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Short communication

Capacitive antibacterial susceptibility screening test with a simple renewable sensing surface

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ABSTRACT

A simple renewable surface for a rapid antibacterial susceptibility test has been demonstrated. The 3-aminophenylboronic acid (3-APBA) modified electrode bind with *cis*-diol groups on the cell wall of both gram positive and gram negative bacteria. The detection of antibacterial susceptibility response by a capacitive system can be done within a short time, 2.5 h for the whole process, with good repeatability of the electrode's preparation. An acid solution, could break the bonding between 3-APBA and the bacteria, which were then easily removed by the fluid flow, renewing the sensing surface for the next test. This modified electrode can be reused up to 35 times. This sensor is useful for testing the susceptibility of bacteria to antibacterial agents that affect their cell wall. Results from the capacitive sensor corresponded well with the antimicrobial information in the literature and to the morphology of the treated bacteria revealed by scanning electron microscopy. Antimicrobial susceptibility to natural products could also be easily tested.

1. Introduction

Common methodologies for antimicrobial susceptibility testing are disk diffusion and broth dilution. These usually take from 1 to 7 days to complete (Poupard et al., 1994). Alternative methods may be more rapid and easier to use. A cell-based biosensor, for example, electrochemically detects bacterial metabolic activity via the dissolved oxygen level (Karasinski et al., 2007) or via a mediator (Mann and Mikkelsen, 2008). Other studies have detected changes of bacterial properties with affinity biosensors using surface plasmon resonance (SPR) (Chiang et al., 2009) and the change in mass using quartz crystal microbalance (Ma et al., 2015). These affinity techniques are reliable, sensitive and take less analysis time than conventional methods and it would be of a great advantage if the surface modification of an affinity biosensor could be easily refreshed for a new test.

In our previous work, we used 3-aminophynylboronic acid (3-APBA) to bind with polysaccharide (with diol-groups) on the bacterial cell wall for a rapid detection of total bacteria (Wannapob et al., 2010). 3-APBA is a chemoselective ligand which is less expensive than biological ligands and more resistant to chemical and biodegradation (Lau et al., 2000). The bond bacteria were easily removed by 10 mM acetate buffer pH 4.50 so, the sensing surface could be reused.

For the detection, the measure of the change in capacitance causes by the alteration at the sensing surface and the electrolyte interface based on a potentiostatic method has proven to be highly sensitive (Berggren et al., 2001; Dawan et al., 2011; Jiang et al., 2003; Suwansaard et al., 2009). An insulated electrode surface is an important requirement of the capacitive system and it can be prepared by a coating of polytyramine (Pty), a non conducting polymer. At the same time tyramine provides free amino groups which can be used to anchor the ligand to the transducer (Yuqing et al., 2004). This research described the fabrication of a reusable surface of 3-APBA on a Pty coated gold electrode surface to bind with different types of bacteria that were then used to test different types of antibacterial and natural products. The use of this reusable surface makes it easy to load different types of bacteria and detect the effect of various antibacterials. The antibiotics and natural products selected for testing, with both gram positive and gram negative bacteria, were those able to inhibit the synthesis of cell walls, cause cell lysis or inhibit protein synthesis. If the antibacterial has effect on the bacteria, its binding to the 3-APBA would

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be affected, thus, changing the capacitance. Residual bacterial cells were removed from the sensor by an acidic buffer and the renewed surface was ready for the next test with a new batch of bacteria. The reusability of the surface and the repeatability of the fabrication process were also demonstrated.

2. Materials and methods

2.1. Materials

Tyramine, glutaraldehyde and 3-APBA were from Sigma–Aldrich (Steinheim, Germany). Nutrient broth and nutrient agar were from Difco™ (Becton, Dickinson and Company, USA). Ceftriaxone was from Millimed Co., Ltd. (Samut Prakan, Thailand), ampicillin was from T.MAN PHARMA Co., Ltd. (Bangkok, Thailand), vancomycin was from Siam Bheasach (Bangkok, Thailand) and tetracycline was from GP PHARMA Co., Ltd. (Bangkok, Thailand). Antibiotics were prepared in 10 mM Tris–HCl buffer pH 7.50. Two natural products (in ethanol), a pure compound of Rhodomyrtone from *Rhodomyrtus tomentosa* (Saising et al., 2008) and a crude extract of the *Quecus infectoria* (*Q. infectoria*) (Voravuthikunchai et al., 2008) were kindly provided by Prof. Dr. Supayang Voravuthikunchai, Department of Microbiology, Prince of Songkla University, Hat Yai, Songkhla, Thailand. All antibacterials were diluted with 10 mM Tris-HCl pH 7.50. Tris-HCl and acetate buffers were prepared with deionized water treated with a reverse osmosis-deionizing system (Pentair, Inc., USA). Before use, buffers were filtered through a nylon membrane filter (Vertical, Albet, Spain, pore size 0.2 μm) with subsequent degassing. Other chemicals used were analytical grade.

2.2. Methods

2.2.1. Preparation of electrode

Tyramine monomer, 50 mM, in 2.0 mM phosphate buffer pH 7.00 and ethanol (3:1 vol ratio) was electropolymerized onto a cleaned gold electrode (diameter 3 mm, 99.99% purity) by cyclic voltammetry for 15 scans, between 0.0 and 0.8 V (vs. Ag/AgCl) with a scan rate of 50 mV s^{-1} (Loyprasert et al., 2010) and rinsed with distilled water. It was then treated with 5.0% (v/v) glutaraldehyde in 10 mM sodium phosphate buffer pH 7.00 for 20 min to activate the N-aldehyde groups of the polytyramine (Pty) that would bind with the amino groups of 3-APBA. After washing, 20 μL of 3-APBA (400 ppm in phosphate buffer pH 7.00) (Wannapob et al., 2010) was dropped onto the electrode, kept at 4 °C for 24 h and rinsed with deionized water before use. Steps of the electrode modification were characterized using scanning electron microscopy (SEM) (JSM-5200 LV, JEOL, Japan, operating at 20 kV) and Fourier transform infrared spectrometry (FTIR) (VERTEX 70, Bruker, Germany (Supplementary data Fig. S1 and Fig. S2).

