Design for Biosecurity

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Lecture 38

World of Electrochemical Biosensors

Welcome back to the third class of this week. In our last two sessions, we covered the foundational aspects of biosensor development. Let me briefly recap for everyone.

We explored the world of electrochemical biosensors, also known as bioelectrochemical sensors, emphasizing their time efficiency, simplicity, sensitivity, and rapid response. These are essential features, especially when dealing with clinical samples and point-of-care devices. We then delved into the definition of electrochemical biosensors and their broad applications in environmental monitoring, healthcare, and biological analysis.

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Next, we discussed two primary types of biosensors: affinity sensors, which rely on

biomolecules like antibodies, nucleic acids, and nanobodies, and biocatalytic devices. We also touched on the first biosensor developed by Clark and Leon in 1962, which employed glucose oxidase as the key enzyme. From there, we examined the basic chemistry behind glucose oxidase, an enzymatic biosensor in which oxygen is reduced to hydrogen peroxide. This peroxide is subsequently converted back into oxygen by applying a voltage of 0.7 volts.

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During this redox (reduction-oxidation) process, two electrons and two protons are generated. These two electrons can be quantified, providing insight into how much glucose is present or consumed in the reaction. We then discussed the roles of the first and second electrode systems in these sensors, as well as the architecture of glucose sensors, which use the enzymatic amperometric method. It is referred to as amperometric because it involves measuring the amount of current generated in the process.

Following this, we reviewed the basics of first-generation glucose biosensors, particularly the YS123A glucose biosensor, examining its historical development and the underlying

theory. However, first-generation biosensors presented several challenges, including the high cost of platinum, the application of a relatively high voltage (0.7 volts), which led to the degradation of other compounds, and interference issues.

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THEORY OF REACTION AND MONITORING

- The YSI 23A biosensor was also based on Clark's electrode scheme. However, it relied on hydrogen peroxide oxidation for glucose monitoring since hydrogen peroxide is also produced by the enzymatic reaction at a concentration that is proportional to the glucose concentration. Briefly, for the construction of the biosensor, glucose oxidase was immobilized between two membrane layers. The first layer was a polycarbonate membrane which was used to permit only glucose molecules to move towards the enzyme layer by blocking the many other larger substances including enzymes and proteins available in whole blood thus, decreasing the interference effect of the species. Therefore, only glucose reached the enzyme layer where it was oxidized. The product hydrogen peroxide from this reaction passed through a cellulose acetate membrane which also acted as a barrier for the larger molecules. Finally, hydrogen peroxide was amperometrically detected at the platinum electrode surface.

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Now, we transition into discussing the second generation of glucose sensors. The prohibitive cost, interference from molecules, and the need for enhanced sensitivity drove the demand for further advancements in the field. This marked the emergence of the second generation of biosensors, a period that coincided with major developments in surface chemistry. This era saw the rise of techniques such as X-ray photoelectron spectroscopy (XPS) and screen printing technologies, borrowed from the field of microelectronics and semiconductor integration.

An important milestone in the second generation was the introduction of synthetic electron acceptors. These electron acceptors, which can be chemically synthesized, were crucial in improving the performance of biosensors by allowing for more precise and controlled electron transfer processes.

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The development of synthetic electron acceptors, especially in the form of redox couples or mediators, represented a major leap forward in the field of electrochemical glucose sensors. These mediators are capable of shuttling electrons between the enzyme's redox center and the electrode surface, enhancing the sensor's efficiency. Let me take a moment to explain this in detail to ensure clarity. Let’s revisit the core reaction taking place.

Imagine this: at the heart of the system, we have the glucose oxidase enzyme. The key reactions, specifically, the oxidation of glucose, occur inside the enzyme's pocket. For this to happen, both oxygen and glucose must reach the enzyme’s core. So, picture this enzyme with a pocket deep within its structure. Glucose must penetrate into this pocket, where the electron transfer occurs. Likewise, oxygen has to make its way into the core as well. Once both are inside the enzyme's pocket, the reaction proceeds, resulting in the production of gluconic acid and hydrogen peroxide.

