ΕΘΝΙΚΟ & ΚΑΠΟΔΙΣΤΡΙΑΚΟ
ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
ΙΑΤΡΙΚΗ ΣΧΟΛΗ

ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ «ΝΑΝΟΪΑΤΡΙΚΗ»

ELECTROCHEMICAL BIOSENSORS EMPLOYING NANOMATERIALS FOR THE DETECTION OF CIRCULATING TUMOR CELLS (CTCs)

ΚΟΝΤΟΜΑΝΩΛΗΣ ΕΜΜΑΝΟΥΗΛ (20201092)

ΤΡΙΜΕΛΗΣ ΕΠΙΤΡΟΠΗ:

ΤΣΟΥΚΑΛΑΣ ΔΗΜΗΤΡΙΟΣ: ΕΠΙΒΛΕΠΩΝ ΚΑΘΗΓΗΤΗΣ

ΚΑΤΗΦΕΛΗΣ ΕΚΤΟΡΑΣ

ΔΕΜΕΤΖΟΣ ΚΩΝΣΤΑΝΤΙΝΟΣ

ΑΘΗΝΑ 2022

Contents

Summary

Biosensors can detect various biomolecules originated from biological systems and convert them into useful signals, voltage or current. In other words, they are analytical devices that convert biological responses into quantifiable and editable signals. The purpose of the biosensor is to provide reliable information to analysts quickly and accurately. Regarding the electrochemical parameter which is used as a method of detection, biosensors are divided into the following categories: (a) ammetric, (b) voltammetric, (c) potentiometric, (d) conductive. Regarding the biological identification element used in the sensor detection electrode, there are different types of biosensors as well. Among these biosensor types, enzyme biosensors are used more often. However they can only be used, provided there are complex samples and large or charged molecules (ions). Obviously, biosensors contribute to the development of diagnostic methods used to examine infectious diseases.

1. SENSORS

1.1 Definition – Operating Principle

Based on the definition proposed by the IUPAC, biosensors are defined:

"A biosensor is a stand-alone device capable of providing specific quantitative or semi-quantitative analytical information using a biochemical receptor that is in direct contact with a converter element. A biosensor should be clearly distinguishable from a bioanalytic system, which requires additional processing steps, such as the addition of reagent. In addition, a biosensor should be distinguished from a bioprobe which is either disposable after a measurement, ie disposable, or unable to continuously monitor the concentration of the analyte".

Biosensors comprise oftwo primary parts associated in arrangement:

The organic identifier and the physicochemical signal exchanger (Thevenot et al., 2001). The signal may be the result of a change in the concentration of protons, the uptake or release of gases such as oxygen and ammonia, the emission of light, reflection or absorption, thermal emission or other mechanisms resulting from the action of sensor molecules. This signal can then be converted from a signal converter to a measurable physicochemical quantity. Both the biological and the electrical signal can then be further utilized by amplification or promotion.

Biological identification element

This element translates biochemical information, analyzing the concentration, into a physical or chemical signal, with specialized sensitivity. This is usually a limit, which can either degrade the substrate, or there may be some structural change or change its biological or physicochemical properties during the bonding process. This material is immobilized on the sensor interface with the sample to be analyzed. Based on the way the biomarker interacts with the analyzer, biosensors are divided into two major categories:

i) Affinity biosensors (chemical touch)

The operation of the biosensor is based on the interaction of the analyzer with the biological material. The recognition and binding of the analyte is due to the reversible binding between receptor-substrate, which exhibits complementary structures, and the restoration of equilibrium. In this class the biological receptor may consist of antibodies, oligonucleotides or lectins. The main disadvantage of these systems is the strong interconnection between receptor-analyte, which does not allow the rapid regeneration of the biosensor.

ii) Catalysis Biosensors

Catalysis biosensors are based on the catalytic action of biological receptors – extremities that are protected in their biological environment (cells, microorganisms, etc.) or have been previously isolated from it, and may also have undergone genetic modification. In catalysis biosensors, the analyzer reacts in the presence of the biomarker to a product (P).

Analyzer S / substrate detection can be based on:

→ In the consumption of substrate S and its consequent reduction signal.

→ In the production of product P and the subsequent increase of the mark.

Signal converter

The physicochemical changes that take place during the interaction between the biological part of the biosensor and the analyzer must be converted into a signal, which will then be properly processed in order to obtain the detailed information. The choice of signal transducer is determined by the result of the biological identification and the character of changes it brings to the system.

Different parameters are changed at the same time, thus allowing the selection and use of different signal exchangers. Biosensors depending on the signal exchanger used are categorized into:

- ✓ Electrochemical (amperometric, potentiometric, voltammetric)
- ✓ Opticians (UV / Vis absorption, fluorescence)
- ✓ Thermal
- ✓ Mass

1.2 Electrochemical Analysis Techniques

Electrochemical analysis techniques involve a wide variety of techniques, each of which is based on a particular phenomenon, performed on an electrochemical element. The experimental electrochemical system consists of the electrolyte, which conducts current, the electrodes, and the measuring circuit or external circuit, which is used to apply and measure electrical signals. Various electrical quantities are measured, such as e.g. current, potential, resistance (conductivity), single or in combination, and based on measured values quantitative or qualitative analysis is performed. In an electrochemical analysis, either the relationship between electrical signal size and activity (concentration) is determined, either the electrical signal is used to determine the end point of a titration or the current is converted to designated chemical entity in a defined form determined either by the station or by the amount of electrical charge consumed.

Definitions

The electrodes of the electrochemical cell are the rise and fall of the cell. Regardless of the type of element, reduction reactions are always performed at the cathode and oxidation reactions at the anode. Indicative electrode (indicator electrode) is the electrode, which shows a potential reliant to concentration of the solution, reference electrode (reference electrode) is the electrode, which shows a constant and repetitive potential. Irrespective of the constitution of the solution and the alterations in current, the working electrode is the one at which the controlled or monitored electrode reaction takes place and auxiliary or counter electrode, which consists of an inert metal, through which current passes, thus avoiding the passage of current through the reference electrode.

Electrode processes

Two types of processes take place at the electrodes, faradaic processes and non-faradaic processes. In Faraday processes, governed by Faraday law (ie that the amount of the substance is oxidized or reduced, is proportional to the amount of electricity passing through the solution) electrons are transferred through the electrode-solution interface (phase) and therefore oxidation or reduction takes place, because it is not possible for the electrode to survive. The electrodes in phantom processes are called charge transfer electrodes and the observed current is called **faradaic current (i_F).** In non-faradaic

processes no electrons are transferred across the interface electrode-solution, because it is not favored kinetically or thermodynamically. Such processes are the adsorption and desorption of substances at the electrode and the change of interface properties during changes in the electrode potential or the composition of the solution. Although no electrons are moving in the non-faradaic processes, currents can still be observed, even if transiently, when the potential or surface of the electrode or the composition of the solution changes. This current is called **capacitive current, i_c.** An interesting example of a faradaic process is the charging of an electrode, in which the capacitive current charges the electrical bilayer on the electrode surface to the equilibrium value corresponding to the electrode potential.

An electrode in which no charge is transferred through the metal-solution interface for any value of potential applied from an external voltage source is ideally called a polarized electrode. Only non-faradaic processes can be performed on such an electrode. If a depolarizer is added to the solution, i.e. an electroactive substance, which can be reduced or oxidized, then a faradaic current is passed and the electrode is depolarized.

When the faradaic current begins to flow, the balance between oxidized and reduced form is disturbed, but it can be continuously restored, as long as the electrode reactions are reversible. If there is a delay, then the actual potential Energy of the electrode is different from the potential Eeq in the equilibrium state. The difference n = E-Eeq is the superconductivity (hypertension) of the electrode (downward or upward). When superconductivity (hypertension) is the condition in which certain materials have zero dc, have a polarizer concentration on the terminal surface lower than its concentration in the ground configuration, due to the moderate polarizer growth towards the terminal, at this point it stops there is concentration polarization. When superconductivity (hypertension) is due to the moderate development of electrons due to inertia of the chemical system, we talk about activation polarization. Both types of polarization are possible coexist.

1.1 ELECTROCHEMIC SENSING DEVICES

Electrochemical biosensors are the main class of biosensors. These sorts of biosensors have a number of preferences that makes them especially appealing to utilize. The

primary to apply potentiometric and non-potentiometric, while followed by inducible field mutant biosensors and conductors. Electrochemical biosensors are the main category of biosensors.In biosensors the measurement of electrochemical properties for extracting information from Biological systems are usually electrochemical in nature and the bioelectrochemical element acts asthe main mutant (transduction element).Although biosensors are being exploitedvarious biosensors, electrochemical detection techniques mainlyenzymes. With the help of nanotechnology mass production is low cost, with durability and quality.

Also:

They can be applied to complex samples.

- ✓ Good sensitivity.
- ✓ They are compatible with several biochemical systems.
- ✓ Simple integration with electronic systems.
- ✓ Reliability and speed during in vivo and in vitro bioassay.
- ✓ Functionality at ambient temperatures (low power consumption, ie ideal for portable systems).

The most restrictions within the utilize of electrochemical biosensors are:

- ✓ The nearness of electroactive substances within the sample, which interfere with the determination.
- ✓ Passivation of the terminal surface (fouling).

The following may be a brief depiction of the principle of operation of the different electrochemical signal exchangers:

Electrochemical cells

Reduction reactions of oxidation or redox occur in electrochemical cells. There are two types of electrochemical cells. Spontaneous reactions occur in galvanic (voltaic) cells. non-spontaneous reactions occur in electrolytic cells. Both cell types contain electrodes where oxidation and reduction reactions occur. Oxidation occurs at the electrode called the anode and the reduction occurs at the electrode called the cathode. Electrochemical cells are used in electrochemical biosensors and as mentioned earlier they consist of 3 main electrodes which will be analyzed below. These electrodes have a significant

function in the presentation of biosensors. The electrode material, its dimensions and the surface treatment significantly affect the detection ability of the sensor. The three types of electrodes are:

Reference electrode: The anode of an electrolytic cell is positive (the cathode is negative) since the anode attracts anions from the solution. However, the anode of a galvanic cell is negatively charged, as spontaneous oxidation at the anode is the source of the cell's electrons or negative charge. The descent of a galvanic cell is its positive terminal. In both galvanic and electrolytic cells, oxidation occurs at the anode and electrons flow from the anode to the cathode. A reference electrode is an electrode which has a stable and well-known electrode potential. The high stability of the electrode potential is usually reached by employing a redox system with constant (buffered or saturated) concentrations of each participant of the redox reaction. There are many ways reference electrodes are used. The simplest is when the reference electrode is used as a half-cell to build an electrochemical cell. This allows the potential of the other half cell to be determined. An accurate and practical method to measure an electrode's potential in isolation (absolute electrode potential) has yet to be developed.

Auxiliary or counter: In a two-electrode system, when an acknowledged potential is placed between a reference electrode and an supplementary electrode, additional variables can be quantified. The supplementary electrode acts as a cathode on the condition that the reference electrode functions as an anode and vice versa.

Working electrode: The working electrode is the electrode in an electrochemical system on which the reaction of interest is occurring. The working electrode is often used in conjunction with an auxiliary electrode, and a reference electrode in a three electrode system. Depending on whether the reaction on the electrode is a reduction or an oxidation, the working electrode is called cathodic or anodic, respectively. The types of electrochemical biosensors are analyzed separated by the nature of the electrochemical alterations detected during a bio – cognitive reaction. (Grieshaber et al., 2008).

1.3.1. Potentiometric Sensing Devices

Potentiometric tools estimate the increase of electric potential at the working electrode contrasted to the reference electrode in an electrochemical cell in the event that the current flowing through them is either zero or relatively insignificant. That is,

potentiometry contributes facts about the behavior of ions in an electrochemical reaction (He, Zhou, He, Wang, & Qin, 2011).

The potential contrast that develops is due to the specific binding of the analyzer to the working anode surface and is proportional to the logarithm of the analyzer action, as described in the Nernst equation.

In the case of biosensors, the transduction of the biological reaction to an electrical signal is done using ion-selective electrodes. Specifically, it contains an immobilized enzyme membrane bordering the probe by a pH estimation, where the catalytic reaction produces or soaks up hydrogen ions. The response that occurs near to the narrow glass membrane detects the alteration in pH that can be counted immediately using the pH screen. Characteristic of the applying of systems is that the electrical potential is regulated in a high degree of opposition permitting virtually zero current flood, without interfering with the reaction (He et al., 2011).

There are three types of selective electrode ions utilized in biosensors:

Glass electrodes for cations (eg normal pH electrodes) in which the detection element is a very thin hydrated glass membrane that produces a transverse electric potential due to concentration-dependent competition between the cations for special binding sites.

Glass pH electrodes which are coated with a membrane through which air passes CO_2, NH3 or H_2S. The spreading of the fumes via this membrane causes pH alteration of a sensory mixture between the membrane and the electrode which is then ordained.

Solid state electrodes where the glass membrane is exchanged by a narrow ionic membrane made of a blend of silver sulfide and silver halide.

1.3.2. Amperometic Sensing Devices

The operation of ammetric biosensors is based on the production of current when a potential between two electrodes. In general, response times, dynamic regions and their sensitivity are similar to potentiometric biosensors.. With a biological perspective, amperometric biosensors are constructed by the detection of electrochemically active substances, which are produced or consumed in the process of biological recognition. These compounds are oxidized or reduced directly to the working electrode and the analytical signal comes from the measurement of the electrons produced. They are the most sensitive system for converting biological recognition into analytical signal, as

recording currents in the order of 10-9 Å requires a reaction of only 10-14 moL / s (for single-electron redox reactions).

