Application of parylene-coated quartz crystal microbalance for on-line real-time detection of microbial populations

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ABSTRACT

A novel technique of applying a quartz crystal microbalance (QCM) sensor to the on-line real-time detection of microbial populations is described. The pQCM sensor was fabricated by depositing di-para-xylene (parylene) over the entire surface of a QCM sensor through a chemical vapor deposition (CVD) process. An electrically insulated film of parylene on the QCM sensor enabled the operation of the sensor in the liquid environment, and the resonance frequency of the pQCM sensor set in the medium of a cultivation flask shifted in response to the microbial population.

The effects of pH, conductivity, and viscosity of the medium on the frequency shift of the pQCM sensor were investigated. Ignorable responses (less than 1% at 10^3 cells) were obtained during an incubation cycle.

The detection limit of the pQCM sensor was identified as 10^2 cells ml^{-1} with a frequency shift of around 2×10^3 Hz. The cell numbers of Escherichia coli cultivated in both the YEM medium and whole milk were detected. A satisfactory correlation ($r^2 = 0.95$) was obtained between the cell number and the response of the pQCM sensor.

Experimental results suggest that the pQCM described here is applicable to the continuous long-term detection of microbial populations during a fermentation process.

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1. Introduction

The presence of bacteria in food, water, environmental samples, etc., could be monitored by measuring the physicochemical changes caused by bacterial growth or metabolism. *Escherichia coli* is an organism that commonly lives commensally in gastrointestinal tracts. It is one of the most important pathogenic bacteria capable of contaminating foodstuffs, thereby causing food poisoning; it can also cause enteric infections in humans, with severe and fatal consequences (Kosorok et al., 1998; Oliver et al., 2003). The cost of such contamination to the dairy food industry is associated with loss of yield, cost of discarded food, waste of herdsmen's time, and extra services of culling (Miller et al., 1993).

Various techniques have been developed for the detection of *E. coli*, such as Gram-staining analysis, enzyme-linked immune-sorbent assay (ELISA) (Louis et al., 1998; Kim et al., 2004), polymerase chain reaction (Xu et al., 2004), and automated detection of food-borne pathogens (Oberst et al., 1998; Sharma et al., 1999), all having been developed to improve upon the currently available technology (conductivity monitoring).

Recently, a variety of biosensors used in the analysis of molecular recognition have been developed for the detection of pathogenic bacteria. These biosensors are a unique combination of a receptor for molecular recognition and a transducer for converting the interaction information into an electrical signal.

For example, in robotic milking systems, mastitis detection is carried out by placing electrodes in the milking system in order to detect changes in the conductivity of milk (electrical conductivity). But, in mastitis detection, there is no evidence to suggest that it is possible to detect all types of changes by a conductivity assay (De Mol and Ouweltjes, 2000). Additional drawbacks of conductivity measurements have been reported such as the failure to detect mastitis caused by *Streptococcus uberis* and a high number of false positive results (Hillerton and Walton, 1991; Milner et al.,

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1996). Furthermore, electrical conductance methods employed for the same purpose are unable to yield an acceptable specificity or sensitivity of clinical, food, or environmental contaminations (Toby et al., 2007).

Surface plasmon resonance (SPR) immunosensors offer high sensitivity, selectivity, speed, and reliability in analyses. However, their use for the *on-line* monitoring of bacteria involves high expenditure and difficulties in their miniaturization (Mullett et al., 2000; Shankaran et al., 2007).

A quartz crystal microbalance (QCM)-based technique is favorable over SPR and impedance technology from the viewpoints of system miniaturization and cost. Following the theory of Sauerbrey, the observed decrease in frequency should be proportional to the change in the mass of the quartz resonator (Sauerbrey, 1959).

Furthermore, researchers have developed immunosensors exploiting QCM as the biosensor for the detection of important bacteria (Fengjiao et al., 1994; Noel and Topart, 1994; Ben-Dov et al., 1997; Spangler and Tyler, 1999; Spangler et al., 2001), including *E. coli* (Park et al., 2000; Spangler et al., 2001). They can also be used to detect precise changes in the oscillation frequency and energy dissipation, which occur when membranes or molecules bind to an oscillating quartz crystal (Keeneth, 2001; Cavic et al., 1999). In a recent research conducted on the hepatitis C virus, it was shown that the real-time kinetics of the interactions occurring on the lipid bilayer is derived from the cells (Cho et al., 2007).

Cell proliferation has been reported from the adherence of cells onto the QCM gold electrode; cell proliferation can be used to monitor cell-cycle-related viscoelasticity (Alessandrini et al., 2006). Moreover, cell fibrillations induce a change in the viscoelastic properties of the QCM during multilayered amyloid deposition onto the electrode surface (Hovgaard et al., 2007).