Now, here’s where the challenge arises. To measure the electron movement during this reaction, the electrode must be positioned very close to the reaction site. However, most

electrodes are flat surfaces, while the reaction occurs deep within a three-dimensional pocket inside the enzyme. This spatial separation naturally lowers the sensitivity of the electrode in detecting electron transfer.

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This is where synthetic electron acceptors, such as inorganic redox couples and organic dyes, come into play. These molecules act as electron shuttles, transferring electrons from the core of the enzyme to the electrode surface. This concept of electron shuttling was revolutionary. These electron mediators are designed to efficiently penetrate into the enzyme's three-dimensional core, extracting electrons and carrying them to the electrode. In doing so, they paved the way for the second generation of glucose sensors.

These synthetic electron shuttles are remarkable because they not only facilitate the electron transfer from deep within the enzyme structure but also do so at much lower applied voltages. This significantly reduces interference from unwanted species, as we no longer need to apply high voltages, like the 0.7 volts used in first-generation biosensors, which often caused degradation of other compounds. Lowering the voltage mitigated this

interference, making the process more efficient and reliable. The reliance on oxygen concentration was also minimized, further improving sensor performance.

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In 1984, the first mediated amperometric biosensor for glucose was introduced, utilizing these synthetic shuttles. Simultaneously, advancements in screen-printing technology were being adapted for biosensor production. This ushered in the era of disposable biosensors, particularly in the field of glucose monitoring. The combination of electron mediators and screen-printed electrodes led to the creation of small, robust, cost-effective electrodes for amperometric biosensors. These innovations culminated in the first successful home-use blood glucose biosensor, which was based on mediators and screen-printed electrodes.

In 1987, these biosensors were commercially launched under the brand name Exactech by Genetics International, a company established between Cranfield and Oxford Universities. This marked the true rise of second-generation biosensors, building upon the first generation that spanned from the 1960s to the early 1980s.

The introduction of mediators was a critical milestone in the development of biosensors,

alongside the screen-printed electrodes. This second generation of biosensors held strong for some time. However, as with all technological progress, the field eventually evolved, giving rise to the need for even more advanced, third-generation biosensors.

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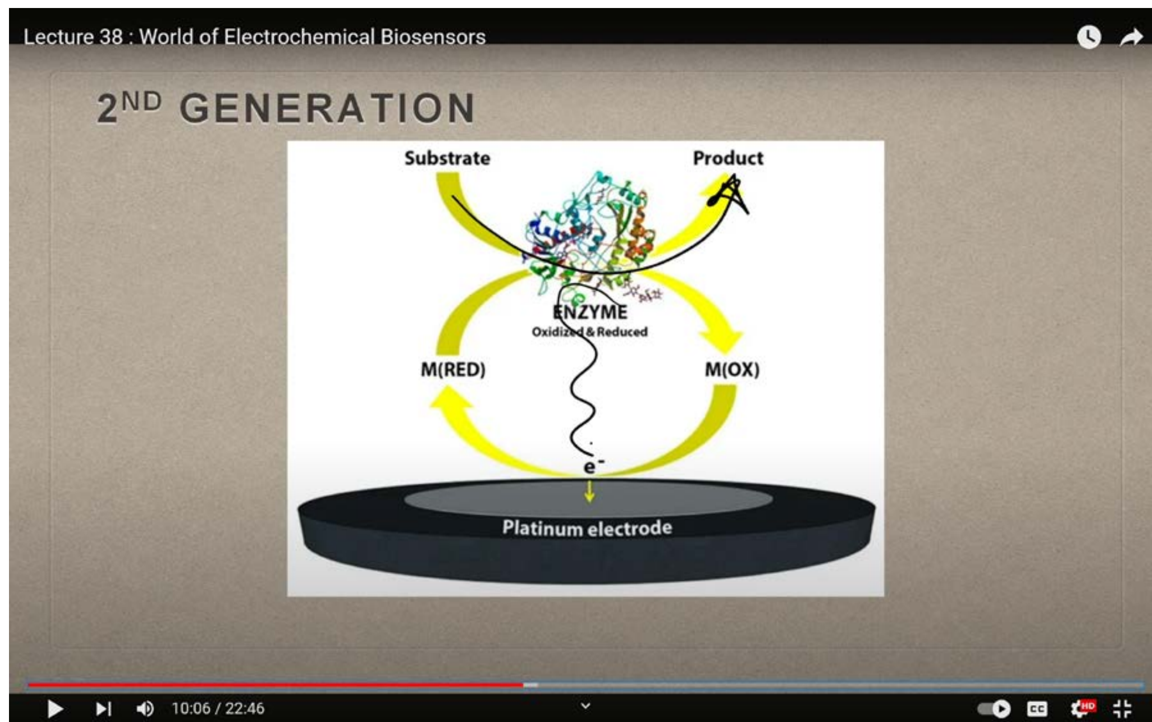