The reactants, one of which is usually the analyte (analyte), are diffused into the enzyme layer, where their conversion to products from the immobilized enzymes is catalyzed. Achieving the measurement requires at least one of the reactants or products to be electrochemically active. Oxidation and reduction at the electrode produces the measured signal (current). If the electroactive substance is one of the products of the reaction, then an increase in current is recorded when the sample is added. If the electroactive substance is a cofactor of the enzymatic reaction, then its amount must be constant and in relative abundance. In this way the enzymatic reaction is affected only by the analyzer, and any change in current is correlated with the amount of analyte in the sample (Fig. 1). The amperometric biosensor for the location of α-ketoglutarate (α-KG) was developed by means of an electrochemical advance, in which the glutamate dehydrogenase (GLUD) was adjusted on the face of diminished graphene oxide-gold nanoparticle composite (rGO-Aunano composite). (Peng et al., 2020). The rGO-Aunano/GCE was moreover found to electrocatalyze the oxidation of β-nicotinamide adenine dinucleotide (NADH) at the top potential of 0.3 V. (Peng et al., 2020) After the modification of GLUD, the GLUD/rGO-Aunano/GCE driven to viable amperometric discovery of α-KG through observing the NADH utilization and shown a linear response within the extend of 66.7 and 494.5 µM, with the location restrain of 9.2 µM. (Peng et al., 2020)

Diffusion and mixing and then diffuse inside the enzymatic membrane, at which point the reaction takes place at a rate determined by the kinetics of the enzyme [108]. The current profile, in relation to time, decreases exponentially and after the restoration of equilibrium (where the current has reached a constant value) its value is given by the Cottrell equation:

$$i = nFADC / \delta$$

where:

n: number of electrons involved in the redox reaction, F: the Faraday constant, D: the diffusion coefficient of the substance, C: the concentration of the substance and δ is a constant related to the thickness of the diffusion layer.
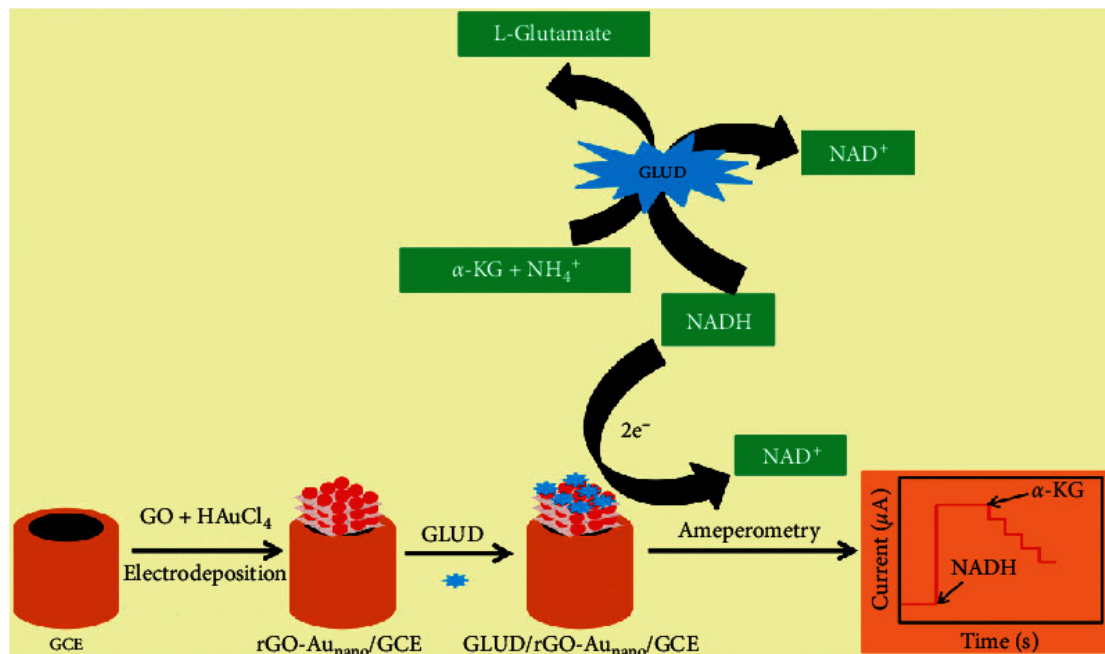
Figure 1. Example of amperometric biosensor. Illustration of the preparation process and the sensing mechanism of the α-KG biosensor. (Peng et al., 2020)

1.3.3. Conductive Sensing Devices

These systems measure the capacity of an analyst (e.g.electrochemical mixture) or a medium (eg nanowires) electrical for the energy between the two electrodes.. In most cases, conductivity devices are connected with chemicals, where the ionic quality, and so the regulation of an arrangement amidst two electrode alterations as a result of an enzymatic response (Koyun et al., 2012).

Conductivity biosensors are determined by the fact that almost all of them are enzymatic and therefore lead to a general change in the ionic composition of the test sample. Biosensors determined by the conductivity have several advantages:

- ✓ Narrow film electrodes are appropriate for shrinking devices and large scale manufacturing applying cheap technology
- ✓ Do not depend upon a reference electrode
- ✓ Signal transformers are not responsive to light
- ✓ The used voltage may be low enough to reduce the loss of energy
- ✓ A wide variety of combination of diverse natures can be determined based on unusual responses and mechanisms.

The analyzed liquids are considered to have a background, which can be easily determined by various factors and therefore the specificity of this method is considered to be low. On the other hand, in the case of an integrated micro-sensor, most of these difficulties can be overcomed, using a differential estimation that compensates the changes in the managing of the background, the effect of changes in temperature and additional factors. Although conductivity biosensors have not been used as much as they should be, there are some examples of their applications, such as the detection of drugs in human urine samples and the detection of pollutants in environmental controls. Entire cells have also been used as bioreceptors in conductivity biosensors for toxic analyzes, immobilizing them in signal electrodes. (Koyun et al., 2012).

1.3.4 Field Effect Transistor (FET) Sensing Devices

Field effect sensors are devices that operate based on semiconductor technology and mainly on metal oxide conductor technology, such as SiO_2, Al_2O_3, etc. (MOS, Metal Oxide Semiconductor). Their principle of operation is determined by the interaction of the sensor surface with the ions of the solution, which affects the resistance of the semiconductor through field effect phenomena. Field effect sensors attached to the surface of a biomembrane are commonly referred to as ENFET (enzyme FET) or IMFET (immunological FET).

1.4 Design of amperometric biosensors

The basic stages in the development and construction of a biosensor are analyzed below:

Enzyme immobilization

The   foremost essential step   in building a biosensor   is   to   immobilize the biological material on the sensory support and join it to the signal transducer (Fig. 2, 3). Immobilization of the enzyme in such a support influences its characteristics (balance, soundness, and catalytic activity), hence, influencing the haracteristics of the biosensor. The immobilization strategy must the availability of the substrate to the

dynamic centers and the quick transport of a mass of substrates and items to and through
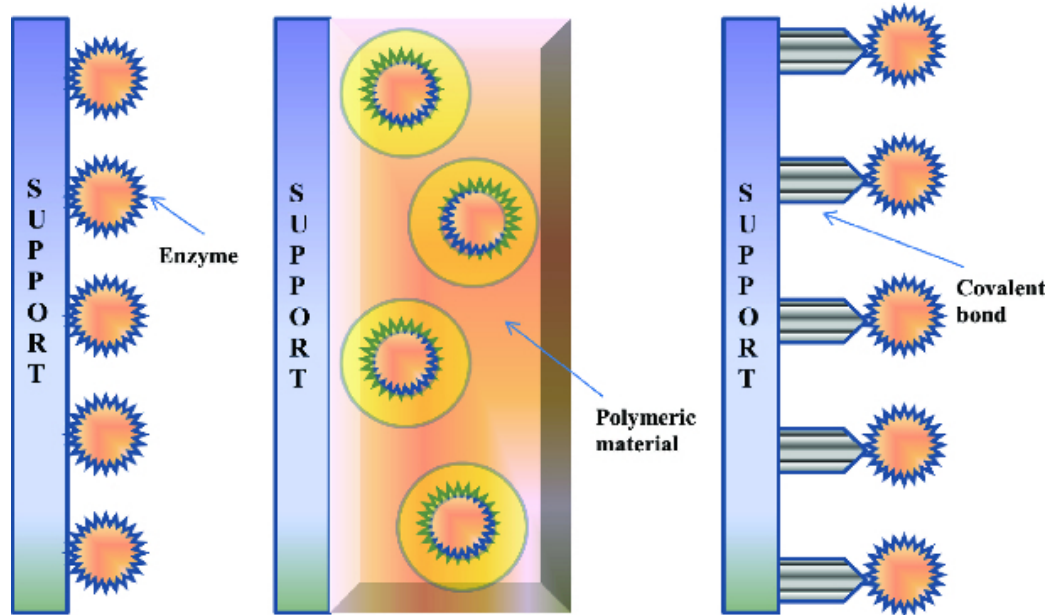

Figure 2. Illustration of schemes of the three most common enzyme immobilization techniques: (A) physical adsorption, (B) entrapment and (C) covalent attachment/cross-linking. (Mohamad et al., 2015)
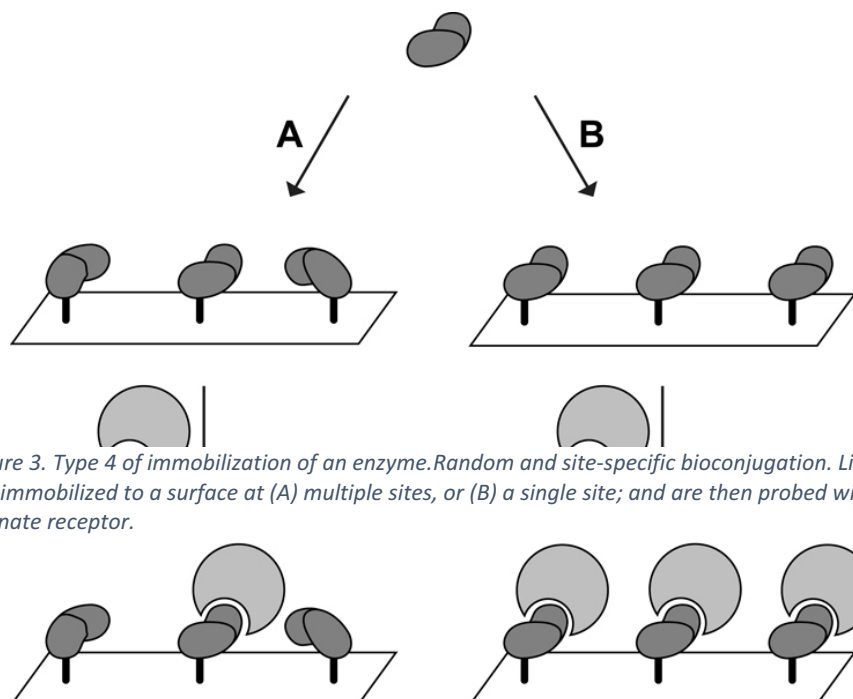
the immobilization layer.

Figure 3. Type 4 of immobilization of an enzyme.Random and site-specific bioconjugation. Ligands are immobilized to a surface at (A) multiple sites, or (B) a single site; and are then probed with a cognate receptor.

1.4.1   **Adsorption.**

Application or infestation is a property of some chemicals to bind another substance to their surface. Offer is the attachment of atoms, ions or molecules of a gas, liquid, or dissolved solid to a surface. This process creates a film of adsorbent material on the surface of the adsorbent medium. This process is different from absorption, in which a liquid passes through a liquid or a solid or dissolves in it. Attacking is a process that takes place on the surface, while absorption concerns the entire volume of the material. The term aspiration includes both processes, while the reverse is secreted. **Trapping.** A thin film of enzyme is placed on the surface of the electrochemical detector and trapped there by means of a polymeric membrane (usually cellulose acetate, collagen, Teflon and polyurethane) permeable to the analyzer. This method was applied to the original constructions of biosensors and has the advantage that it reduces the contamination of the enzymes by components of the sample, which at the same time is maintained in its normal state. The main disadvantage of the method is the low stability.

1.4.2   **Cage.** The enzymes are mixed with soluble solutions, which are then multiplied under appropriate conditions encapsulating the biomarker. Commonly used polymeric membranes include polyacrylonitrile as well as sil-gels. In recent years, multicomponent conductors, such as polypyrrole, have also been used to achieve faster transport of electrons from the enzyme to the electrode face. The main disadvantages of the method are the long response time, due to the slow diffusion of the substrate through the polymer as well as the short life time which is mainly determined by the stability of the polymer lattice.

1.4.3   **Biological status.** The biological boundaries are attached directly to the electrode surface by bonds formed between active electrode surface functional groups and active amino acid residue groups on the outer surface of the enzyme. The method favors the immobilization of enzymes in a controlled orientation. The enzymes are kept in close contact with the electrode favoring the rapid transfer of electrons, while also there are no barriers to the diffusion of the substrate. However, this method leads to biosensors with low mechanical stability, while care is required so that the binding is done through amino acids that are not necessary for the catalytic action.

1.4.4 **Cross-border Connection.** The enzymes bind to the surface of the electrode or support material with the aid of reactive reagents, such as glutaraldehyde and carbadioid. The enzymes bind either to each other or to other inactive proteins to form cross-linked bonds. The main drawback of the process is the large loss of enzymatic function, due to the utilization of chemical reagents and organic solvents.

1.4.5 **Creation of Self-Assembled Monolayers.** In recent years, a method of immobilization that has been developed is based on the creation of self-constructing structures. This process is determined by the automatic organization of smaller boundaries into boundary structures. In contrast to the traditional organic composition, where the various synthetic stages involve the dissolution and formation of bonds and take place under kinetic control, the creation of SAMs is based on the development of weak bonds (van der Waals, hydrogen bonds, etc.) and the process is done under thermodynamic control.

To date, no immobilization method has been developed that is generally applicable to all biological materials and all support bases. The main problems encountered are the stability and repeatability of the systems. The immobilization method is optimized accordingly for each biological limit.

