Electrochemical sensors and biosensors for influenza detection - update 2012-2013

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Ifluenza virus

Viral Nucleic Acid

PB1, PB2, PA (transcriptase complex)

M1 (matrix protein)

NP (nucleocapsid)

M2 (ion channel)

Lipid Bilayer

NS2

HA (hemagglutinin)

NA (neuraminidase)

5 examples of influenza detection strategy
Sialic Acid (SA) Moieties

Galactose Terminals of Glycoprotein

Hemagglutinin

Influenza Virion

Live System

Self-assembling of 1-octanethiol and octyl-galactoside layers, the latter being further functionalized with sialic acid groups by using the appropriate type of sialyltransferase enzyme.

Constructed System

Gold Electrode

Impedance detection

Carboxyphenyl groups modified electrode

CV voltammograms of the probe DNA/GCE (a), the electrode hybridized with two-base mismatched DNA ($1.0 \times 10^{-11}$ M) (b), and target DNA ($1.0 \times 10^{-11}$ M) (c) in 1.0 mM $\text{K}_4\text{Fe(CN)}_6/\text{K}_3\text{Fe(CN)}_6$ (1:1) containing 0.1 M KCl with a scan rate of 50 mV/s. When the probe DNA of the E-DNA biosensor was hybridized with two-base mismatched DNA sequences (b), the probe DNA/GCE exhibited redox peaks that correspond to the $\text{Fe(CN)}_6^{3-/4-}$ electron transfer processes with a small reduction in peak current due to partial hybridization between the probe and two-base mismatched DNA. On the other hand, when the probe DNA of the E-DNA biosensor was hybridized with complementary target DNA, the decrease in peak current of $\text{Fe(CN)}_6^{3-/4-}$ redox process is due to the hybridization interactions between the probe and targeted DNA sequence (c). The targeted DNA concentrations could be monitored with the change in the $\text{Fe(CN)}_6^{3-/4-}$ redox peak current.
A magnetic bead-based bienzymatic electrochemical immunosensor

This strategy avoided the accumulation of H$_2$O$_2$, which could cause enzyme inactivation, and allowed a low detection potential with the use of hydroquinone (HQ) as mediator for DPV detection.

Quinones undergo one-electron reductions to hydroquinone radicals, or two-electron reductions to hydraquinones.

A "four-ferrocene" modified stem-loop structure as a probe for sensitive detection of DNA.

Electrochemical response upon hybridization with complementary short sequence (30-base length) and long sequence (50-base length) strands was observed by differential pulse voltammetry.

The longer the sequence, the greater the decrease in current.

5′Fc-Fc-Fc-GATCGGGAAGACGTCCAAAAACTCGATCGT-O-C6H12-S-3′

bisfunctional ferrocene containing phosphoramidite and dimethoxytrityl (DMT) groups

1-[3-O-Dimethoxytritylpropyl]-1’-[3’-O-(2-yanoethyl-N,N-diisopropylphosphoramidityl)propyl]ferrocene

Fig. 7. NiDPV signal decrease observed at 3 h hybridization reaction with the PCR product analogs 5 (complementary target) and 6 (non complementary target). The electrochemical response of the biochip is normalized using the equation \( Ni_{DPV} = (I - I_0)/I_0 \). Results are the mean values of three experiments with 5 and four experiments with 6.

Impedance

\[ Z = \frac{U}{I} \]

Resistance

\[ Z = R = \frac{U}{I} \]

Resistance is a special case of impedance under application to direct current (DC).

Alternating current (AC) circuit

\[ Z = f (\text{magnitude, phase angle}) \]

direct current (DC) circuit

\[ R = f (\text{magnitude}) \]

\[ \text{phase angle} = 0 \]

\[ Z = R + jX = |Z| \cos \varphi + |Z| \sin \varphi \]

\[ u(t) = U_m \sin(\omega t + \Psi_u) \]

\[ i(t) = I_m \sin(\omega t + \Psi_i) \]

\[ \varphi = \psi_u - \psi_i \]

\[ Z = \frac{U}{I} = \frac{U_m}{I_m} = \frac{U_m}{I_m} \cdot e^{i(\psi_u - \psi_i)} = Z \cdot e^{i\varphi} \]
\[ Z = R + jX \]

**Real Part - Resistance**

**Capacitive Reactance**
- electrostatic storage of charge induced by voltages between conductors.

A DC voltage applied across a capacitor causes positive charge to accumulate on one side and negative charge to accumulate on the other side; the electric field due to the accumulated charge is the source of the opposition to the current.

**Inductive Reactance**
- apparent resistivity of element with inductance when passing an alternating electric current.

**Inductance** is the property of a conductor by which a change in current flowing through it "induces" (creates) a voltage in both the conductor itself (self-inductance) and in any nearby conductors by magnetic fields of currents.
The experimental protocol consisted of three elements: electrode surface modification, AIV detection and RBC amplification.

Polyclonal antibody against N1 subtype was immobilized on the surface of the microelectrode to specifically bind AIV H5N1 to generate more specific impedance signal and chicken red blood cells (RBC) were used as biolabels to attach to AIV H5N1 captured on the microelectrode to amplify impedance signal. RBC amplification was shown to increase the impedance signal change by more than 100% compared to the protocol without RBC biolabel.

RBCs were used as biolabels to amplify the antibody-virus binding due to their larger diameter (7–12μm) compared to the virus (80–120 nm), and strong and specific binding by virus hemagglutinin to sialic acid linkages found on the cell surface.
Thank you for your attention.