Now, let's delve into the third generation of glucose biosensors. Despite the advancements achieved with mediated biosensors, significant challenges remained, particularly concerning the wear and modification of enzymes. Here's the concept: imagine an enzyme with a reaction happening at its core. Ideally, any electrons generated from this reaction should be efficiently funneled out to the electrode. However, achieving this requires extensive chemical modification of the enzyme to integrate a molecular wire that facilitates electron transfer. This concept leads us to the use of molecular wires in third-generation glucose biosensors.

Even with the considerable success of mediated glucose biosensors, issues persisted with the biochemical structure of the glucose oxidase enzyme, as well as the solubility and toxicity of the synthetic mediators. These mediators, though effective, introduced a degree

of toxicity, and their instability affected the performance during extended use. Consequently, research shifted towards developing sensors based on direct electron transfer between the enzyme's redox center and the electrode.

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The challenge here is achieving direct electrical contact with the enzyme's redox center. The enzyme's redox center is often deeply embedded within a thick, electrically insulated protein shell, making it difficult to establish a direct electrical connection with the electrode surface. Therefore, minimizing the distance for electron transfer is crucial for enhancing sensor performance. This presents a molecular structural challenge.

One significant breakthrough came from Heller's research group, which made remarkable progress in this area. They developed a novel approach to establish communication between the glucose oxidase active site and the electrode. This involved using a long, flexible polymer, specifically polyvinyl pyridine (PVP) or polyvinyl imidazole, which served as a molecular wire. These polymers were embedded with a dense array of linked osmium complex electron relays.

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GENERATION 3: PROGRESS

- Heller's group reported one of the first smart routes to establishing this communication between the **glucose oxidase active sites and the electrode using a long, flexible poly(4-vinlypyridine) (PVP) or poly(vinylimidazole) polymer backbone which had a dense array of linked osmium-complex electron relays**

PVP

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GENERATION 3

Extremely small flexible electron

- In this way, **the redox polymer penetrates and binds the enzyme and forms a three-dimensional network. This immobilization process—wiring the enzyme to the surface—significantly decreases the distance between the both redox centers of both the polymer matrix and the enzyme. Due to the permeable nature of the applied polymer, glucose and the product of the reaction are easily transferred between the matrix and the electrode.**

MOLECULA

16:01 / 22:46

This innovation allowed the redox polymer to penetrate and bind to the enzyme, forming a three-dimensional network. This breakthrough significantly altered the landscape of glucose biosensor technology. By using a molecular wire to bridge the gap, the redox polymer reduced the distance between the polymer matrix and the enzyme's redox center. The porous nature of the polymer facilitated easy transfer of glucose and the reaction products between the matrix and the electrode. This method effectively brought the active site of the enzyme into close proximity with the electrode, allowing for efficient electron transfer and a more accurate signal.