Cyclic Voltammetry

Voltammetry is a process by which information about the analyzer is acquired by the changing of the potential and then counting the resulting current. It is therefore an amperometric technique. But since there are several ways to change the potential, there are several ways of voltammetry. Some examples are polarography, linear, pulse and reverse pulse voltammetry. The most widely used technique, however, is cyclic voltammetry and is used to obtain information on redox potential and electrochemical response rates in analyte solutions. In this case, the voltage is scanned between two values at a constant rate, but when the voltage reaches its maximum value, the scan is reversed and returns to its original value. A critical value in cyclic voltammetry is the scan rate, as the duration of the scan must give adequate time for the chemical response to take place. Therefore, changing the scan rate results in a change in performance (Grieshaber et al., 2008).

These days, fast scanning circular voltammetry is getting to be increasingly common, through which the quick flow of neurotransmitters are checked, which seem not be observed with systems.The high affectability of the method uncovered that the fast discharge of dopamine amid intracranial self-stimulation is broken down into smaller release sums, emphasizing that dopamine is not the remunerate signal.
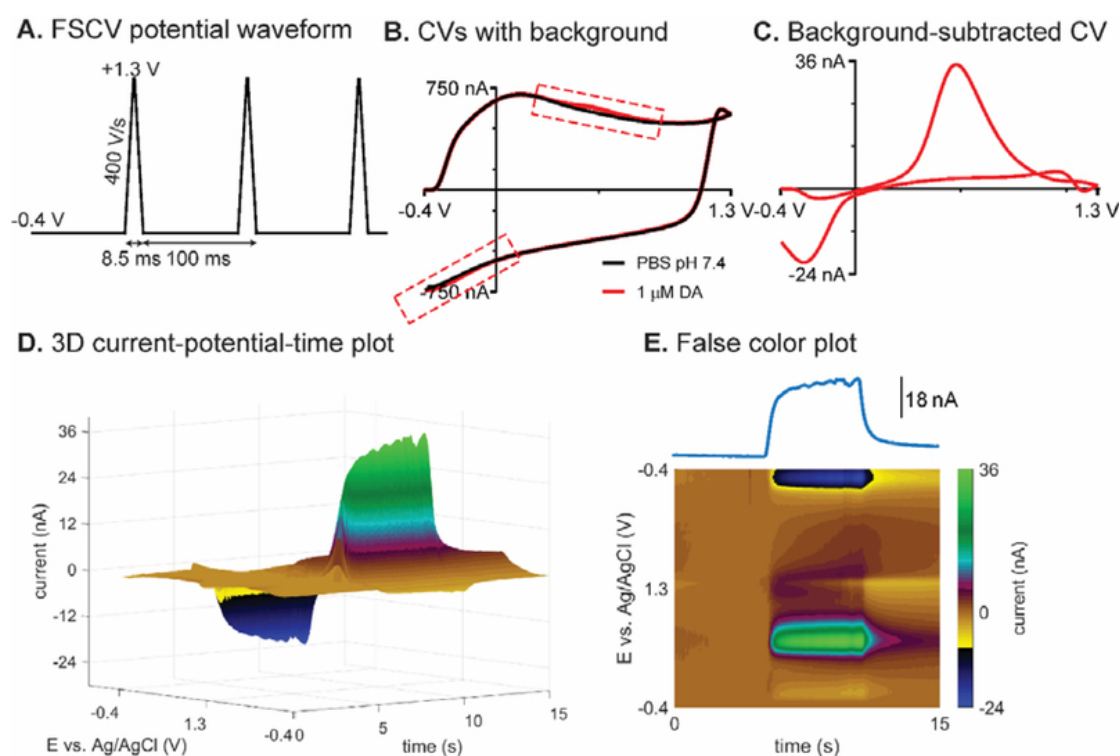

Figure 4. FSCV of dopamine. (A) Connected potential waveform utilizing –0.4 V holding potential, +1.3 V exchanging potential, 400 V/s check rate, and 10 Hz reiteration rate. (B) Illustrations foundation streams: clear (PBS pH 7.4) (dark) and buffer with 1 µM dopamine (ruddy). Dashed boxes emphasize the distinction between them. (C) Background-subtracted CV of 1 µM dopamine. (D) Three-dimensional current-potential-time plot and (E) Customary untrue color plot with anodic top current-time follow of 5-s bolus infusion of 1 µM dopamine. (Puthongkham et al., 2021).

1.5 Electroanalytical techniques (amperometry and potentiometry)

Potential is practiced to the working electrode and the coming current is recorded during the time. Between the disintegrant and there's a dissemination layer on the surface of the cathode. The concept of this layer was proposed by Nernst. Dissemination regulates the exchange of the analyte from the most of the solution to the higher concentration range near the electrode. There is therefore a concentration step from the solvent on the electrolyte surface. This situation can be

described by the Cotrell equation, which defines the current-time base for linear diffusion regulation at an electrode (Grieshaber et al., 2008).

$$i = \frac{nFAc_j^0 \sqrt{Dj}}{\sqrt{\pi t}}$$

I = current, in A (units), n = number of electrons (to oxidize or reduce one molecule of analyte j, for instance), F = Faraday constant, 96485 C/mol, A = area of the planar electrode in cm^2, D_j = diffusion coefficient for species j in cm^2/s, t = time in s.

A significant practice of chronoammetry is real-time detecting of electrochemical neurotransmission by Grieshaber et al., 2008. They supply a technical description and comparison of temporal and chemical analysis of chronoammetry, rapid scan cyclic voltammetry, and high-velocity time-aminometry in researches with different cells, brain fragments, and intact animals. In chronoammetry, potential is counted as an activity in the time in response to a constant or square current wave. This technique was used to investigate the uptake of human serum albumin (HSA) in SAMs (Grieshaber et al., 2008).

1.5 Electrochemical impedance Spectroscopy

Electrochemical impedance (impendance), ie the impedance of a system, is more often than not counted by practicing nonstop potential to an electrochemical cell and a following current measurement. Within the case of sine wave potential, the reaction may be a coordinate current. This signal can be analyzed as a whole of sine functions (Fourier arrangement). By changing the excitation frequency f of the connected potential over a recurrence extend, one can calculate the impedance, as the sum of the real and imaginary impedance components, of the system as a work of frequency. In this manner, EIS combines the investigation of both real and imaginary components of impedance, ie electrical tolerance and reaction (Grieshaber et al., 2008).

For electrochemical location, impedance strategies are valuable for observing changes in electrical properties coming about from bioassay occasions on the surfaces of altered electrodes. Changes in final conductivity can be measured due to protein immobilization and antigen antibody responses at the electrode surface. With the EIS

can be utilized to develop an arrangement of crosslinked electrodes and screen antibody antigen responses within the holes among the electrodes.

2. ELECTROCHEMICAL BIOSENSORS

2.1 Introduction

A biological identification component, or bioreceptor, comprises of an immobilized biomolecule capable of identifying the target to be analyzed. Immobilization of biomolecules on a surface is the method of joining a biomolecule to a substrate. This comes about within the loss of mobility but without losing its usefulness. Immobilization can cause auxiliary changes within the biomolecule which will inactivate it by a rate. The biomolecule can be immobilized within the off-base heading with the result that its dynamic center is blocked or "covered up" by the bolster fabric and the analyzers to be recognized not have get to to it. To join a natural substance to a surface, it is vital to relate a bunch of the organic substance with a gather of the surface. Moreover, an critical parameter is the creation of fittingly altered surfaces by immobilizing biomolecules in such a way as to attain affectability, selectivity and life span. (Perumal & Hashim, 2014).

Illustrations of such biomolecules are basically antibodies, enzymes, cells and nucleic acids. The interaction among the analyzer and the bioreceptor causes a chemical alteration including the mass of additional chemicals, the release of warm, the flow of electrons and pH changes. One of the fundamental alluring and essential characteristics of the biosensor is the sensitivity and selectivity towards the specific and as it were target that you simply need its recognition, in arrange to dodge the impedances of a distinctive substrate from the sample to be analyzed. The bioreceptor or the component of biological identification could be a characteristic that decides the type of biosensors. In general, they can be sorted according to the form of component they use to get the biological signal. The more common categories are referred in the picture underneath (Perumal & Hashim, 2014).
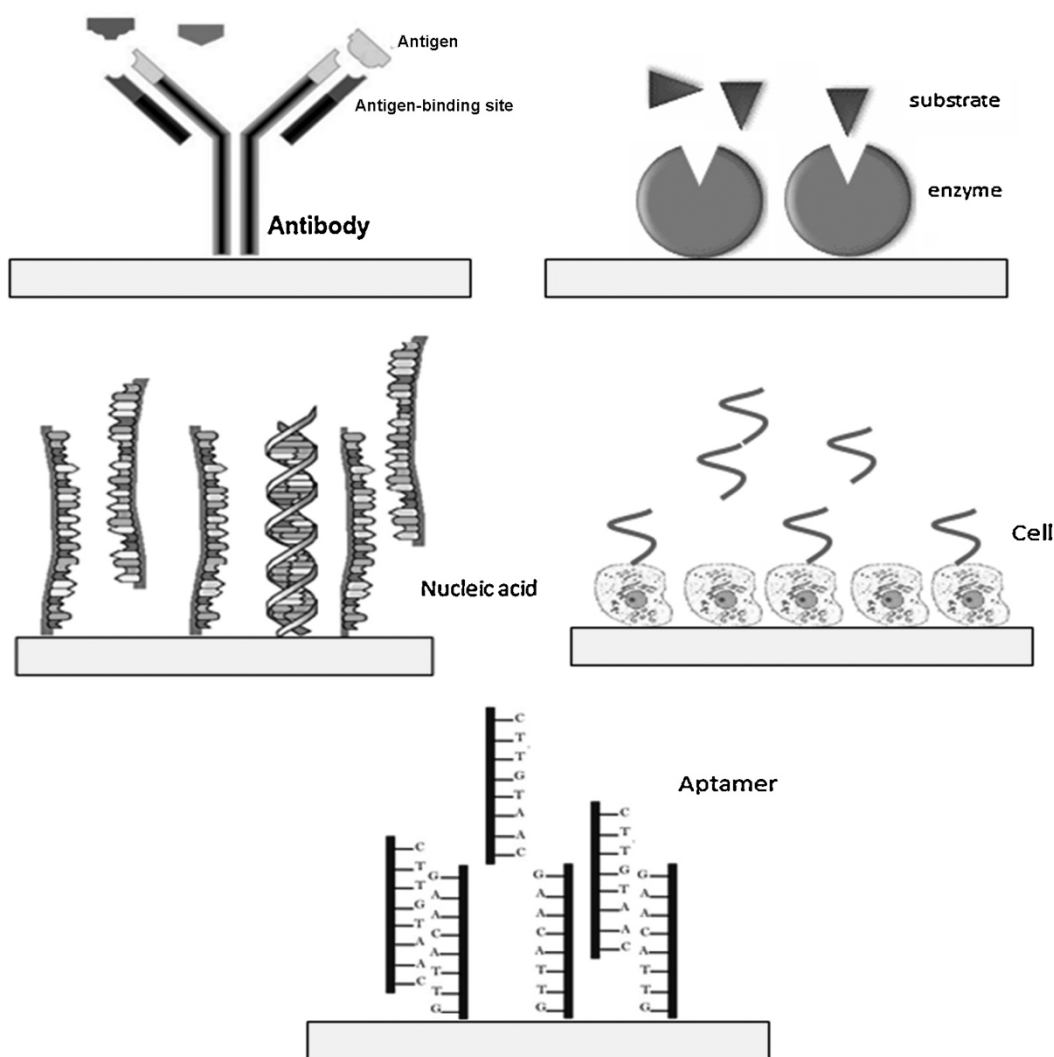
Figure 5. Methods of biosensing with various biological signal mechanism: (a) antibody/antigen; (b) enzyme catalyze; (c) nucleic acid; (d) cell-based; (e) biomimetic. (Perumal & Hashim, 2014)

2.2 Electrochemical DNA Biosensors

The high and selective affinity of the binding two single stranded DNA (ssDNA) chains that create double stranded DNA (dsDNA) is the basis of nucleic acid sensors as an element of biological recognition. The method of operation of this type of biosensor is determined by the acknowledgment of the supportive strand of ssDNA to create steady hydrogen bonds among the two single-stranded nucleic acid up dsDNA. To realize the process, an immobilized single-stranded ssDNA is utilized as a test in a bioreceptor in which the base grouping is matching with the target area. Introduction of the mark to the test comes about in hybridization of the assistant ssDNA to dsDNA designation, coming about within the generation of a

biochemical response that allows to intensify the flag into an electrical one. Concurring to the literature, the nearness of a ligand such as thiomol or biotin is vital within the endeavor to paralyze the ssDNA at the discovery side. An significant possession of DNA is that nucleic corrosive ligands can be destroyed in invert and recovered by regulating the mass of the administrative ion. The nucleic acid layer as an element of biological recognition that integrates into the biosensory organ is easily synthesized, provides high specificity, and is reused after thermal melting of DNA. Moreover, this biosensor has an important of measuring the concetration of single molecular species in a composite solution. The DNA-based biosensor has potential applications in clinical diagnoses for the detection of viruses and diseases. The designation of an electrochemical biological DNA sensor has received much attention in the latest years due to its rapid response, high sensitivity and selectivity (Perumal & Hashim, 2014).

Most of the strategies utilized for DNA location for diagnostic purposes are PCR and Q-PCR. Numerous E-DNA Biosensors utilize inverters that have been created for real-time discovery without an enhancement step. They contain conductive materials competent of changing their electrical properties after the hybridization response on their surface. Conductive polymers (CP) are broadly utilized as converters for biological interactions. They are polymers with an electronic structure that gives them characteristics such as low ionization potential, high electron exchange capacity and optical properties. This can be related to their electrical, electrochemical properties, as well as their capacity to immobilize on a micro-sized surface by electropolymerization. The location of DNA hybridization based on CP has been accomplished by measuring their electrical properties through amperometric strategies, impedance, redox markers. Combining them with graphene or carbon nanotubes has been appeared to progress detection performance.

In DNA electrochemical biosensors, DNA is immobilized on the surface of the electrode and its electrochemical behavior is studied in relation to compounds that show adhesion to it.

