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Bacterial biofilm-based water toxicity sensor

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ABSTRACT

Cell-based toxicity bioassays harbor the potential for efficient detection and monitoring of hazardous materials. However, their use in the field has been limited by harsh and unstable environmental conditions that shorten shelf-life, introduce significant noise, and reduce the signal and signal-to-noise ratio; such conditions may thus decrease the probability of correct decisions, increasing both false positive and false negative outcomes. Therefore, there is a need for a stable cell-on-chip integration that offers long-term storage and resilience to environmental factors. The use of intact microbial biofilms as biological elements in a whole-cell biosensor, and their integration into specialized biochips, holds promise for enhancing sensor stability as well as providing an innovative platform for biofilm research. We report here for the first time on the integration of a bacterial biofilm as the sensing element of a whole-cell biosensor, as a means to stabilize and preserve reproducibility, viability and functionality of the bacterial sensor cells. We have employed a genetically engineered *Escherichia coli* sensor strain, tailored to respond to the presence of genotoxic (DNA damaging) agents by the induction of a reporter enzyme, alkaline phosphatase, and tested its functionality in colorimetric and electrochemical assays. Three different bacterial integration forms were examined: planktonic cells, electronically deposited sessile cells, and biofilms. For all integration forms, a clear dose-dependent positive response to the presence of the model toxicant nalidixic acid was observed, with biofilms displaying higher current density and detection sensitivity than planktonic and sessile cells. We present the electrode apparatus and methods and biochip characterization of such chips, e.g. signal vs. time and induction factor, and discuss the advantage and potential problems of the new biofilm-biochip technology.

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1. Introduction

Integration of diverse chemical and biological processes onto a microchip, often referred to as a micro total analysis system (micro-TAS) or "lab on a chip", is currently generating major interest due to the potential for functional integration with other technologies and miniaturization, leading to portability, high throughput usage, and low cost mass production. Over the last few years, microchips' footprint decreased and their complexity increased, due to advances in micro- and nano-fabrication and cross disciplinary micro and nano system technology. Biochips integrate diverse biological components on a chip; in a subset of this field, microbial cells are integrated into micro-environmental systems and their reactions to the tested samples are monitored on-chip [1–4]. In these bio-micro-electro-mechanical-systems (bio-MEMS) sensors, live microbial cells convert a chemical, physical or a biological signal into an electrical one [5]. Microbial sensors can be genetically engineered [6] to detect very complex series of reactions that can exist only in an intact, functioning cell [7]. Microbial biosensors have been proposed for usage in diverse applications including monitoring glucose [8], microbial growth rate [9] and response to biocides [10]. They are also used for environmental monitoring including the detection of toxicity, genotoxicity, and the presence of specific groups of chemicals [11–15]. Adaptation of whole-cell biochip technology into a field-compatible format would provide real-time information about contamination sources and allow field use and on-site analysis. It will also reduce the costs involved in sample transfer and maintenance, thus significantly reducing the overall response time. Towards this aim, we propose to integrate the sensor cells into the hardware platform in the form of a permanent biofilm, generating an environmentally stable whole-cell biochip.

As the development of technologies for the construction of low profile biochips is a relatively new concept, it should be thoroughly

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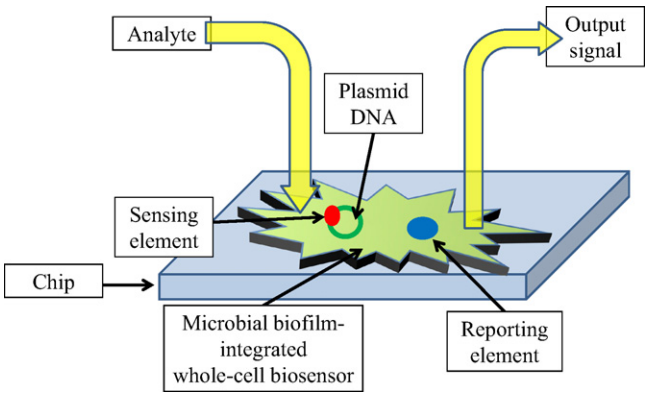

Fig. 1. Scheme of the microbial biofilm-integrated whole-cell biosensor concept.

investigated before it is applied. We expect it to improve biochip performance in cases where the signal is low and the sensitivity is compromised. Effective integration of the biological material into a solid-state platform will stabilize the bio-electronic system and improve the signal-to-noise ratio for rapid and sensitive biosensing.