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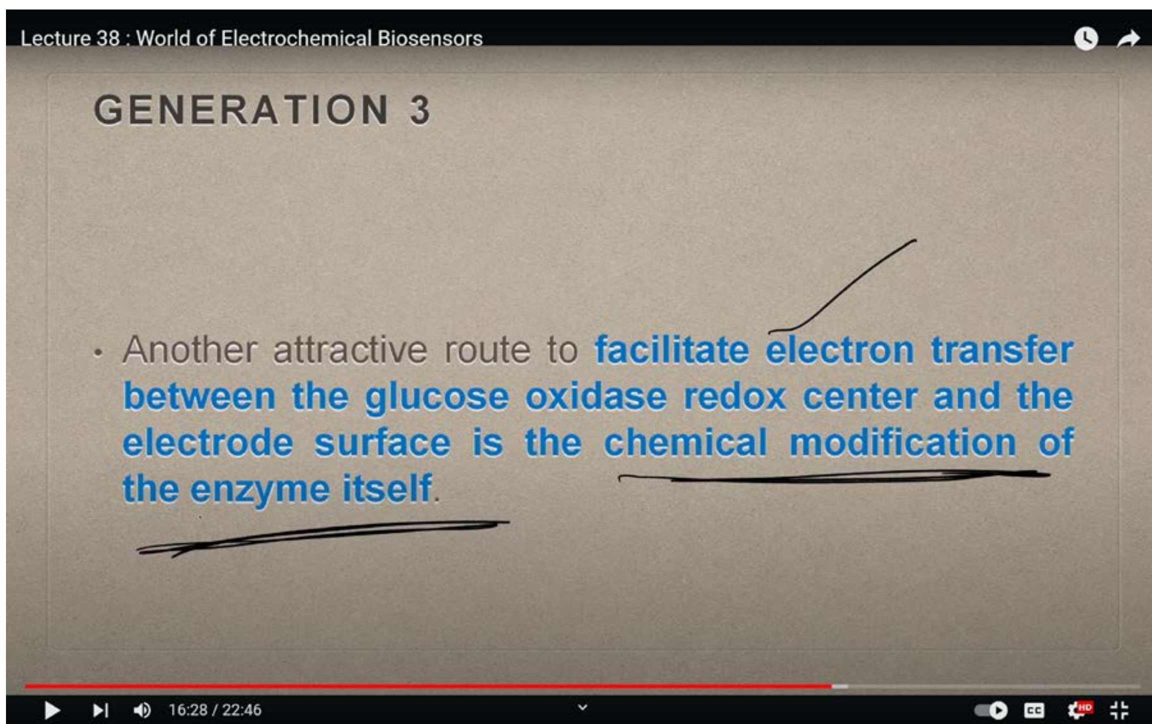

So, when we discuss third-generation glucose biosensors, we are talking about extremely small and flexible electrodes that can penetrate effectively. These are what we refer to as molecular wires, conductive molecules designed to access specific sites within the enzyme and extract signals from there.

Another promising approach to enhance electron transfer between the glucose oxidase redox center and the electrode surface involves the chemical modification of the enzyme

itself. This method was explored after the second generation of biosensors, particularly around 1987. In 1987, Degani and Heller demonstrated a significant advancement by covalently attaching ferrocene carboxylic acid, a notable electron transfer molecule, to glucose oxidase using carbodimide chemistry.

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GENERATION 3: DEGANI AND HELLER

- In 1987, Degani and Heller demonstrated the covalent attachment of ferrocenecarboxylic acid to the glucose oxidase via carbodiimide chemistry. When a sufficient amount of covalent attachment of ferrocenecarboxylic acid molecules to the enzyme was achieved, an enhanced electrical communication was obtained between the redox center of the enzyme and the electrodes (gold, platinum and carbon electrodes).

FERROCENE CARBOXYLIC ACID

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By achieving a sufficient level of covalent attachment, they were able to enhance electrical communication between the enzyme's redox center and the electrode, whether it was gold, platinum, or carbon. Notably, when carbon electrodes were used, it marked a significant shift towards more economical and sustainable biosensors. These breakthroughs marked a crucial evolution in the third generation of glucose sensors.

Then, in 1995, Wilner's group introduced an elegant approach to improve electrical contact. They treated glucose oxidase with electron relays, specifically modifying the enzyme's redox center, flavin adenine dinucleotide (FAD), with ferrocene. This modification involved removing the FAD from the enzyme, attaching it to ferrocene, and then reconstructing the apoenzyme with the ferrocene-modified FAD.

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GENERATION 3: WILNER'S WORK

- In 1995, Willner's group reported a highly elegant approach to improve the electrical contact by treating the glucose oxidase with electron relays. In this study, the redox center of the glucose oxidase—flavin adenine dinucleotide (FAD)—was removed and modified with ferrocene. This was followed the reconstruction of the apo-enzyme with the ferrocene-modified FAD.