- In DNA only nucleic acids and in particular bases are electroactive and produce electrochemical signals.

- Based on the type of interaction between DNA and the compounds or drug, two categories of electrochemical DNA-biosensors can be distinguished:
  - ✓ Electrochemical biosensors for DNA damage detection or structural biosensors.
  - ✓ Electrochemical biosensors for detecting hybridization in DNA or hybridization biosensors.

Structural biosensors utilize the following phenomena:

- ✓ The change in the electrochemical signal of DNA bases and especially guanine is an indication of DNA damage.
- ✓ Many compounds that interact with DNA produce their own electrochemical signal and this is an indication of their binding to DNA.
- ✓ With the mercury electrode it is possible to detect the "break" of the polynucleotide chain.

The interaction between DNA and the various compounds is carried out in the ways mentioned above.

The most common technique for detecting DNA damage is the gel electrophoresis technique. The disadvantages of this technique are the long total analysis time and the low sensitivity, as it is unable to detect minor damage due to ionizing radiation, exposure to chemicals, etc. Electrochemical biosensors have many advantages such as relatively inexpensive organology, speed, selectivity, analytical sensitivity, and can be used in screening methods of compounds that are DNA-resistant or have anticoagulants in the DNA. Structural biosensors can also be used to identify DNA or RNA traces (ng levels), to electrochemically characterize ssDNA, dsDNA, microRNA, and to identify different types of genetic materials.

2.3 MicroRNAs Biosensors (miRNAs)

MiRNAs are a small category of non-coding RNAs, ie they do not translate into protein, but have an important part in gene expression at the transcription. This occurs by inhibiting transcription or leading to regulation of the RNA sequence. Most of these are in exon and intron regions and are transcribed by RNA polymerase (Shabaninejad et al., 2019). The creation of the miRNA consists of three important steps, the first step taking place in the nucleus, where the PRI-miRNA is transcribed from the genome whose ends are cut by a nuclear enzyme called Drosha (Shabaninejad et al., 2019). This

results in the formation of a 60 to 70 nucleotide intermediate loop called Pre-miRNA, which is transferred to the cytoplasm and processed by the Dicer complex (Shabaninejad et al., 2019). A fragment of the dual latent fragment of the original is formed, which eventually converts to an RISC complex and leads to suppression of transcription or degradation of the target mRNA depending on their homology to it (Shabaninejad et al., 2019).

The application of various enzymes that catalyze oxidation-reduction responses can be useful in miRNA electrochemical biosensors, in which the generation of the final electrochemical signal is done by the enzymes (Paniel, Baudart, Hayat, & Barthelmebs, 2013). It should be noted that these enzyme-based biosensors require perfectly ideal conditions to maintain the stability and activity of the enzymes. An alternative approach to electrode modification is the application of conductive polymers (CP), whose polymers and their derivatives accelerate the transfer of electrons to the electrodes (Paniel, Baudart, Hayat, & Barthelmebs, 2013). In addition, the use of nanoparticles in biodetection may lead to the reduction of electrochemical biosensors and the improvement of their analytical data (Paniel, Baudart, Hayat, & Barthelmebs, 2013).
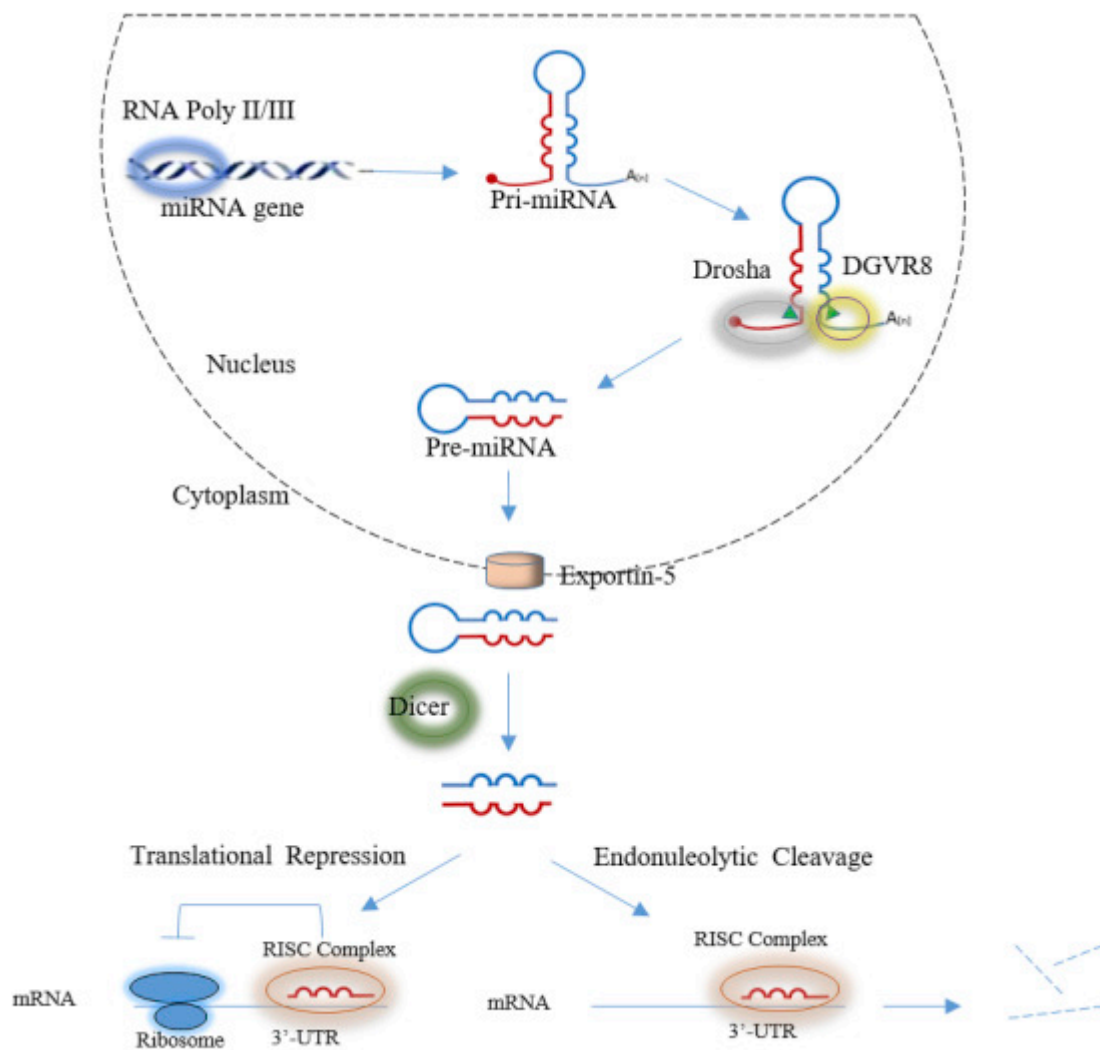
Figure 6. Generation of miRNA starting from the nucleus and continuation of processing in the cytoplasm by DICER, eventually forming the RISC complex causing interruption of translation or destruction of the mRNA due to conjugation (Shabaninejad et al., 2019)

The most usual techniques for their detection are northern blotting, microarray analysis and RT-PCR which unfortunately have high costs and complex handling. For this reason, to be used in diagnostic tests, highly sensitive techniques are required that require a small sample volume, able to detect a small number of cells and have costs that do not make them impossible (Paniel et al., 2013). However, many researchers have mentioned that certain miRNAs are significantly altered during development and in the event of disease, such as certain forms of cancer, heart diseases and diabetes. Therefore, miRNAs that have been altered during the course of the disease are of research interest. It is worth noting that miRNA expression is a dynamic process and

the application of nucleic acid can be a cost-effective and non-invasive tool for disease control and evaluation at an early stage. To date, it has been shown that certain miRNAs, such as miRNAs-141, miRNAs-155, Let-7, and miRNAs21, are either elevated or decreased in cancer cells. They therefore provide an alternative to highly sensitive and specific biomarkers for tumor sorting and prognosis (Crivianu-Gaita & Thompson, 2016; Shabaninejad et al., 2019).

In a classical miRNA electrochemical sensor, the switching element is a solid electrode, mainly of gold or graphite on which a small single-stranded nucleotide sequence, a probe, is immobilized (Zhang et al., 2006). The DNA probe and the miRNA target interact and affect the performance of the electrode (Zhang et al., 2006; Shabaninejad et al., 2019). Often, an electrochemically active type of control changes the properties of the electrodes due to hybridization with miRNA and leads to its signal rendering. Examples of such electrochemically controls used in miRNA monitoring in biosensors are hydrogen peroxide (H_2O_2), glucose oxidase, alkaline phosphatase, methylene blue and hydroquinone (Zhang et al., 2006; Shabaninejad et al., 2019).
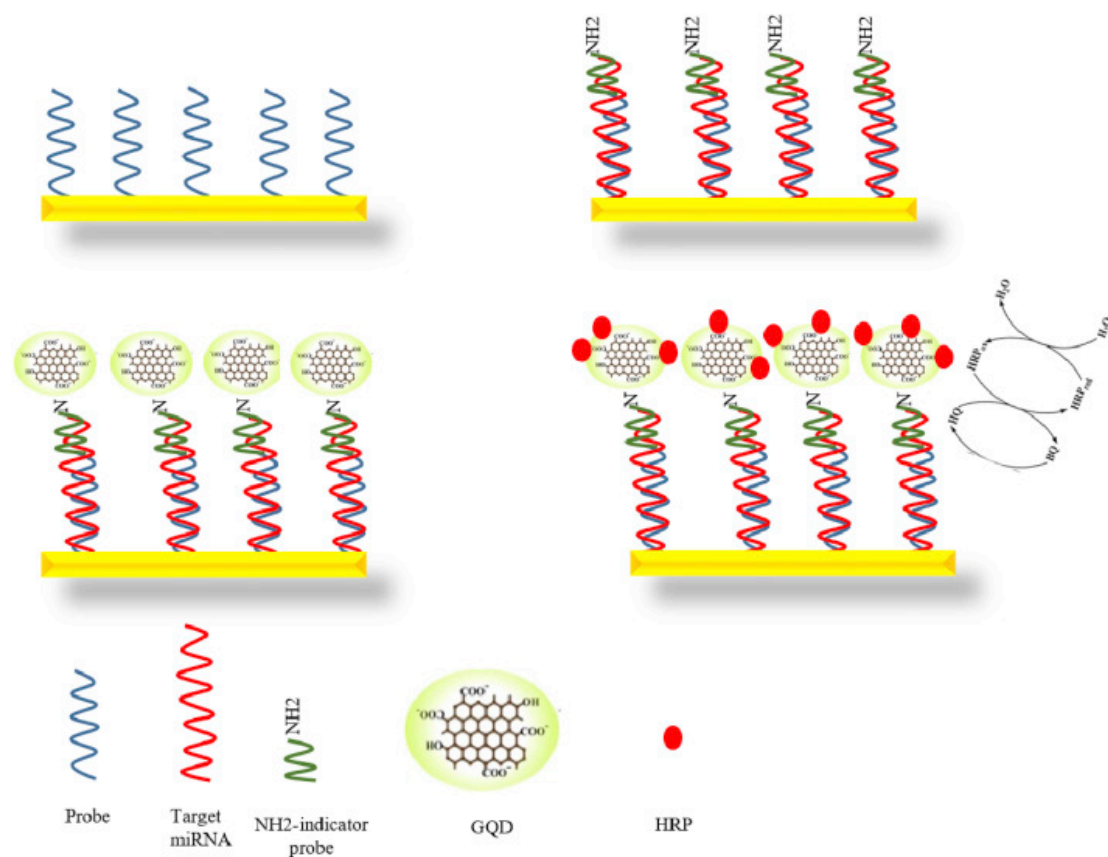

Figure 7. An electrochemical biosensor with miRNA as a biological reading element (Shabaninejad et al., 2019).

In hybridization biosensors, a particular sequence is immobilized on the electrode surface (DNA probe) which may have a length of 15 to 20 nucleotides, and then the DNA-shaped electrode is immersed in a solution containing the DNA, which contains the target DNA. (Josephs et al., 2013) If the base sequence of the target DNA is exactly complementary to that immobilized on the electrode surface, then a hybridization product is formed on the electrode surface. (Josephs et al., 2013) In case of non-complementarity, no hybrid is created. (Josephs et al., 2013) There are two very important steps in the whole process: (a) the formation of the hybrid and (b) the conversion of the above phenomenon into a measurable electrical signal. Exposure of the sensor to a solution containing an electrochemical marker (e.g., an oxidation cationic metal complex) reacts strongly and reversibly with the hybridized DNA. Increase in the electrochemical signal due to the redox of the electrochemical indicator, which is connected to the newly formed double helix, serves as an analytical signal to monitor hybridization. (Josephs et al., 2013)

Hybridization biosensors are mainly used to identify genes.

2.4 Enzyme Based Sensors

Enzymes are known to be exceedingly proficient biocatalysts, which have the capacity to specifically distinguish the substrate and catalyze its transformation. (Perumal & Hashim, 2014; Zhu et al., 2019) These properties of enzymes are promoted in a device. Their operating rule is based on the catalytic activity and the official imaginary results for the reason of the specific discovery. (Perumal & Hashim, 2014; Zhu et al., 2019) Such bioreceptors comprise of chemicals that distinguish the target to be analyzed from a test. Particularly, the parameter identified is the alteration within the concentration of a component coming about from a catalytic response or the emission of light. (Nguyen, Lee, Lee, Fermin, & Kim, 2019). Within the case of fondness sensors, the association between the receiver and the target to be analyzed is recorded. (Perumal & Hashim, 2014; Zhu et al., 2019) The key-lock official and actuated adjustment speculation can

be connected to clarify the instrument by which protein movement is (Perumal & Hashim, 2014; Zhu et al., 2019).

