Experimental Nanobiotechnology

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Lecture 12: Electrochemical Nano-Biosensor

Hello everyone, today we are going to learn about electrochemical nanobiosensors. In today's lecture, we are going to learn what is a biosensor, what are the various components of biosensor, and we are also going to learn how an electrochemical biosensor works, both theoretically and practically, through a demonstration. Let us see what is a biosensor. A biosensor is a sensor that integrates a biological element

with a physiological transducer to produce an electronic signal proportional to an analyte concentration, which is conveyed to a detector. In today's lecture, we are going to learn all these in detail in the subsequent slides. Let us briefly learn the history of biosensors. Dr. Clark was the first one who introduced the concept of a biosensor, in which he incorporated an enzyme as a bioreceptor to an electrode, where he could sense the analyte based on electrochemical change.

Originally, it was designed to detect oxygen, which we also call the Clark electrode. With further development, he also developed it for glucose sensing and based on this pioneering work, we can now see the most successful commercialized product: the glucose biosensor. In this picture, you can see that the glucose oxidase enzyme has been immobilized on an electrode surface, and when the glucose analyte comes in contact, a reaction takes place on the anodic as well as the cathodic electrodes, which generates an electrochemical change to detect the glucose.

Let us see the development of biosensors. In 1962, the first glucose biosensor was developed. Followed by that, you can see that the immunosensor was developed in 1985. In 2000, wearable and implantable biosensors were developed. And from 2010 onwards, you can see the role of nanotechnology in biosensors.

From this slide, you can clearly understand the development of biosensors starting from the oxygen electrode that was developed in 1956, followed by the glucose biosensor in

1962, which is one of the most successful biosensors to date. And there has been a lot of development in this field, especially it started in 1975 when the immunosensor was developed, and in 1998, the DNA-based biosensor. And presently, you can see here there are many sensors like contact lens biosensors, colorimetric sweat biosensors

and flexible and wearable biosensors. And presently, we are using these AI and ML-based biosensors. Let us see the characteristics of nanobiosensors. The first one is sensitivity, that means it detects small changes in analyte concentration, and the next one is specificity, that means its ability to detect a specific analyte distinguishes the target analyte from similar ones.

And the third one is linearity, which is the direct proportionality between response and concentration. And the fourth one is the response time, which means the rapid time to reach a stable signal. And the fifth one is reproducibility, which is the consistency of results across tests. And the last one is the dynamic range, which means the range of reliable analyte concentrations. Let us learn about the various components of a biosensor.

The first one is the target analyte, which represents the substance of interest. And the bioreceptor, which is a specialized molecule or system designed to specifically recognize and bind to the target analyte. So you can see here, for example, it can be an antibody specific for a particular antigen. So this can come and bind. to the bioreceptor.

This analyte can come and bind to the bioreceptor, and this bioreceptor is immobilized onto the transducer, which is responsible for detecting and converting the biological interaction into a measurable signal. So, once there is an antigen-antibody complex, it will measure the signal, and this signal is subsequently amplified by the amplifier, and the signal processor then refines and quantifies the amplified signal, which is finally displayed as a measurable output on the display unit.

In the following slides, we will go deeper into each of these components to better understand their roles and functions. Let us see the first component of a biosensor, which is the target analyte. Malfunction at the cellular level triggers the release of specific molecular markers, which serve as early indicators of disease. As the condition advances, these biomarkers accumulate, and their concentration rises significantly.

Therefore, early detection of these biomarkers is critical to ensure timely diagnosis and intervention. As I mentioned earlier, any disease starts at the molecular level. At the molecular level, you get signals in femtomolar, picomolar, or nanomolar concentrations.

To detect these signals, we have to use a very sensitive biosensor. with the progress in the disease, what happens is, the biomarkers also accumulate

There will be a rise in the biomarkers, but if you are able to detect the disease in the later stage at the organ level or the system level, it is very difficult to cure the disease. However, if you are able to detect the disease at an early stage, at the molecular level, using this sensitive biosensor, then we can overcome these diseases. The next component is the bioreceptor. A bioreceptor specifically engages with the target analyte, and the bioreceptor ensures the sensor's selectivity and specificity.

by binding or reacting with the analyte through biological recognition mechanism. So these are the various bioreceptors. The first one is enzyme based. So here we can use the enzyme as a bioreceptor. Example is glucose oxidase for glucose sensing.

And next one is we can use the antibody. These antibodies are highly specific for the particular biomarker. For example, if you are using the antibody specific for the lung cancer, it will detect the lung cancer. And if you are using the antibody specifically for the breast cancer, it will specifically detect the breast cancer. The next one is we can also use the DNA or RNA as a kind of bioreceptor.

