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Single-cell-based sensors and synchrotron FTIR spectroscopy: A hybrid system towards bacterial detection

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Abstract

Microarrays of single macrophage cell-based sensors were developed and demonstrated for potential real-time bacterium detection by synchrotron FTIR microscopy. The cells were patterned on gold electrodes of silicon oxide substrates by a surface engineering technique, in which the gold electrodes were immobilized with fibronectin to mediate cell adhesion and the silicon oxide background was passivated with polyethylene glycol (PEG) to resist protein adsorption and cell adhesion. Cell morphology and IR spectra of single, double, and triple cells on gold electrodes exposed to lipopolysaccharide (LPS) of different concentrations were compared to reveal the detection capability of this cell-based sensing platform. The single-cell-based system was found to generate the most significant and consistent IR spectrum shifts upon exposure to LPS, thus providing the highest detection sensitivity. Changes in cell morphology and IR shifts upon cell exposure to LPS were found to be dependent on the LPS concentration and exposure time, which established a method for the identification of LPS concentration and infected cell population. Possibility of using this single-cell system with conventional IR spectroscopy as well as its limitation was investigated by comparing IR spectra of single-cell arrays with gold electrode surface areas of 25, 100, and 400 μm^2 using both synchrotron and conventional FTIR spectromicroscopes. This cell-based platform may potentially provide real-time, label-free, and rapid bacterial detection, and allow for high-throughput statistical analyses, and portability.

Keywords: Biosensors; Cell adhesion; FTIR; BioMEMS; Cell-based sensors

1. Introduction

Study of single cell behavior in a specified chemical or biological environment holds important implication in cell biology, biochemistry, and development of cell-based sensors, as it reveals a spectrum of responses from each individual cell under stimulation (Chiou et al., 2005). In a multi-cell system, critical information may be lost or submerged in averaged bulk cell measurements (Teruel and Meyer, 2002). Particularly, in a cell-based sensor array, the signal generated by a multi-cell sensing element in response to an analyte is embedded with the interferential signals (noises) resulted from cell–cell interactions in the cell cluster. Furthermore, variations in conformation of cell clusters on multi-cell electrodes of any array may result in a different response even when they host similar number of cells. Thus, reducing or eliminating the interference from cell–cell interactions represents a major challenge in development of cell-based sensors.

Cell-based sensors are hybrid systems (biology + device) that use cells' remarkable abilities to detect, transduce, and amplify very small changes of external stimuli (Lorenzelli et al., 2003). They offer new opportunities for many biomedical applications, including biothreat detection, drug evaluation, pollutant identification, and cell type determination (Bashir, 2004). They are generally constructed by interfacing cells to a transducer that converts cellular responses into signals detectable by electronic

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or optical devices. Recent years have witnessed a substantial growth in application of planar microelectrode arrays in cell-based biosensors (CBBs) (van Bergen et al., 2003; Wang and Li, 2003; Yang et al., 2003), because they can be easily interfaced with electronic, optical or chemical detecting mechanisms (Miller et al., 2002). Major advantages of these sensing arrays over conventional biosensors include rapid and inexpensive analyses, much smaller sample size requirement, low sample contamination, high throughput and sensitivity, and portability. Among cell-based sensors, single-cell-based sensors are of particular interest; with an array of virtually identical single cells as sensing elements integrated with real-time data acquisition technology, it is possible to experimentally study cellular pathways without interference from other cells, thereby eliminating the uncertainty incurred by states of neighboring cells (Elowitz et al., 2002). Statistical analysis of cell behavior, a topic extensively pursued in cell biology, requires closely identical cell sites (Hyden, 1995), and a single-cell-based system may ideally serve the purpose.