Due to the enzyme-substrate relationship and the enormous constant catalysis of kcat, enzymes are an essential and broad biosensor lesson. But beyond specialization, this catalytic activity offers a breakthrough. In a perfect world the catalytic action can be affected by different variables such as substrate concentration, temperature, nearness of competitive and non-competitive inhibitor and pH (Perumal & Hashim, 2014; Zhu et al., 2019).

2.4.1 Amperometric enzyme biosensor

These sorts of biosensors are among the finest examined sensors due to their points of interest, such as the capacity to function with a little test volume, with complex networks and their durability. Cases of widely utilized enzyme amperometric biosensors are for the assurance of glucose or liquor levels utilizing oxidases, which oxidize the substrate to deliver hydrogen peroxide which is recognized at the electrode. (Nguyen et al., 2019; Zhu et al., 2019).. At first, in first-generation biosensors, the chemical is immobilized on the surface and the substrate to be analyzed is identified by measuring the item of the chemical response (H2O2, NADH, etc.) or by checking the utilization of a cofactor such as O2. As specified the sensitivity of these biosensors in combination with the short response time may be a critical advantage. (Nguyen et al., 2019; Zhu et al., 2019).

In order to avoid oxygen dependence, the transition from the electron collector and not from the oxygen is used, in order to transfer the electrons to the electrode. It could be a little dynamic redox atom (such as ferrocene and derivatives, conductive organic salts, quinones), which responds with the dynamic portion of the enzyme and after that with the electron surface. Hence, a signal is delivered that's corresponding to the concentration of the substance to be analyzed that's identified. Agreeing to their rule of operation, the go between is decreased due to the exchange of electrons amid the enzymatic response and after that oxidized by exchanging the electrons to the electrode. Of course, this reliance and the intercession of the mediator moreover brings drawbacks to the strategy. For this reason, the third era biosensors were created to perform coordinate electron exchange without the intercession of a arbiter or cofactor amid the catalytic change of the substrate into a master. Third-generation biosensors offer expanded selectivity since they work at a potential that is similarly to the redox potential of the enzyme (Nguyen et al., 2019; Zhu

et al., 2019). They comprise of three components, the enzyme as a bioidentification component, an oxidizing polymer or nanowire (performance enhancement) and the electrode to guarantee signal engendering and an electrode as a catching surface (Rocchitta et al., 2016).
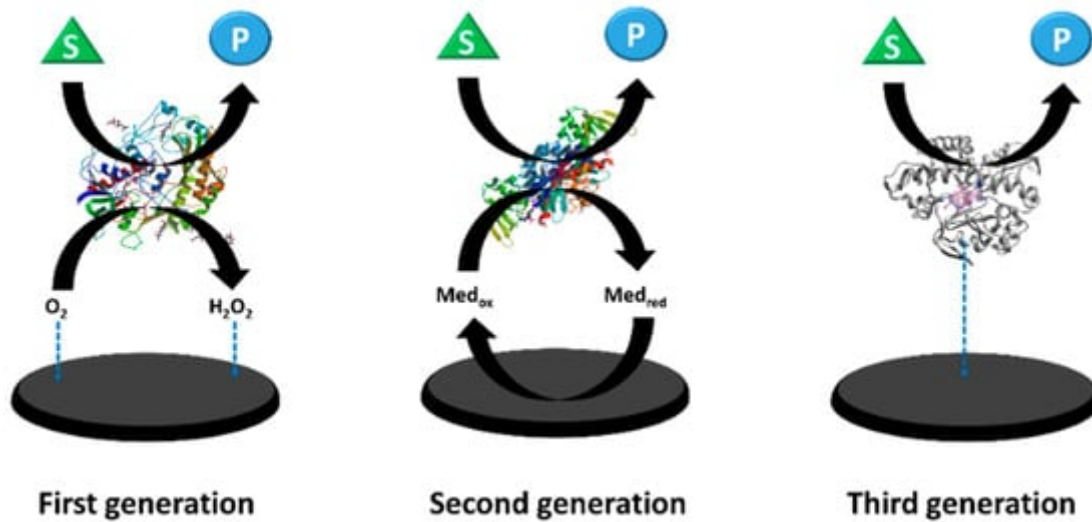

Figure 8. Schematic illustration of the primary, second and third generation enzyme biosensors (Nguyen et al., 2019)

2.4.2 Voltametric enzyme biosensor

Cyclic voltammetry is the foremost common strategy of voltammetry and is frequently connected to enzyme biosensors. As detailed by Zhu et al. (2019), Cyclic Voltammetry was performed to confirm the electrochemical reaction of some heme proteins, which compares to the decrease of dissolved oxygen (Zhu et al., 2019). Besides, the alteration of the Pt terminal with zinc oxide nanocuboids and the strategy of cyclic voltammetry driven to a delicate location of acetylcholinesterase (Throb), which appeared great conductivity and biocompatibility (Zhu et al., 2019). In arrange to extend the sensitivity of the biosensor and to further facilitate an electron exchange the conductivity of the working electrodes must be taken into consideration. Differential Pulse Voltammetry (DPV) is a potentiostatic method that offers some advantages to common techniques like Cyclic Voltammetry (CV), in that the waveform is a series of pulses increasing along a linear baseline. The way in which the current is measured at each pulse aids in minimizing the measurement of background (charging) current. (Zhu et al., 2019).

2.4.4 Potentiometric enzyme biosensor

As a few enzymatic responses include the discharge or utilization of hydrogen particles, coming about in changes in ionic concentrations, an ion-selective electrode seem distinguish and screen these forms (Grieshaber et al., 2008). In a potentiometric biosensor, the potential distinction between the reference electrode and the working electrode is the signal measured beneath equilibrium conditions, ie no current so as not to cause signal impedances (Grieshaber et al., 2008). The measured signal is related with the target to be analyzed with a logarithmic connection to be measured (Nguyen et al., 2019; Zhu et al., 2019).

Ion selective electrodes (ISEs) are utilized to change over the action of a specific ion within the test arrangement to potential, to be measured with a pH meter or voltmeter. The electrode as a rule comprises of two components: an indo-selective membrane that gives specific penetrability to particular ions from a arrangement to be analyzed, comprising different ionic shapes, and a reference electrode, isolated or coordinates.

After particle penetrability is accomplished an electrochemical equilibrium is made and the result is the contrast between

the possibilities of the two stages, the arrangement and the inner arrangement. Depending on the membrane potential, the potential discrimination is controlled by specific ions at these stages. (Rocchitta et al., 2016)
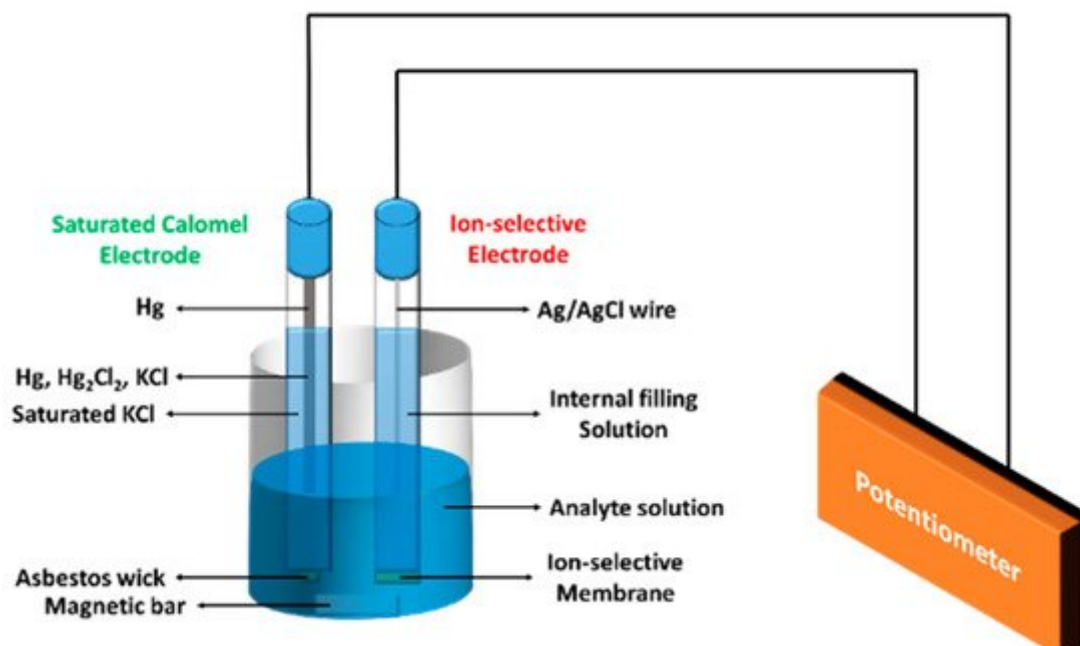

Figure 9. Schematic representation of an ion-selective electrode (Nguyen et al., 2019).

Another class of potentiometric biosensors are chemical sensitive transistors (Protein Field-Effect Transistors). These are semiconductor devices that react to the surface electrical gradient of the gate electrode. Coupling a thin enzyme membrane on the entryway surface with an ion-sensitive transistor makes the chemically sensitive transistor. At first, such sensors are inferred from a pH-sensitive discovery in which the enzyme catalytic response is sensitive to pH. As a result, the concentration of protons is corresponding to that of the substrate and not as it were subjective but too quantitative examination is performed. The collection of cargo carriers at the gate happens in understanding with the catalytic response until the substrate particles are expended. The change within the electrical signal of the source can be caused by the enzymatic response. The foremost common sensor of this type is

for the estimation of glucose changed over to hydrogen peroxide concurring to the reaction (Nguyen et al., 2019; Zhu et al., 2019):

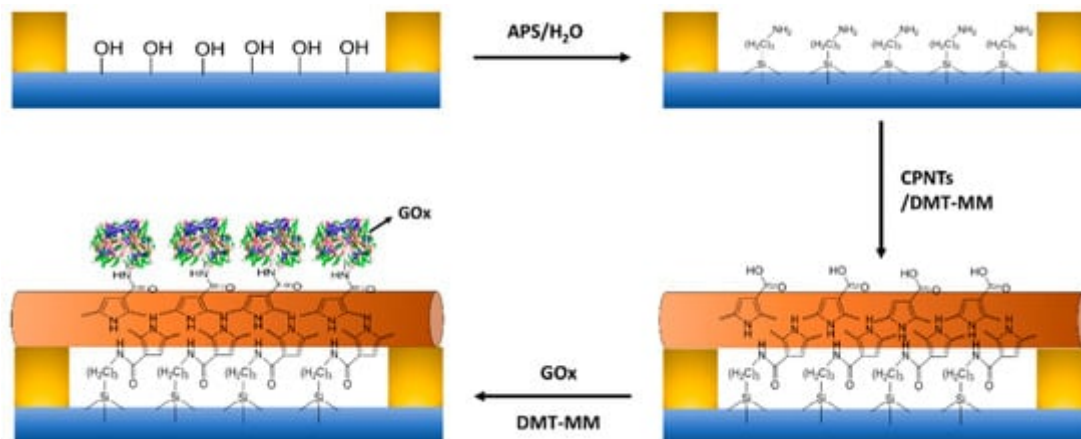$$glucose + O_2 + glucose\ oxidase \rightarrow H_2O_2 + gluconic\ acid$$
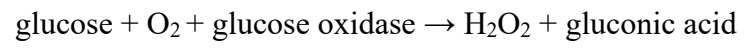
Figure 10. Schematic diagram of polypyrrole nanotube FET processing steps, including immobilization of nanotubes on substrate and immobilization of glucose oxidations (GOx) (Nguyen et al., 2019)

2.4.5 Conductive enzyme biosensor

The change in ionic concentrations, in addition to changes in pH, also causes alterations in the conductivity of the electrolyte solution, which can be counted by applying a different potential among the electrodes. Thus, the mobility of ions increases due to the movement of the negatively charged to the upward and the positively to the downward. The conductivity of electrolyte solutions depends on the concentration of ions and their mobility, so it is in cases where no electrochemical reaction takes place at the electrodes (Nguyen et al., 2019; Zhu et al., 2019).

2.4.6 Enzyme impedance biosensor

Impedance estimation at an electrode can be utilized to identify changes in interfacial properties as a result of interaction with bioreceptors. The impedance range can be utilized to measure electrochemical parameters (Nguyen et al., 2019; Zhu et al., 2019). This strategy isn't so common in chemical bioreceptors due to the time required to get the range in a recurrence extend. As of late, such a sensor has been created based on lactase dehydrogenase and pyruvate oxidase layers. It appeared high stability, specialty and low monitoring constrain (Nguyen et al., 2019; Zhu et al., 2019).
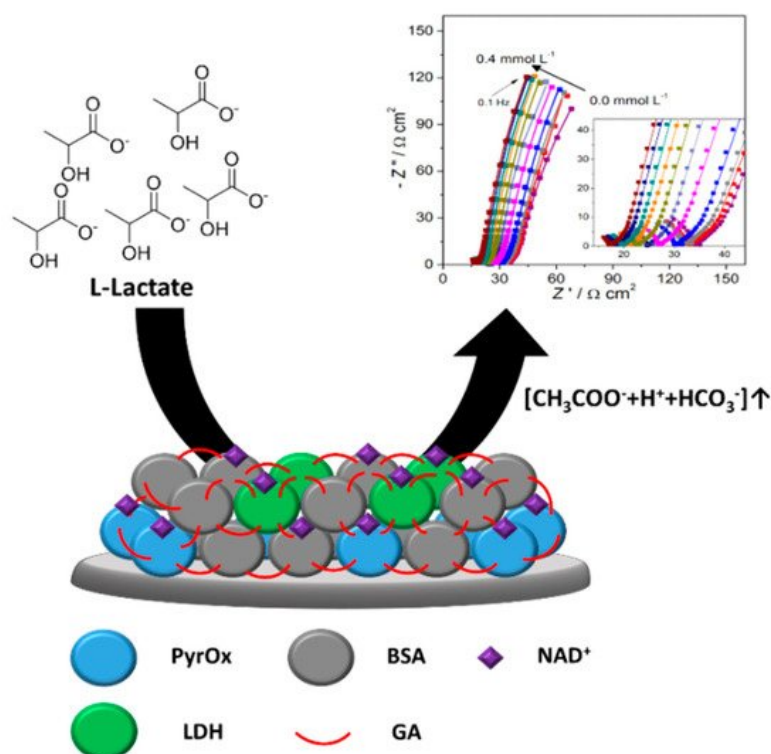
Figure 11. Imaging of an L-lactate selective impedance biosensor with layers of lactase dehydrogenase (LDH) and pyruvate oxidase (PyrOx), BSA: bovine serum albumin, GA: glutarynaldehyde. (Nguyen et al., 2019)

2.7 Immune biosensors

These biosensors are synthesized by an antibody or antigen as an element of biological recognition and are a key tool for clinical diagnosis. The antibodies are Y-shaped and are immunoglobulins with two heavy chains (H) and two light chains (L) (Perumal & Hashim, 2014). However, some human antibodies form a dimeric or pentameric configuration using disulfide bonds and a J binding protein J. Each chain comprises a fixed and a variable moiety. The variable moiety is specific to the antigen attached to it, offering high sensitivity and specificity. Therefore, an immunosensor that uses an antigen as a bioreceptor utilizes its ability to bind to the corresponding antibody in a very stable, specific, and flexible binding. (Perumal & Hashim, 2014) The specificity of the antibody for binding to the antigen is achieved thanks to its amino acids. The most widely used types of detectors are optical and electrochemical, with optics having low sensitivity and half-life when used as a radioimmunoassay. Of course, recent technological developments reveal the possibility of construction automated instruments and are considered important in the development of such detectors. Instead,

electrochemists provide a simple, fast, and economical detection method that overcomes such problems. Immunosensors for the monitoring of pathogens and bacteria is a promising method due to the application of POC. More recent research reveals their potential for detecting tumors and cancers at an early stage (Perumal & Hashim, 2014).