Here the DNA or RNA will bind to the complementary sequence of the target nucleic acid that is the DNA probes which can be useful for genetic testing as well as the pathogen detection. For example, if there is a viral infection or bacterial infection and if you do not have antibody for specifically for that particular virus or bacteria, we can use this DNA or RNA and most of you know the DNA work based on the complementary sequences. And if you have the complementary sequences, it will bind and it will produce the signal.

And that can be measured using this nucleic acid-based sensor. And the fourth one is an aptamer. This aptamer is a synthetic oligonucleotide or peptide. And that binds specifically to targets like proteins or small molecules. So we can use these aptamer-based sensors mainly for identifying small drug molecules.

The next one is immobilized cells or tissue, where we can immobilize living cells that detect specific environmental changes such as toxins or biological substances, and that can be detected by using this whole-cell biosensor by immobilizing these whole cells. The next component is the transducer. The transducer is one of the most crucial

components of the biosensor. which transforms the biological interaction between the analyte and bioreceptor into a measurable signal.

And we have several types of transducers. The first one is the electrochemical transducer. It measures changes in electric properties such as current, voltage, or impedance caused by biochemical reactions. The next one is the optical transducer. It measures changes in light properties like intensity, wavelength, or phase.

or the polarization of the light due to an analyte and receptor interaction. So when there is an interaction, for example, if you are getting a fluorescent signal, it can be fluorescent on or it can be fluorescent off. So it can quantify and detect the particular analyte. The third one is the acoustic transducer.

It detects changes in sound wave properties such as frequency and amplitude. And the fourth one is the piezoelectric transducer. It measures changes in mechanical properties such as mass or resonance frequency caused by the interaction. And the last one is the thermal transducer. It detects temperature changes

in terms of heat generated during the biochemical reaction. So, if there is heat generated, that can be detected using this thermal transducer and if there is a mass or resonance frequency change, the mass or resonance frequency that can be detected using this piezoelectric transducer. From this slide, you can clearly understand some examples of transducers as well as their applications.

So, under the electrochemical transducer, the examples are amperometric, potentiometric, and impedimetric. So, applications like glucose sensors or pH meters can be used, and under the physiologic transducer, examples include QCM, and applications can include gas sensors or pathogen reduction. Under the acoustic transducer, we have examples such as SAW sensors, which can be useful for environmental monitoring and food safety. Optical transducer examples include fluorescence and SPR, which is surface plasmon resonance.

Applications include DNA sensors and biomarker detection. The last one is the thermal transducer, and the example is a colorimetric biosensor. Applications include metabolic studies as well as food testing. Let us see the classification of biosensors. Biosensors can be classified based on bioreceptors.

They can be enzyme-based, antibody-based, aptamer-based, whole-cell-based, or DNA-based, as I already explained. They can also be classified based on detection technology.

They can be nanomaterial-based biosensors or SPR-based. Biosensors can also be magnetic-based or classified based on the transducer, such as optical, thermal, acoustic, or gravimetric.

And again, based on modern development, we can use advanced materials and reduce the size. We can have small-sized and wearable devices. And we can also integrate IoT, AI, and ML to advance this biosensor. Let us see how an electrochemical biosensor works. Electrochemical biosensing is the study of the relationship between voltage, resistance, or capacitance change

and the identifiable chemical or biological change upon analyte interaction. That means once the analyte interacts with the particular receptor, there will be changes. So that can be capacitance, voltage, or resistance. The capacitance change means it demonstrates changes in stored electrical charge with analyte presence. In the case of resistance change, it reflects alterations in the material conductivity upon analyte binding.

And voltage change indicates a shift in electrical potential due to analyte interaction. Let us learn about the fundamentals of electrochemical biosensors. The foundation of electrochemical biosensors lies in redox reactions. According to redox nomenclature, a pair of species involved in electron transfer is referred to as a redox couple. Redox reactions, in which one chemical species obtains electrons and another gives up electrons, are the basic building blocks of electrochemical biosensors.

In this picture, you can see here that the target analyte undergoes diffusion to reach the biorecognition element. which is immobilized on the biosensor surface. The biorecognition element specifically interacts with the target analyte, triggering a change in the redox reaction. This reaction generates a flow of electrons, which is then captured and translated into an electrical signal by the transducer. The magnitude of this signal is directly proportional to the concentration of the analyte, as shown in this graph.

The behavior of these redox reactions and the resulting signal can be mathematically described using the Nernst equation.

$$\textbf{\textit{Nernst Equation}};\ E_{cell} = \mathrm{E}^\circ - \frac{\mathrm{RT}}{\mathrm{zF}} \ln \frac{[\mathrm{red}]}{[\mathrm{ox}]}$$

R = Universal Gas constant
T = Temperature
F = Faraday Constant
Z = Number of electrons transferred

This equation explains how the potential generated during the redox process depends on the concentration of the redox species and other system parameters. By monitoring this potential or the resulting current, we can quantitatively detect the target analyte. So, this is the fundamental principle of electrochemical biosensors.