In this study, a cell-based sensor platform was established by combining a microarray of single macrophage cells with synchrotron FTIR spectromicroscopy for real-time potential bacterial detection, and its sensing capability was demonstrated through a comparison study with multi-cell sensor systems. Using a previously established technology (Veiseh and Zhang, 2006) silicon oxide substrates were patterned with an array of gold square electrodes and surface modified to host a single or a group of macrophage cells. Conventional technologies for detection and identification of bacteria, including immunoassay, genetic markers, and cell culturing, use reagent-based tools, which are slow and/or costly due to their reliance on expensive consumables. For example, *Salmonella* detection takes 3–4 days for presumptive results and another 5–7 days for confirmation (Andrews, 1992). The technique introduced in this study may potentially allow for rapid detection of bacteria in a few hours. Lipopolysaccharide (LPS) was used as our model analyte in view of its effects on macrophages. LPS is a major structural component of gram-negative bacterial cell wall and a potent activator of the macrophage cells. LPS is also a major pathogenic factor causing septic shock syndrome and death in critically ill patients (Cohen, 2002; Fujihara et al., 2003; Raetz, 1990; Ulevitch and Tobias, 1995). The syndrome is primarily caused by an overproduction of pro-inflammatory cytokines after macrophage cells have been activated by lipopolysaccharide (Akashi et al., 2000; Kirkley et al., 2003; Rovida et al., 2001; Schumann et al., 1990; Soler et al., 2001; Triantafilou and Triantafilou, 2003; Zhang et al., 1997). Macrophage activation by LPS and its products are both dose-dependent and heterogeneous (Frevel et al., 2003; Hamilton et al., 1986; Wiklund et al., 1999). Using synchrotron IR spectroscopy and DIC reflectance imaging we investigated and compared LPS-induced responses of cells in isolated (single cell) and communicating (colony of the cells) states. To illustrate how the light source quality would affect sensitivity and spatial resolution of the cell-based sensors, the spectra generated by the synchrotron was compared with those generated by a conventional FTIR source.

2. Experimental

2.1. Materials

The following materials and chemicals were used as received: silicon wafers of (1 0 0) orientation (Wafernet, CA), Nanostrip 2× (Cyantek, Fremont, CA), 11-mercaptoundecanoic acid 95% (11-MUA), 3-mercaptopropionic acid 99% (3-MPA), N-hydroxysuccinimide 97% (NHS), 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDAC) (Sigma, St. Louis, MO), 2-[methoxy(polyethyleneoxy)propyl] trimethoxysilane (M_w = 460–590 Da) (Gelest, Morrisville, PA), fibronectin protein, Trypsin–EDTA, Sigmacote and lipopolysaccharide (*E.-coli* 0111:B4, endotoxin unit: 500,000) (Sigma, Milwaukee, WI). Nanostrip 2× was purchased from Gelest (Morrisville, PA). All the solvents including toluene, triethylamine, and dimethylformamide were purchased from Aldrich (Milwaukee, WI). Absolute ethanol was always deoxygenated by dry N_2 before use. RAW264.7 cells (murine monocyte/macrophage) were purchased from American Type Culture Collection (Manassas, VA). The following cell culture reagents were purchased from Gibco (Carlsbad, CA): Trypan Blue, Fetal Bovine Serum, HBSS (Hanks balanced Salt Solution), DMEM (Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose).

2.2. Substrate preparation

The 4″ p-type silicon substrates of (1 0 0) orientation were cleaned with piranha (hydrogen peroxide/sulfuric acid 2:5, v/v) at 120 °C for 10 min, dipped in HF, and thoroughly rinsed with DI water. A layer (1.1 μm) of positive photoresist was then coated on the surface, and patterns were formed on the substrate upon exposure to ultraviolet light through a mask with square patterns of three different sizes (25, 100, and 400 μm^2). A titanium (Ti) layer (10 nm) was then deposited on the photoresist-developed substrates at a deposition rate of 0.3 Å/s. A gold film of 100 nm thickness was subsequently deposited on the Ti at a deposition rate of 5 Å/s. The photoresist was dissolved in acetone and the remaining metal film was lifted off. After lift off, the surface was exposed to buffered oxide etch (HF/NH4F 5:1, v/v) for 60 s and rinsed with DI water to remove native oxide on silicon before oxidation. The surface oxidation was performed under a dry oxygen flow for 6 h at 400 °C. The gold-patterned silicon oxide substrates were then cut into slides of 8 mm × 8 mm. To prevent surface contamination and scratches, the silicon oxide wafers were coated with a 2 μm layer of photoresist on their polished sides before cutting.

2.3. Surface modification

The surface was modified following a previously established procedure with minor modifications (Lan et al., 2005; Veiseh et al., 2002; Veiseh and Zhang, 2006). The protective photoresist layer on gold-patterned silicon substrates was removed by sonication for 10 min in acetone, 2 min in ethanol, and 2 min