Antibodies are expansive proteins delivered by the immune system with amazingly high fondness and target specificity. .Each antibody comprises of two parts where each comprises a heavy and a light chain held together by disulfide bonds. The amount of bonds in the region of their compound depends on the type of antibodies and their course. As appeared in Figure 5, the antigen or analyte binds to the beat of the chains and in specific to the variable moiety (V_L, V_H). Each antibody, in any case, comprises of two parts (Fab), each of which comprises of a variable portion and a consistent of both the light and heavy chains (V_L, V_H, C_L, and C_{H1}) and, as raised, are held together by a disulfide bridge. Removals are performed in two ways: by recombinant mixture or by proteolytic cleavage of the original antibody. Within the case that they also contain a disulfide bridge made of thiols, Fab 'is symbolized, the nearness of which facilitates the immobilization on the surface of the sensor. Even smaller are the Fv fragments which consist only of the variable segments VH and VL and result only by recombinant synthesis. Their significantly smaller size allows them to be immobilized at a higher density on the surface and consequently lead to a higher sensitivity and lower limit of detection. (Crivianu-Gaita & Thompson, 2016)
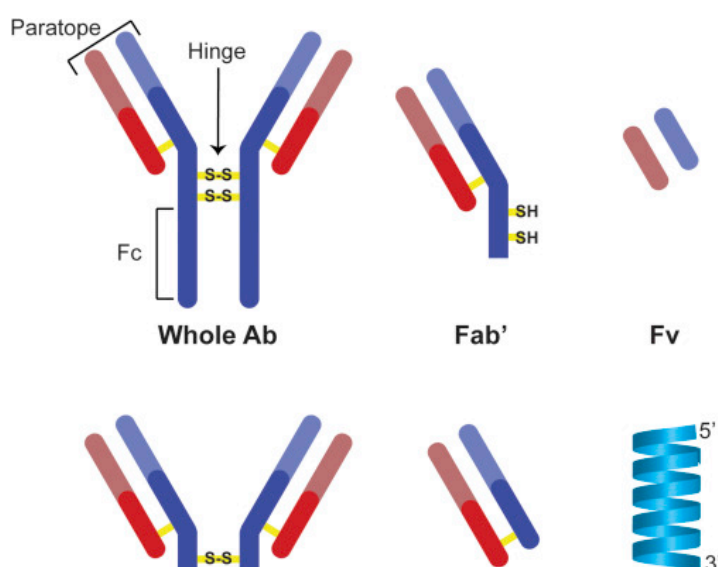

Figure 12. Imaging of whole antibody (Ab), Fab ', Fab, Fv and aptamer fragments (its size and shape varies, while it does not have to form a helix) (Crivianu-Gaita & Thompson, 2016)

Fv fragments are unstable due to their non-covalent bonding. For this reason a linker peptide, 15 to 30 amino acids, is usually added between the variable segments of the two chains (scFv fragment). The most common peptide is $(Gly_4\text{-}Ser)_3$ due to its flexibility, but some modifications to the amino acids can be incorporated to increase solubility. Fragmentation of the fragments involves head to tail binding and most likely the N end of V_L and the C end of V_H are involved in antigen binding. Non-covalent dimers are difficult to form ternary and higher molecules but some dimerization of the scFv fragment and subsequent disulfide bridge formation can lead to affinity similar to that of the whole antibody. For the synthesis of scFv fragments the best expression is shown by bacterial systems where chemical modifications are avoided, such as E.coli, while Pichia pastoris shows unwanted glycosylation. (Crivianu-Gaita & Thompson, 2016; Truong, Hsieh, Truong, Jia, & Hammond, 2018)

To create more durable surfaces, scFv fragments are immobilized by covalent bonding. The simplest method is to attach the thiols of the C-terminus of the fragments directly to the surface of the gold by adding a chemical agent to prevent alteration of the antibody from the surface, reducing the interaction of the two. Furthermore, modification of the fragment by the addition of cellulases to the C end can lead to covalent bonding to a gold side. In any case, the presence of thiols contributes to the oriented immobilization of the fragments. (Crivianu-Gaita & Thompson, 2016; Truong et al., 2018)

3. Nanomaterials

3.1 ALLOTROPIC TYPES OF CARBON

The nanotubes are concentric graphite cylinders, closed at each end by five-membered

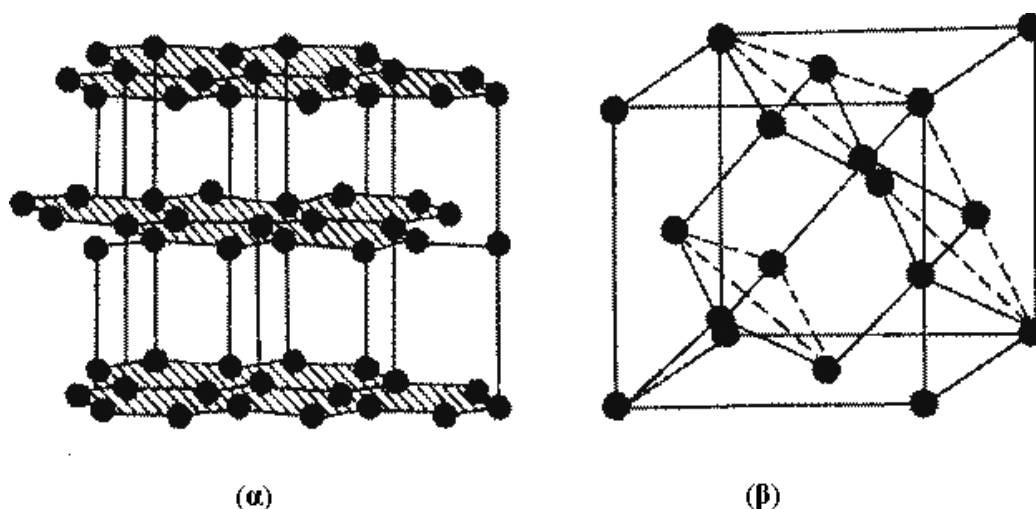

(α)   (β)

Figure 13. (a) Graphite and (b) Diamond.

rings. The nanotubes are concentric graphite cylinders, closed at each end with five-membered rings. Carbon exists in nature in two allotropic crystalline forms, as graphite and as diamond. The graphite consists of levels of carbon atoms in sp2 hybridization that form the characteristic structure of Figure 13a. At this stage it is strong and rigid making a material that can remain in a steady state (absence of oxygen) Up to 3300oC. The bonds, on the other hand, that bind carbon atoms between two levels are vulnerable. Diamond is composed of carbon atoms in sp3 hybridization creating bonds that are equivalently rigid and high strength (Figure 13b). It is able to withstand up to 1800oC beyond which it is converted to graphite, due to the high energy stability of sp2 hybridization beyond this temperature

Fullerene, a spherical carbon structure, was discovered in 1985 and was discovered by Harold Croto and his colleagues. They discovered that when graphite is evaporated under the influence of a laser beam in a stream of helium, molecules are formed, quite stable, consisting of a large (32-90) number of carbon atoms. The most stable was the C60, and the scientists who discovered it thought it was in the shape of a soccer ball. They named it buckball or buckminsterfullerene after the architect Buckminster Fuller who had built similar structures. The collection of all C60s was called fullerenes. The fullerene boundaries consist of a network of carbon atoms in the sp^2 state in pentagons and hexagons resulting in the formation of a spherical boundary. The most common

fullerene limit is the one consisting of 60 carbon atoms (C_{60}) and shown in Figure (14). In 1991, during research on fullerenes, another new allotropic form of carbon, carbon nanotubes, was discovered.
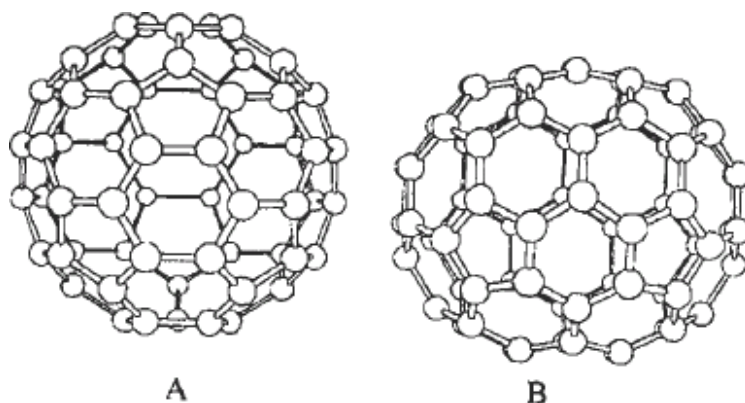

Figure 14. Fullerenes A) C_{60} and B) C_{70}.

3.2 DISCOVERY OF CARBON NANOTUBES

The discovery that carbon can be formed into other stable forms besides toner and diamond has prompted researchers around the world to look for new forms of carbon. The discovery of tubular allotropic forms of carbon with a large length / diameter ratio was called a nanotube. This research gave new impetus when in 1990 it was shown that the C60 could be produced by a simple extruder, via an electric arc and be readily
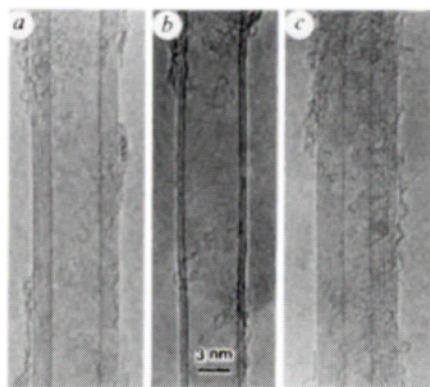

Figure 15. First images of multi-walled carbon nanotubes by Sumio Iijima, published in the journal Nature in November 1991.

available in all laboratories. Using one such atomizer, Japanese scientist Sumio Iijima discovered carbon nanotubes in 1991.

3.3 Structure of carbon nanotubes

3.3.1 Structure of single wall carbon nanotubes (SWNTs)

Each single-wall nanotube (SWNT) (M. S. Dresselhaus et al., 2000) can be schematically illustrated by a graphene sheet, which is wound into a cylinder by paying attention to the hexagonal graphene rings. When closing the cylinder to come into coherent contact with each other. The ends are closed with the fullerene limit hemisphere having the appropriate diameter. There are different ways to wrap graphene in a wall-mounted nanotube (Springer Handbook of Nanotechnology, 2010). Some nanotubes have symmetry parallel to and perpendicular to the nanotube axis, such as zig-zag and armchair, while others do not, such as helical or chiral nanotubes.
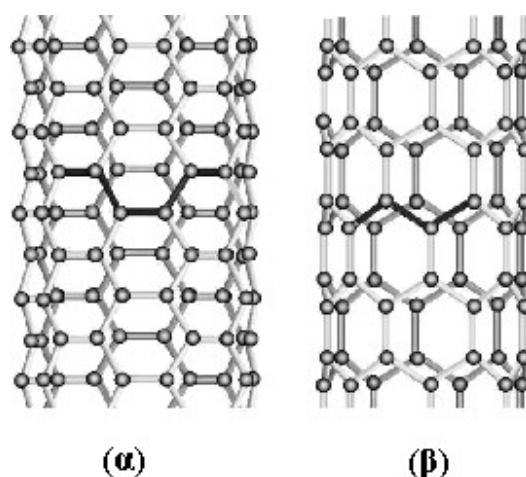

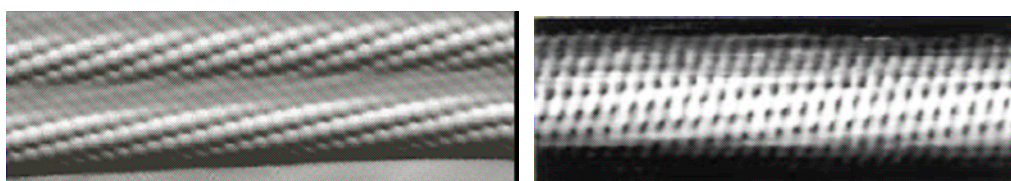*Figure 16. a) Armchair type nanotube b) zig-zag type nanotube.*

Figure 17. Images from STM showing the structure of helical nanotubes.