Let us now explore the electrochemical workstation and the key components of the three-electrode system, which is commonly used in biosensor applications. An electrochemical workstation is a sophisticated instrument designed to control and measure electrochemical reactions in a precise and controlled environment. So, it comprises several components, including a potentiostat, which regulates the voltage between electrodes.

And this potentiostat governs the interaction between these electrodes by applying a controlled voltage and measuring the resulting current. This dynamic interplay provides critical information about the concentration and nature of the analyte, and we have a computer interface. To monitor and analyze the resulting data. At the heart of this system, we have this three-electrode setup, often integrated into a screen-printed chip or electrode for compactness. And ease of use.

It consists of a working electrode, a counter electrode, and a reference electrode. The working electrode is the electrode where the electrochemical reaction of interest occurs. It interacts with the analyte and measures the resulting current or potential changes. The next one is the counter electrode, also known as the auxiliary electrode. It completes the electrical circuit by allowing current to flow between itself and the working electrode.

The third one is the reference electrode. This is an electrode that can maintain a constant potential under changing experimental conditions. It is used as a reference point to measure and control the potential of the working electrode. Let us learn the role of cyclic voltammetry in biosensing. Cyclic voltammetry in biosensing is an electrochemical technique used to analyze the redox behavior of biomolecules and detect biological targets.

This cyclic voltammetry involves applying a cyclic potential sweep to the working electrode while monitoring the resulting current. The applied potential is varied linearly with time in a triangular waveform, as shown in this graph. During the scan, the potential causes oxidation and reduction of the analyte at the electrode surface. These electrode reactions generate current responses that are recorded to produce a characteristic plot

known as a cyclic voltammogram. This cyclic voltammogram is a plot of current versus potential.

It provides vital information about the electrochemical properties of the analyte. Here, we have this anodic peak corresponding to the oxidation process, which means the loss of electrons, and we have the cathodic peak corresponding to the reduction process, which is the gain of electrons. The separation between the anodic and cathodic peaks provides insights into the reaction kinetics, while the peak intensities are directly related to the analyte concentration.

In biosensing, CV is often used to study the interaction between the target analyte and the biosensor's biorecognition elements, such as antibodies or enzymes. For example, the schematic here demonstrates the redox reaction of ferrocyanide and ferricyanide, which is frequently used as an electrochemical probe. It is also called a redox couple. When a target analyte interacts with the biorecognition layer, it modulates the redox behavior of the probe, causing measurable changes in the voltammogram.

Let us learn how to fabricate an immunoelectrode for biosensing applications. So, it has three steps. The first one is electrode modification. We will be using a screen-printed electrode. The working electrode of the screen-printed electrode will be modified using a nanomaterial.

Here, we are going to use functionalized graphene, which will improve conductivity and surface area. You can deposit the functionalized graphene by the drop-casting method. Once the working electrode is modified, you can see that the CV curves show an increase in peak current, indicating enhanced electron transfer. The next step is functionalization with antibodies.

Now, we have to conjugate the antibody on the working electrode surface using a coupling agent, EDC-NHS. We are going to add the anti-CEA antibody, which is the antibody against carcinoembryonic antigen. This will be immobilized on the graphene surface, and after this, you can see that The CV result shows a slight shift, indicating the successful conjugation of the antibodies. The third step is blocking with BSA, that is, bovine serum albumin.

In this step, non-specific binding sites are blocked using BSA, and the CV response here ensures that the electrode remains selective for the target analyte. It also minimizes background noise. By blocking with BSA, we prevent non-specific binding and improve

the efficiency of this biosensor. Now, let us learn how to interpret the CV results from this graph.

Whenever we fabricate an immunoelectrode for biosensing applications, each step must be confirmed by measuring the CV. From this graph, we can observe that when using a bare electrode, there is baseline electrochemical activity. When using this functionalized graphene-coated electrode, there is a significant current increase, reflecting enhanced electron transfer kinetics. When immobilizing the anti-CEA antibody on this f-graphene electrode,

there is a slight current reduction due to the insulating nature of antibodies. When using BSA as a blocking agent, there is a further current reduction. This indicates successful blocking of non-specific sites. In today's practical demonstration, we will learn this in more detail. Before learning about immunosensor biosensing studies, let us briefly discuss differential pulse voltammetry (DPV).

So, DPV is an advanced electrochemical technique widely used in biosensor studies for its high sensitivity and low detection limit. Compared to CV, DPV applies a series of pulses over a linear potential ramp. The current is measured just before and after each pulse, allowing for the separation of background currents and highlighting the faradaic process associated with the analyte. Let us see the biosensing ability of the fabricated immunosensor.