Classical QCM technology involves studies that require the removal of samples from the liquid environment of a fermentor by pumping out samples; subsequently, investigations are carried out by using spectrophotometers, QCM microscopes, etc., as well as conductive analyses. These removal methodologies can aid in the precise removal of a small-volume sample from a bioreactor by using a fluid control pump; however, these methodologies are plagued with problems induced by sample removal or the rinsing process, which can alter microorganisms. These technologies are not suitable for large-scale experiments. Besides, QCMs are fabricated by coating a metal electrode on both sides such that when they are used in liquids, the electrodes are shortened. This in turn would decrease the effective surface due to the above-mentioned reasons.

Parylene is biocompatible, hydrophobic, and highly transparent; it does not have pinholes and it lowers dynamic capacity and inductance. Furthermore, it offers high resistance against acids, organic solvents, and inorganic solvents, and it is an insulator.

In this paper, we present a QCM biosensor device that is modified by parylene. This device is referred to as *p*QCM; it can potentially be used for an organic transfer in medical treatment. The *p*QCM sensor can directly analyze the microorganisms in a fermentor, and the two electrodes aid in more mass loading.

In addition, it may prevent any risk posed by toxins produced due to surface modification. *p*QCMs are inexpensive and can easily be used for on-line tests performed for contamination determination. The fabrication details and performance of the device are provided later in this manuscript. The application of the device is demonstrated, and the results are discussed at the end of the manuscript.

2. Materials and methods

2.1. Sensor fabrication and characterization

Parylene coating allows *E. coli* to deposit but not to conjugate on the sensor surface. This specificity of parylene coating thus efficiently helps to protect electrodes for extended use when compared to other rough ways (such as piranha solution) to remove *E. coli.* Even more, it provides more chemical stability.

The base of the *p*QCM is a polished surface, which is a 10-MHz AT-cut quartz crystal (Taitien Electronics Co., Ltd., Taiwan) with gold electrodes that are 8 mm in diameter; the surface roughness of the electrodes is approximately 2 nm. The device is pretreated by piranha solution at 60 °C for 5 min and rinsed by deionized water; then, it is dried in a stream of nitrogen, treated with O_2 plasma, and placed in a deposition chamber in which it is coated with parylene (PDS-2010, Samco, USA). The thickness of the parylene film is approximately 1 μm at room temperature, and the pressure in the chamber is −30 Torr; the thickness of the parylene film is investigated by an α-stepper meter (SE4000, Kosaka, Japan). We also investigated the following characteristics related to the *p*QCM: hydrophobicity, surface roughness, mass loading of liquid, the effect of temperature, and the effect of pH.

The hydrophobicity of parylene is investigated by an instrument for measuring the surface contact angle (FTA 125, First Ten Angstroms, USA); the water contact angle is found to be approximately about 110°, and the roughness, measured by an atomic force microscope (AFM; XE-100 SPM, Park Systems Corp., Korea) is found to be approximately 10 nm.

The frequency losses are affected by the thickness of parylene coating: 0.0297 MHz for 0.1 μm, 0.030 MHz for 0.5 μm, 0.048 MHz for 1 μm, and 0.111 MHz for 2 μm. The increase of coating thickness leads to the increase of mass loading and the decrease of frequency shifts, matching exactly Sauerbrey equation. There is no significant change in terms of the quality factor when QCM is modified as *p*QCM (when coating thickness reaches 1 μm), investigated by an Impedance Analyzer.

2.2. Data acquisition system and pQCM measuring cell

A frequency counter (HP 5313A) and a Colpitts oscillator operating near the first harmonic are used to measure the frequency shift of the *p*QCM, as shown in Fig. 1a. All systems were developed in our laboratory (Chou et al., 2002a,b; Chang et al., 2003).

We constructed an aqueous measuring cell, which was a *p*QCM device mounted on a cell cultivation flask (25-cm² tissue culture flask, USA); the entire *p*QCM device was immersed into a liquid. The effect of temperature on the oscillation frequency was determined for the *p*QCM crystal. The temperature coefficient for an AT-cut quartz crystal at 25 °C was approximately 0.5 ppm/°C; after being coated with the parylene film, the temperature coefficient increased to 10 ppm/°C.

To determine the sensitivity of the *p*QCM sensor, a dilution experiment was performed for various quantities of *E. coli* cells; sodium chloride and BSA were detected on the surface. When the concentration of *E. coli* suspension was successfully adjusted from 10^1 to 10^4 cells ml^{-1} (cells counted by microscope), 40 ml were directly injected into the measuring cell to detect the frequency shifts after the interval of 5 min. A slope of 4 Hz/mm² ng^{-1} was used to compute accumulated dry mass of the detected sodium chloride under air conditions, and a slope of 14 Hz/mm² ng^{-1} was used to compute the aqueous mass loading of the detected BSA under liquid conditions (40 ml).

We have shown that the *p*QCM system has good sensitivity, similar to that of a conventional QCM. In a recent research on the QCM,