3.3.2 Multi-wall carbon nanotubes (MWNTs)

The construction of multi-walled nanotubes is more complex, as it has to do with the various ways in which graphene is organized and depicted in a filamentous morphology. The easiest way to display an MWNT (Multiwall Nanotube) is to concentrate multiple SWNTs in different diameters, as shown in Figure 18 (c-MWNT). The number of walls can be any, starting with two. The distance between the walls is approximately the same as the distance between the layers of graphene in laminated polyamorphic solids and is equal to 0.34 nm. (the distance between the graphite layers is 0.35 nm), as due to the porosity of the nanotubes, the carbon atoms are not represented in the same way as in the graphite. Each wall has its own helix, while the walls are held together by van der Waals forces. Another type of MWNT is the herringbone type (hMWNT), in which the graphene form an angle with respect to the axis of the nanotube, as shown in Figure 18B (Springer Handbook of Nanotechnology, 2010). The angle varies and ranges from 0 ° (where we have c-MWNT) to 90 °, where the tube now loses its tubular shape and transforms into nanofibers.
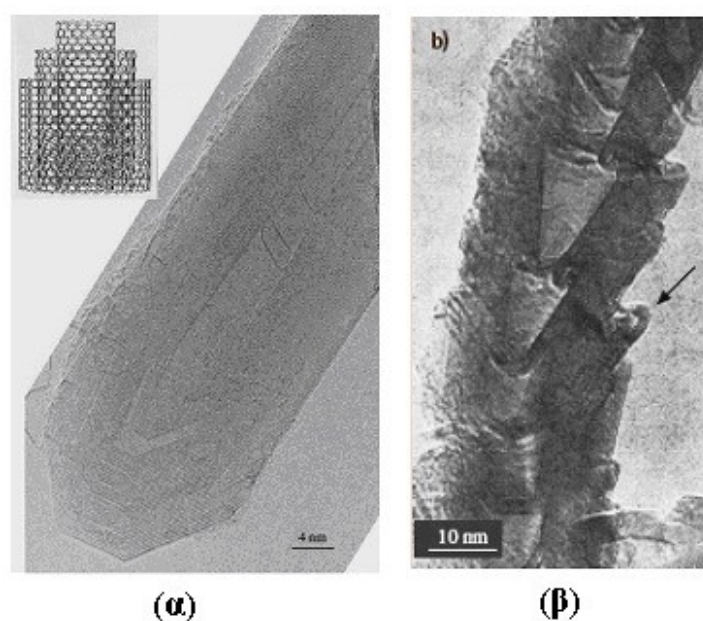

(α) (β)

Figure 18. a) TEM image of c-MWNT nanotube prepared by the electric arc method. b: PCS image of herringbone type nanotube.

4. PROPERTIES OF CARBON NANOTUBES

4.4.1 Electrical properties

Carbon nanotubes can be metal or semiconductor. In the case of single-walled nanotubes, all armchair types have a metallic behavior, the rest can be either conductors with very low energy gaps (or metals) or conductors. This categorization is obviously directly related to the conductivity of the material. The conductivity in metals is due to the free electron of the outer layer, which has the ability to move inside the material. However, due to the movement restrictions that exist inside the material, it has scattering, which also causes its temperature to rise (Springer Handbook of Nanotechnology, 2010). In the case of nanotubes, only one of the four atoms of the outer layer of the carbon atom moves in the lattice. Due to the geometry of the tube, the possibility of electron movement is limited only in the axial direction of the tube, which greatly reduces electron collisions (scattering), resulting in increased conductivity of the nanotubes and at the same time an increase in their anticorrosion the Bells. (Argyriadis, 2012)

4.4.2 Thermal properties

Equally interesting in electrical properties are the thermal properties and generally the thermal behavior of nanotubes. In this case, too, the phenomena take place along the axis of the CNTs and therefore, as we saw in the electrical properties, the thermal conductivity in this direction is much higher than in any other.

The heat energy in the nanotubes is essentially transferred to the cells. In materials that are good conductors of heat, these waves move very fast in a one-dimensional direction. Heat cells, which carry heat energy, were found to move inside the nanotubes at a speed of 10000m / sec, which is consistent with very high thermal conductivity.

4.4.3 Mechanical properties

In graphite and carbon nanotubes, three types of forces between carbon atoms are responsible for their elastic properties: the σ and π bonds that develop between the intra-C = C bonds, as well as the weak cross-sectional interactions. Since the bond is the strongest chemical bond in nature, a carbon nanotube is the strongest fiber in terms of the force along its axis. Experimental calculations, which agree with the proposed

theoretical calculations, show that carbon nanotubes are probably the hardest material in nature, perhaps even harder than diamond. (Koromilas, 2012)

5. CELL RECEPTORS – APTAMERS

Microorganisms such as bacteria and fungi can be used as biosensors to detect specific molecules or to assess the overall state of the environment (Perumal & Hashim, 2014). In addition, proteins included in cells can also be used as bioreceptors to detect a specific analyte (Perumal & Hashim, 2014). In essence, the living cell-based biosensor is the only biosensor as opposed to additional types of biosensors containing materials extracted from living things (Perumal & Hashim, 2014).

However, the most important limitations of the cell biosensor are the stability of the cell, which depends on various conditions such as sterilization, longevity, biocompatibility (Perumal & Hashim, 2014). Another issue that can limit the success of cell sensors depends mainly on poor selectivity due to the ability of intact cells to respond to multiple stimuli. Despite these disadvantages, the cell-based biosensor is still preferred by researchers because of its advantages over those based on enzymes (Perumal & Hashim, 2014). They are less sensitive to solvent inhibition and are more tolerant of non-optimal pH and temperature values than those based on the enzyme. However, they should not exceed a narrow range due to the possibility of cell death (Perumal & Hashim, 2014).

One way to overcome the problem of instability of biological molecules is to replace them with artificial receptors or bio mimics. A biomimetic biosensor is a synthetic sensor that mimics the function of a natural biosensor. It may comprise as an element of biological identification an artisanal nucleic acid sequence, aptamers (Springer Science and Business Media LLC, 2010). For this reason, it is chemically related to the nucleic acid probe, but more closely resembles the behavior of antibodies (Springer Science and Business Media LLC, 2010). Aptamers are synthetic nucleic acid strands that can be designed to identify amino acids, oligosaccharides, peptides, and proteins and show astonishing flexibility compared to other bioassay elements (Springer Science and Business Media LLC, 2010). Therefore, as an element of biological recognition, antibodies are superior in relation due to the high binding efficiency, less complex and the use of animals is avoided (Perumal & Hashim, 2014).

Aptamers are single inactive DNA or RNA chains made to imitate the selectivity and specificity of antibodies. Nano, pico and comparable however, these nucleic acid chains can be generated for a surprisingly large array of particles Still, these nucleic acid chains can be created for an amazingly wide run of particles. The advancement prepare starts with a enormous random library of RNA or DNA, with the objective of finding the arrangement that permits it to be coupled to the analyte. An analyte is at that point included and the nucleic acid chains that tie the analyte are effectively disconnected, opened up and taken after by an extra improvement cycle (PCR procedure). Different cycles of this process (8 - 15 cycles) result within the exponential increment of the nucleic acids that show the greatest official connections with the examiner. At that point the analyte and are those aptamers that lead to coupling and after that increase the their expression utilizing the PCR strategy and are encourage inspected for its optimization coupling with the analyzer.

This repetitive process is called "systematic evolution of ligands by exponential enrichment" or SELEX and an example is illustrated in the Figure below (Springer Science and Business Media LLC, 2010).
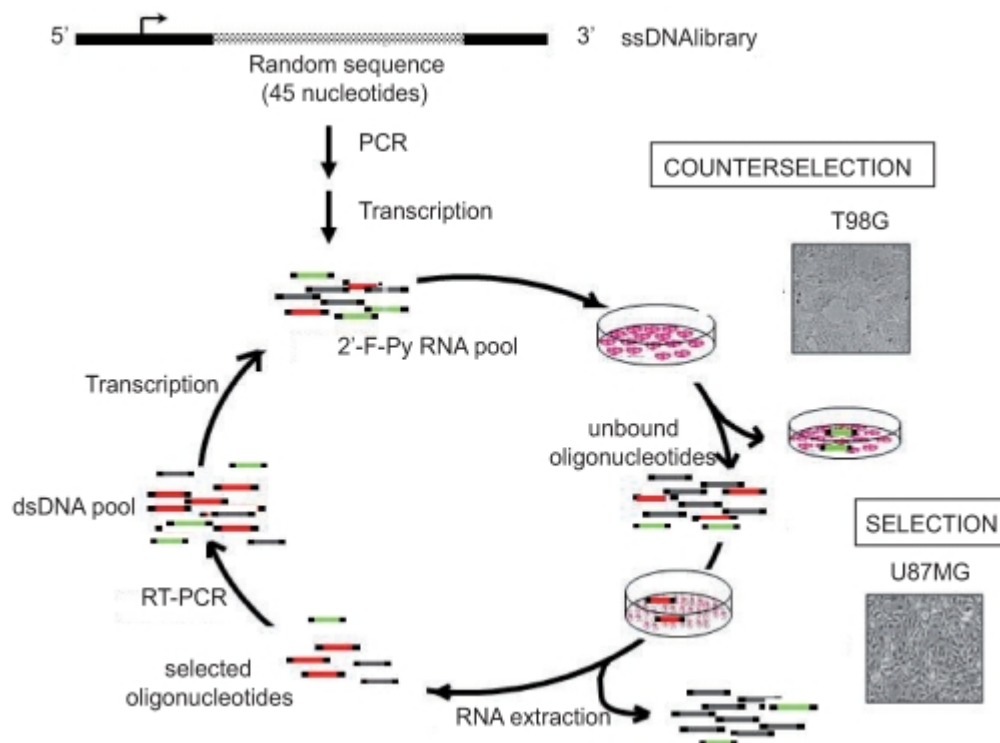
Figure 19. Choice of U87MG cell-specific aptamers. A pool of 2'F-Py RNAs was hatched with ineffectively tumorigenic T98G cells (Counterselection) (Springer Science and Business Media LLC, 2010). Unbound groupings within the supernatant were recuperated and hatched with tumorigenic U87MG cells for the choice step (Determination). Unbound arrangements were disposed of by washings and bound groupings were recouped by add up to RNA extraction. Groupings enhanced by the determination step were increased by RT-PCR and in vitro translation some time recently a unused cycle of determination. (Cerchia et al., 2020)

Electrochemical biosensors using aptamers are based on the changes caused by their coupling configuration. This coupling results in the transfer of electrons between the electrode surface and the control redox molecule (Guy et al., 2016).

6. DETECTION OF CTCs

Tumor metastasis causes about 90% of cancer mortality (Zhi-Fang et al., 2021). Circulating tumor cell (CTCs) were discovered in 1869, (Zhi-Fang et al., 2021). (CTCs) are cells that have been shed in blood vessels or lymphatics by a primary tumor and carried throughout the body by the bloodstream (Zhi-Fang et al., 2021). They are precursors of metastasis. Currently, there is an FDA-approved method for detecting CTC, CellSearch, and it helps detect a variety of cancers such as breast, prostate, lung and colon. Careful examination and special assurance of CTCs in marginal blood tests contribute to the timely conclusion, prediction and evaluation of cancer treatment. This has led to many efforts to develop effective methods for capturing and detecting CTC. However, the abundance of CTC in the blood is extremely low and there are also large

numbers of blood cells (Zhi-Fang et al., 2021). Due to the complexity of genuine tests and CTCs, various strategies have been used. Normal improvement based on its characteristic biophysical properties (such as measure, deformation and thickness) can be a device for attractive separation strategies (Zhi-Fang et al., 2021). The serum cancer cell count is consistently higher than the normal and white blood cell count (Zhi-Fang et al., 2021). This distinction makes it possible to divide these cells by high-precision filtration.

Due to the excretion of lactic anions within the glycolytic pathway, the negative surface charge of cancer cells can be utilized as an compelling biophysical marker for the division and location of CTC (Zhi-Fang et al., 2021) In addition to their dissimilar biophysical properties, protein biomarkers are continuously overexpressed on the surface of CTCs, which can be used as targets for the division and investigation of CTCs into blood cells between protein layers and individual assays, including antibodies and peptides (Zhi -Fang et al., 2021).

For the epithelial cell adhesion molecule (EpCAM) is generally communicated on the surface of different epithelial cancers (Zhi-Fang et al., 2021). In this way, bio-reading components are selected for which they depend on the cell phenotype for the detection of cancer cells. Between them, antibodies as the foremost utilized tests are for the most part utilized to create immunoaffinity-based strategies with

antibodies adjusted attractive globules (MBs) and nanostructured microfluidic chips. Rapid detection and early detection of cancer, numerous optical and electrochemical procedures have been developed for the sensitive and accurate detection of CTC (Shao et al., 2008 . Shen et al., 2017). In the last two decades, various advanced nanomaterials and nanostructures with excellent physical and chemical properties have been developed (Zhi-Fang et al., 2021) for the construction of hybrid biological / nanostructured cell sensors to enhance the signal and increase sensitivity (Pantel & Panabières 2019 . Afreen et al., 2020 . Cathcart & Chen 2020 . Fattahi et al., 2020). For example, nanomaterials with a high surface to volume ratio and excellent conductivity, such as carbon nanotubes (CNT) and graphene oxide (GO), are used to modify electrodes, improve CTC bonding, and promote electron transfer (Zhi-Fang et al., 2021). Nanomaterials with interesting enzyme-like catalytic properties are utilized to catalyze redox responses in optical and electrochemical tests (Zhi-Fang et al., 2021). Due to the near surface resonance of the plasmon (LSPR), metal nanoparticles (NPs)

have been used as optical flag converters and as Raman flag intensifiers. In this way, combining these highly customizable nanomaterials with different positioning strategies can take drastic steps in terms of adequacy constraint and site impact capability.Electrochemical biosensors have pulled in much consideration in later a long time due to their tall affectability, straightforward operation and quick reaction time (El - Aamri et al.,2020). Bio-interface and signal responder are two basic variables for electrochemical biosensors (Zhi-Fang et al., 2021). According to the scheme of the invention, electrochemical biosensors can be classified as biosensors based on the sensory principle by which the measurable quantity is detected on time and on sandwich-like cytosensor biosensors. (Zhi-Fang et al., 2021).