So, once we modify the working electrode with antibodies specific for CEA, then you can incubate the working electrode with CEA antigen at different concentrations. And you can see here, with respect to the concentration, the current goes down. If there is no antigen, that is at 0 nanograms per mL, you can see here the baseline current corresponds to the unbound immunosensor. And you can see, with respect to the increasing concentration of CEA antigen, the current is going down.

By using this technique, we can quantify even up to 10 femtograms per mL. So, that is the advantage when we are able to detect very, very low quantities. So, this biosensor can be useful for early disease diagnosis. In this case, we are using CEA, which is a carcinoembryonic antigen. So, this can be useful for

early detection of cancer. So, this relationship between the current and concentration demonstrates the sensor's ability to quantitatively detect the CEA levels with high

sensitivity. I hope you got the overall idea about the electrochemical nanobiosensor. Let us go to the lab and learn this technique more in detail.

To fabricate an electrochemical biosensor platform, we need CEA antigen and its monoclonal antibodies. EDC, NHS, BSA, PBS, amine graphene powder, potassium ferrocyanide and potassium ferricyanide. This is a portable potentiostat from Metrohm. which is connected to a laptop and is controlled by a software. The potentiostat has a connector for the screen-printed electrode.

This is the screen-printed electrode which has a counter, reference and working electrodes. The middle portion is the working area where we have to deposit the transducer material. First, we will weigh 20 milligram of amine graphene powder Add this to 20 ml of 70 percent ethanol to make 1 mg per mL solution. We will sonicate the solution for 15 minutes for proper dispersion of graphene.

You can see that the solution is homogenized properly. Now we will drop cast the solution on the area of the electrode. The electrode is covered with a film to only expose the working area. Drop 5 microliter of the material on the working area. and dry this electrode overnight in an oven at 37 degree Celsius.

You can see that the electrode has been modified using amine graphene. For immobilization of the antibodies, we need to first activate the antibody using EDC NHS chemistry. For that, we require 50 micrograms per mL stock of antibodies, 4 molar EDC and 1 molar NHS. First, add 10 microliters of antibody to an empty vial. Then add 5 microliter of EDC and 5 microliter of NHS to make a ratio of 2 is to 1 is to 1.

We will keep this for incubation at 4 degrees Celsius for 45 minutes. After incubation, the antibodies are now activated. We will drop 5 microliters of the activated antibodies to the 3 modified electrodes. Electrodes are then kept in a humidified chamber and place it in the fridge for one hour at 4 degree Celsius. After immobilization, we will move on to the washing step.

PBS is used to wash the electrode surface. Washing is done to remove the unbound antibodies. After washing, we'll block the surface with a 2 percent BSA solution. Take 5 microliters of BSA and add it to the working area of the modified electrode. Again, keep this in the humidified chamber for a 2-hour incubation.

After blocking, we need to wash off the excess BSA with PBS. Now, using cyclic voltammetry studies, we will compare the current response of modified and unmodified

electrodes. For this, we need a redox couple solution, which is made up of 5 millimolar potassium ferricyanide and 5 millimolar potassium ferrocyanide dissolved in PBS. The parameters of CV (cyclic voltammetry) have been applied in the software.

Here, we have the unmodified screen-printed electrode, the screen-printed electrode modified with amine graphene, and the modified screen-printed electrode with antibody immobilized onto it. First, connect the unmodified electrode and add 50 microliters of redox solution to the working area. Run the CV. You can see the graph on the screen.

Similarly, we will check the CV of the modified screen-printed electrode. You can see that in the modified electrode, there is an increase in the peak current. This is a modified screen-printed electrode with antibodies, further blocked with BSA. Here, you can see a potential shift in the graph, which is due to the antibody immobilization on the screen-printed electrode. Here is the comparison of the CVs of the three screen-printed electrodes.

Next, we move on to the electrochemical response study of the electrodes. For this, we will perform differential pulse voltammetry. First, we will take the response of the modified electrode without the antigen. You can see the response on the screen. Now, let us see the response of the electrode when we add the antigen onto it.

We will incubate the CEA antigen on the electrode for about 10 minutes. Add 10 microliters of the antigen on the surface and incubate for 10 minutes. Wash the unbound antigen using PBS. Add 50 microliters of redox solution to the surface and take the response. You can see there is a clear decrease in the current when the electrode is presented with the antigen.

Denoting an immune complex formation. You can further analyze this by incubating different electrodes with various concentrations of antigen and recording the sensor's response. As a summary, in today's lecture, we learned about what is biosensor, what are the various components of biosensor, and we also learned how an electrochemical biosensor works, both theoretically and practically, through a demonstration.

Thank you for your kind attention. I will see you in another interesting lecture.