are immobilized in agar and located inside wells in the PDMS. The flow channels are above the bacteria wells.

RESULTS AND DISCUSSION

A comparison of the SPAD performance relative to a Photomultiplier Tube (PMT) is given in Fig. 3. Here the sample consists of bacteria responding to 16ppm of NA without flow, where the bacteria are suspended in LB. The blue curve is the PMT signal, the red curve is the SPAD signal and the black curve is the SPAD signal shifted down by its dark current. It can be seen that although the SPAD has a much larger dark current, its quantum efficiency and its signal are larger. The use of SPAD allows very sensitive solid state platform operating at signal to noise level superior over standard PN diodes. The SPAD has higher shot noise than the PMT due to its higher dark count. The signal can be improved using a matched digital filter. Using a digital filter we can detect the rise of the signal in 40 minutes.

Measurement of bacteria bioluminescent response to a toxin flow is shown in Fig. 4. The immobilized bacteria were exposed to 10 ppm of NA with flow rate of 0.22 mL/min. The bioluminescence has been measured by a PMT. Comparison of the response of the bacteria in agar vs. its response in LB shows minor time shift.
CONCLUSIONS
A novel microbial whole-cell on-line sensing system for water toxicity has been developed. The biosensor is based on genetically engineered E. coli bioluminescent cells and designed in a format of a whole-cell array. The system is portable with response time of 40 minutes.

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