Biofilms can be defined as communities of microorganisms attached to a surface. Microbial biofilms may be comprised of one or more microbial species, and may be formed on a range of biotic and abiotic surfaces. The sessile microbial cells grow in matrix-enclosed structures interspersed between open water micro-channels. Biofilms predominate in diverse environments, ranging from natural surfaces to biofouling of flow systems, body surfaces and medical devices [16,17]. Studies indicate that biofilms are a stabilized interim stage in a biological cycle including initiation, maturation, maintenance, and dissolution. Biofilms can preserve stable communities of bacteria over long periods of time under harsh environmental conditions [18]. The thickness and the coverage of mature biofilm varies between a few tenths of microns to hundreds of microns and it is dependent with disparate variables: (1) the physical and the chemical properties of the substrate, (2) conditioning films upon the substrate, (3) flow hydrodynamics in the environment, (4) characteristics of the aqueous medium, (5) cell properties, (6) and cell-to-cell signaling (quorum sensing) [18]. Furthermore, the physiological responses of the microbial cells comprising the biofilm which are not homogenous throughout a biofilm, together with diffusional limitations and porosity variations, result in concentration gradients of nutrients, signaling compounds, bacterial waste, and analytes within the biofilm [17].

In this work we demonstrate a new method for the integration of genetically modified *Escherichia coli* biofilms as the sensing element of whole-cell sensor biochips (Fig. 1). Using a model genotoxicity sensor strain and a model genotoxicant, we demonstrate that the deposition of bacterial biofilms as whole-cell biosensors preserves the reproducibility, the viability and the functionality of the bacterial cells on the chip. The proposed chrono-amperometric biosensor is based on the detection of the electrochemical signal generated by the sensor cells following exposure to the model toxicant. An enzymatic reporter is produced by the exposed genetically engineered cells, which in the presence of enzymatic substrate an electro-active species is generated. The electrochemical current generated by the oxidation of the produced electro-active species is measured and it is proportional to the toxicant concentration in the water sample [14]. Three different bacterial integration forms were tested: planktonic cells, electronically deposited sessile cells, and biofilms. Our results indicate for all integration forms a clear dose-dependent positive response to the presence of the model toxicant nalidixic acid (NA); mature biofilms displayed the highest current densities and sessile cells the lowest. Moreover, biofilms demonstrated a higher induction factor sensitivity to NA value than planktonic and sessile cells.

2. Materials and methods

2.1. Bacterial strain culture preparation

E. coli strain AB725 [19] cells harboring a *sulA::phoA* fusion were used for the toxicity detection experiments. The cells were grown overnight in Luria-Bertani (LB) broth containing 0.1 mg/ml ampicillin with shaking at 37 °C. The overnight culture was diluted ×1/150, and regrown to an optical density (600 nm) of 0.2 prior to their use in the experiments. Cells of this strain synthesize alkaline phosphatase in response to the presence of genotoxicants. Nalidixic acid (NA, FW 254.22, Sigma) served as the model toxicant. Depending upon the substrate used, enzyme activity can be monitored either colorimetrically or electrochemically as describe below.

2.2. Colorimetric detection

For the colorimetric assay, the substrate used was p-nitrophenyl phosphate (pNPP, FW 461.4, Sigma), transformed by alkaline phosphatase activity to p-nitrophenol, with an absorption peak at 405 nm. For assaying the activity of suspended bacteria, refreshed bacteria were washed twice and resuspended in MOPS [20] (pH 8.02) containing 0.1% yeast extract at 4 °C. NA was added to a final concentration of either 5 or 10 μg/ml, and following further incubation for 2 h with shaking at 37 °C, aliquots of 200 μL were introduced to a 96-well microtiter plate and pNPP was added to a final concentration of 0.4 mg/ml. Bacterial biofilm assays were performed with 100 μL aliquots of an overnight cell suspension introduced to individual wells of a 96-well microtiter plate, and incubated for 2 h at 25 °C to allow for cell settlement and attachment to the bottom of the wells. Next, the wells were rinsed twice with MOPS containing 0.1% yeast extract to remove unattached cells, and the biofilm was grown for 24 h at 37 °C. Subsequent rinsing of the wells with MOPS containing 0.1% yeast extract was followed by the addition 100 μL MOPS containing 0.1% yeast extract and NA (5 or 10 μg/ml final concentration). Following further incubation for 2 h, pNPP was added to a final concentration of 0.4 mg/ml. The optical density (405 nm) of the exposed samples was measured every 10 min for 2 h at 37 °C (SPECTRAmax 190, Molecular Devices).

Control samples were prepared with either NA or pNPP replaced by equal volumes of growth medium. All colorimetric experiments were performed in quadruplicate.

To depict the response of the bacteria to the genotoxicant tested we calculated the activity factor as follows:

$$\Delta OD = OD_{450\text{nm}(sample)} - OD_{450\text{nm}(blank)} \quad (1)$$

2.3. Electrochemical parallel plate flow chamber

A parallel plate flow chamber, previously shown to be an effective tool for the study of bacterial adhesion kinetics to diverse materials [21–23], was used for this study. An elliptical flow chamber (major radius 10.5 mm, minor radius 1.5 mm, height 3 or 8 mm), was etched in a transparent polymer (Perspex™) body by Computer Numerical Control (CNC) machining. On both bottom and top of the chamber, a rectangular groove (10 mm × 30 mm) was carved to accommodate two parallel polished and passivated float glass plates coated with indium-tin-oxide (ITO, 10 mm × 40 mm × 1.1 mm, Rs = 15–25 Ω/square, Delta Technolo-

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