2.2.2. Bacterial standards

Pure cultures of *E. coli* ATCC 25922, *S. thyphi*, *P. aeruginosa* ATCC 27853, *S. epidermidis, S. aureus* ATCC 25923 *and B. subtilis*, provided by the Department of Microbiology, Prince of Songkla University, Hat Yai, Thailand, were grown in 250 mL conical flasks containing 100 mL of nutrient broth for 24 h at 150 rpm (Heidolph unimax 1010 and incubator 1000, Germany). They were collected by centrifugation at 6000 rpm for 10 min (DUPONT Instruments, USA), washed three times by resuspension in 10 mL of saline solution (0.9% NaCl, w/v) and centrifuged again under the same conditions. The culture was spectrophotometrically quantified (600 nm) and viable cell number was determined by the conventional plate count. These procedures yielded a bacteria stock suspension of approximately 1.0×10^8 cfu mL^{-1}.

2.2.3. Capacitance measurement

Measurements were taken in a flow injection system (Fig. 1) with a 3-APBA/Pty modified gold working electrode, a custom made Ag/AgCl reference electrode, and a stainless steel tube auxiliary electrode (diameter 0.5 mm) connected to a potentiostat (EA161, EDAQ, Australia). The detection principle has been reported in detail elsewhere (Wannapob et al., 2010). In brief 50 mV potential step pulses (pulse width 6.4 ms) were applied on the working electrode, one pulse per min. The totally insulated electrode surface acted like a simple RC circuit (Berggren et al., 2001) and the current response, i(t), decayed exponentially with time. By plotting ln i(t) versus time the capacitance was obtained from the slope of the regression equation. The capacitance versus time was then plotted and shown on the display monitor.

To obtain the baseline capacitance for the analysis, 10 mM Tris–HCl pH 7.50 was introduced at 50 μL min^{-1}. The injection of 300 μL of bacteria, which then bound to the 3-APBA, produced a thicker dielectric layer on the electrode/electrolyte interface and the capacitance decreased ($\Delta C_{bacteria}$). In this step 20 min was allowed to elapse before the $\Delta C_{bacteria}$ was taken (Fig. 1). Then 3.0 mL of an antibacterial solution was flowed through the bacterial surface during which time (60 min) the capacitance increase because of the drug. In this step antibiotic that can inhibit cell wall synthesis or lysis the cell wall caused the bacteria to lost their capability to bind with 3-APBA and detached from the electrode surface. Then 3.0 mL of buffer was passed through to wash the drug away (60 min) and revealed the capacitance of the surface where the detached bacteria caused the increase of capacitance. The response related to bacterial susceptibility was obtained from the measured capacitance before and after the flowing of the antibacterial agent ($\Delta C_{antibacterial}$). Finally, a 300 μL regeneration solution (10 mM acetate buffer pH 4.50) was applied to clear the remaining bacteria from the surface followed by a continuous flow of running buffer for 60 min, after which a renewed surface baseline was recorded for a new test cycle.

3. Results and discussion

3.1. Capacitance change from different bacteria

To obtain a maximum response, the surface should be fully loaded with bacteria. To ascertain that this could be done with one injection of 300 μL of 1.0×10^8 cfu mL^{-1}, three such volumes of bacterial cells were injected into the flow system. After each injection, 20 min was allowed to elapse before the capacitance was measured, followed by the next injection of bacterial cell. After the third injection, cells were removed by the regenerating solution. For each of the bacterial cell types the second and third injections did not produce notable additional responses (Supplementary data Fig. S3 and Table S1). Thus, one injection could provide the maximum loading of bacterial cells.

The effect of different cell types on the capacitance was then investigated. Gram negative bacteria, *E. coli*, *P. aeruginosa* and *S. thyphi*, of the same size (1–2 μm) produced nearly the same responses ($\Delta C_{bacteria}$ 93.1 ± 1.4, 89.6 ± 2.0 and 91.7 ± 2.3 nFcm^{-2}, respectively, Supplementary data Fig. S3). Similarly, the same size (0.5–1 μm) gram positive bacteria, *S. aureus* and *S. epidermidis*, also gave similar responses (74.2 ± 1.2 and 75.8 ± 1.6 nF cm^{-2}). Comparing similarly-sized gram negative and gram positive bacteria, *E. coli* (1–2 μm) and *B. subtilis* (1–4 μm), the capacitance change of the gram negative (93.1 ± 1.4 nF cm^{-2}) was slightly greater than that of the gram positive (85.3 ± 1.7 nFcm^{-2}) bacteria. This was likely because the gram negative bacteria have lipopolysaccharide which extended from the outer membrane making it easier to bind on the 3-APBA (Wannapob et al., 2010). In the case of the gram positive strains of different sizes, the capacitance change of the larger rod type *B. subtilis* (85.3 ± 1.7 nF cm^{-2}) was slightly higher than the smaller cocci *S. aureus* (74.2 ± 1.2 nF cm^{-2}) (Supplementary data Fig. S4). Thus, gram negative bacteria provided larger responses than gram positive, with better responses from larger cells.