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NANO TECHNOLOGY

miniaturization

1990

Glucose sensors

POC

Wilner's work, along with Heller's, laid the foundation for the third generation of glucose sensors. Looking ahead, we'll explore how nanotechnology, emerging in the late 1990s, further transformed the field. This advancement has had a profound impact, particularly in the miniaturization of point-of-care glucose sensors, significantly enhancing their performance and practicality.

So far, we have focused exclusively on enzymatic glucose sensors. Moving forward, we'll also delve into non-enzymatic glucose sensors. The shift to non-enzymatic sensors brings a significant reduction in cost since they don't require the pure glucose oxidase enzyme. Instead, these sensors utilize inorganic systems. We'll also explore the market for these sensors, which ties into last week's discussion on insulin. This exploration is particularly relevant given that India is considered the diabetes capital of the world.

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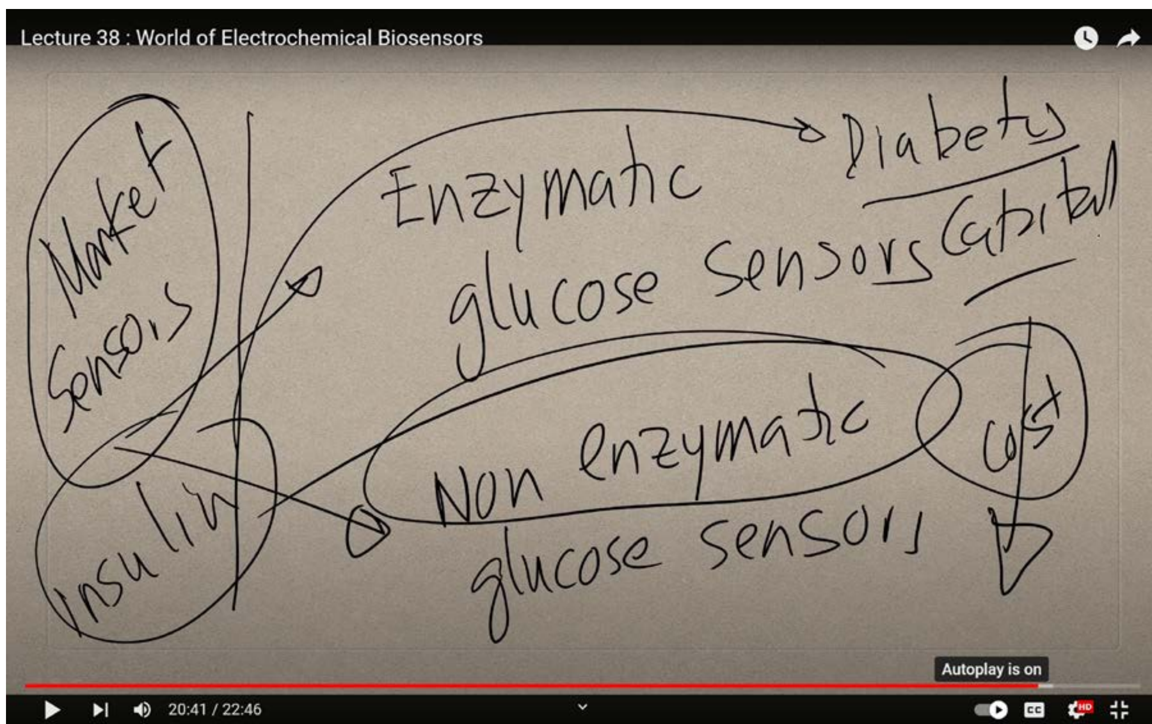

The glucose sensor market has evolved significantly over the past 60 years. Initially, we began with a basic reaction: glucose plus oxygen producing gluconic acid and peroxide. The first sensors quantified the oxygen consumption during the oxidation of glucose, as

measured by Clark's electrode. From these early beginnings, we have advanced considerably.

Wilner’s research highlighted the potential to fully utilize enzyme interactions, marking the start of a deeper understanding of electron transport chains and how electrons move between points. As we conclude today’s discussion, our next class will continue exploring both enzymatic and non-enzymatic glucose sensors and their growing popularity. We will also dive into the architecture of these sensors, examining the exact reactions occurring at the cathode and anode, the concept of polarimetry, and applied voltage. This exploration may extend into next week as we further unpack these concepts. Thank you.