6.1. Early detection

As a non-invasive discovery strategy, electrochemical impedance spectroscopy (EIS) has potential for cell-related applications (Zhi-Fang et al., 2021) in a wide range of applications recovered for single operation. (Shen et al., 2016 . Keyes et al., 2013). Cells captured by the sensor terminal can piece the exchange of electroactive substance electrons into arrangement, which increments the charge exchange resistance (Zhi-Fang et al., 2021). Ordinarily, expansive surface nanomaterials with tall electrical properties can be utilized to alter the cathode as backing materials, hence progressing conductivity and affectability (Zhi-Fang et al., 2021).

Carbon nanotubes (CNTs) have pulled in impressive consideration within the improvement of electrochemical biosensors due to their tall electrical conductivity and fabulous assimilation capacity (Liu et al., 2014). For case, β-cyclodextrin-modified MWCNTs were utilized to distinguish cancer cells by tetrahydiaphene subordinate adjustment (Zhao et al., 2010; Zhi-Fang et al., 2021). Adversely charged SWNTs caused the get together of the iron-positively charged multifunctional (ethyleneimine) cathode to the anode surface by the layer-by-layer strategy, which permitted FR-mediated location of HeLa cells (Liu et al., 2013; Zhi-Fang et al., 2021). Xu et al. created a 3D-MWCNT cluster cytosensor based on neighboring dithiol-containing proteins (VDPs) (Xu et al., 2015). As showed up in Figure 20A, the MWCNTs were immobilized on the Indium Tin Oxide (ITO) terminal as a nanostructured surface (Zhi-Fang et al., 2021). MWCNT-modified 2-p-aminophenyl-1,3,2-dithiarsenolane

(VTA2) reacted particularly with the neighborhood dithiol to a diethiol-containing protein (VDP), which overexpressed in cancer cells. Polydopamine (PDA) was utilized to exemplify CNTs and immobilize FA for cellular affirmation (Zheng et al., 2012). HA conjugated to the surface of decidedly charged CNTs with electrolytic interaction poly-soluble methylammonium may especially recognize CD44 by ligand-protein interaction (Zhang et al., 2019; Zhi-Fang et al., 2021).

Graphene, a two-dimensional (2D) single sheet composed of sp2-hybridized carbon particles, has been associated inside the biosensing (Zhi-Fang et al., 2021). The
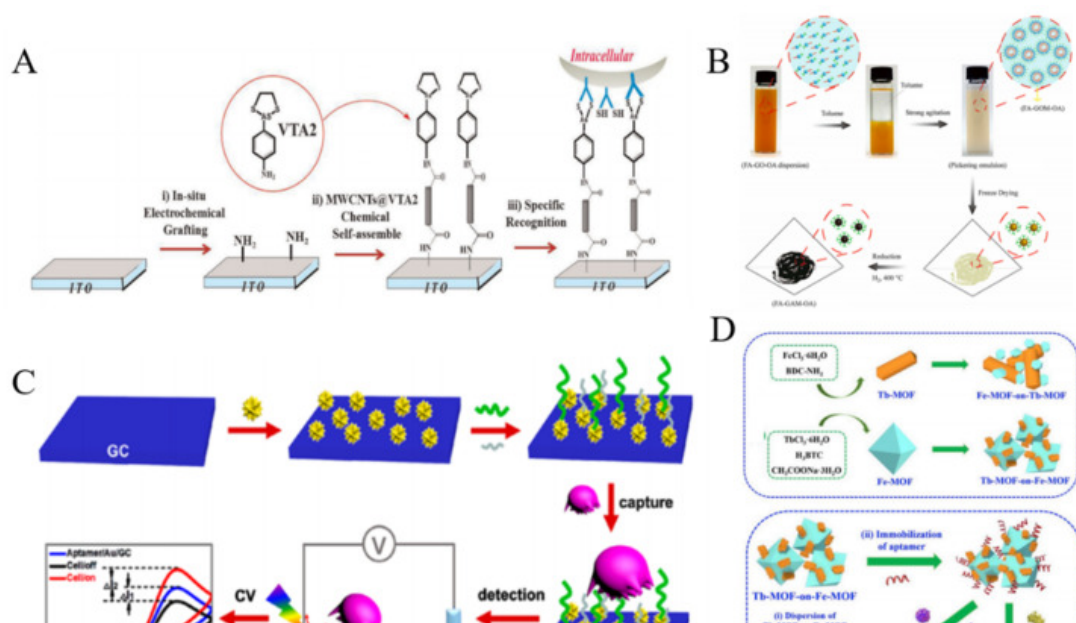

Figure 20. (A) Outline of f collecting forms of 3D-MWCNTs@VTA2 cytosensor for discovery of VDPs overexpressed tumor cell. Xu et al., 2015 . Copyright 2015 Elsevier B.V. (B) Outline of the strategy for blend of FA-GAM-OA Ruiyi et al., 2018 . Copyright 2018 Elsevier B.V. (C) Schematic representation of the procedure for ultrasensitive and label-free location of CTCs by the DPEE instrument (Wang et al., 2019). Copyright 2019 American Chemical Society. (D) Schematic representation of the planning of Fe-MOF-on-Tb-MOF and Tb-MOF-on-Fe-MOF nanostructures and the manufacture strategy of aptasensor based on two sorts of TbFe-MOFs (Wang et al., 2019B). Copyright 2019 Elsevier B.V

poly-L-lysine/GO-modified terminal has been utilized for the grasp and revelation of leukemia K562 cancer cells through the electrostatic interaction. Li et al. nitty gritty an electrochemical biosensor for the area of cancer cells based on FA and octadecylamine-modified graphene aerogel microspheres. As shown in Figure 20B, GO sheets inside the emulsion were self-assembled into graphene oxide gel microspheres on the water/toluene meddle (Zhi-Fang et al., 2021). After freeze-drying and reducing by H2, the formed FA-GAM-OA shown a sphere-like structure with bounty of open-pores and FA bunches (Zhi-Fang et al., 2021). At that point, the

microspheres were kept on the terminal for CTCs area with FA as the capture test (Zhi-Fang et al., 2021).

6.2 Sandwich-Like Detection

As one of the foremost broadly utilized discovery designs, sandwich-like cytosensor appears tall affectability and selectivity. For the foremost part, proteins electroactive particles and useful nanomaterials are widely used as identifiers (Sheng et al., 2015). For case, Yu et al. proposed a fundamental dissolvable phosphatase (ALP)-based electrochemical aptamer cytosensor for the innovation of cancer cells (Yu et al., 2017; Zhi-Fang et al., 2021). Ferrocene-labeled concanavalin A was utilized for recognizing K562 cells bolstered the lectin–carbohydrate interaction (Xue et al., 2010; Zhi-Fang et al., 2021). In any case, the flimsiness of chemicals and frail electrochemical flag of little particles constrained their applications. To beat these issues, different utilitarian nanomaterials are created to upgrade the exhibitions of electrochemical cytosensors. Concurring to the parts in flag intensification, these nanomaterials are often classified into three bunches: carriers for flag particles, nanoelectrocatalysts and electroactive tests (Zhi-Fang et al., 2021).

It could be a common approach to attain proficient flag enhancement by stacking a extraordinary number of flag tests or chemicals onto nanomaterials. Ding et al. utilized Con A and HRP-labeled AuNPs to identify CTCs based on the glycan-lectin interaction (Ding et al., 2010; Zhi-Fang et al., 2021). As showed up in Figure 21A, single-walled carbon nanoforms (SWNHs) were utilized to alter the cathode for advancing electrical arrange, which were help functionalized with arginine-glycine-aspartic acid-serine tetrapeptide to recognize K562 cell (Zhi-Fang et al., 2021). Con A on the AuNPs partner with mannose oligosaccharide on the cell surface, and HRP on the AuNPs catalyzed the reaction to form a strong electrochemical flag (Zhi-Fang et al., 2021). In expansion, tumor debasement factor-related apoptosis-inducing ligands and aptamers have been stacked on HRP-modified AuNPs for the disclosure of CTCs (Chen et al., 2014; Zhi-Fang et al., 2021). Nanohybrids of AuNPs with other nanomaterials were utilized to carry HRP and HRP-mimicking DNA chemical (hemin/G-quadruplex) for CTCs area, such as ZnO nanorods and MOFs (Chen et al., 2018). Ou et al. made a sandwich-type cytosensor for capture, revelation and release of breast cancer cells based on HRP and PtNPs-loaded MOF (Ou et al., 2019; Zhi-Fang et al., 2021). As appeared in Figure

21B, twofold aptamers (AS1411 and MUC1) were immobilized on the terminal with tetrahedral DNA nanostructures (TDN) (Zhi-Fang et al., 2021). MOF PCN-224 was decorated with PtNPs and HRP iotas. Within the intervals, two capture tests comprised of the course of action of AS1411 or MUC1 aptamer and G-quadruplex were changed on the MOF (Zhi-Fang et al., 2021). Inside the closeness of hemin and K+ particles, the catalytic HRP-mimicking G-quadruplex/hemin (GQH) DNA ezymes were made (Zhi-Fang et al., 2021).
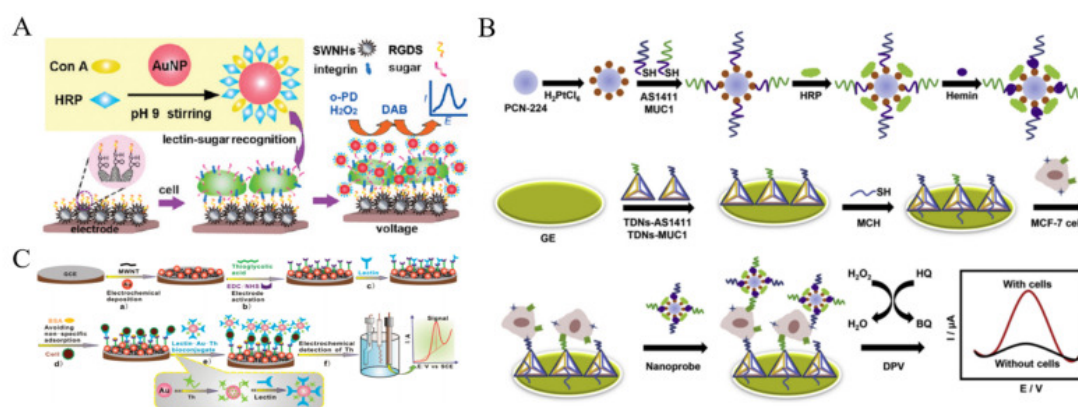

Figure 21. (A) Schematic representation of nanoprobe gathering and electrochemical technique for in situ discovery of cells and mannose bunches on living cells (Ding et al., 2010). Copyright 2010 American Chemical Society. (B) Schematic representation of creation strategies of PCN-224-Pt/HRP/dual-aptamer/GQH nanoprobe and the electrochemical dual-aptamer cytosensor creation handle [Zhang et al., 2010]. Copyright 2019 Elsevier B.V. (C) Schematic representation of the lectin-based biosensor for EC Investigation of cells (Zhang et al., 2010). Copyright 2010 American Chemical Society.

After the arrangement of sandwich-like structure, multifunctional half breed nanoprobes containing PCN-224-Pt, HRP and GQH DNA ezyme catalyzed the era of benzoquinone (BQ) from the oxidation of HQ with H_2O_2 (Zhi-Fang et al., 2021). In expansion to chemicals, electroactive little particles can too be altered on nanomaterials to distinguish CTCs (Doo et al., 2019; Zhi-Fang et al., 2021) For occasion, Zhang et al. detailed a lectin-based biosensor for the tests of A549 cells based on thionine (Th)-labeled AuNPs (Zhang et al., 2010; Zhi-Fang et al., 2021). As outlined in Figure 21C, lectin might particularly recognize sialic corrosive which is over-expressed on the surface of cancer cells (Zhi-Fang et al., 2021). The normal sum of sialic corrosive on the surface of single cell has been assessed by the biosensor (Zhi-Fang et al., 2021).

A novel electrochemical cytosensor was made for the quick and high-sensitivity certification of drug-resistant leukemia K562/ADM cells based on the P-glycoprotein (P-gp) expression level on a cell film (Zhang et al., 2014). The nanocomposite interface of the gold nanoparticles/polyaniline nanofibers (AuNPs/PANI-NF) was chosen to orchestrate the biosensor for electrochemical revelation (Fig. 22) (Zhang et al., 2014). Au/PANI-NF-based cytosensors coated with anti-P-glycoprotein (anti-P-gp) (Zhang et al., 2014) particles show up give a biomimetic interface for the immunosensing of cell surface P-glycoprotein, and in this way might capture the over-expression P-gp cells (Zhang et al., 2015). Transmission electron microscopy (TEM) illustrated that the gold nanoparticles were reliably tied down along the structure of the PANI-NF surface, appearing fibrillar morphology with a breadth of ~70 nm, and nuclear drive microscopy (AFM) help shown the morphology of the nanocomposite film (Zhang et al., 2014). Owing to the high liking of anti-P-gp for leukemia K562/ADM cells of the propounded detecting stage, the proposed biosensor shown amazing expository execution for leukemia K562/ADM cells, extending from 1.6×102 to 1.6×106 cells per mL with a discovery constrain of 80 cells per mL. Recuperation tests illustrated that the affectability nitty gritty here is sensible for commonsense application (Zhang et al., 2015). The cell surface P-gp expression level was inspected by stream cytometric tests, which confirmed the over recognized result (Zhang et al., 2015). This strategy helps to ensure the recognition of cancer cells and cell surface receptors that lead to the recognition of cancer. (Zhang et al., 2015).
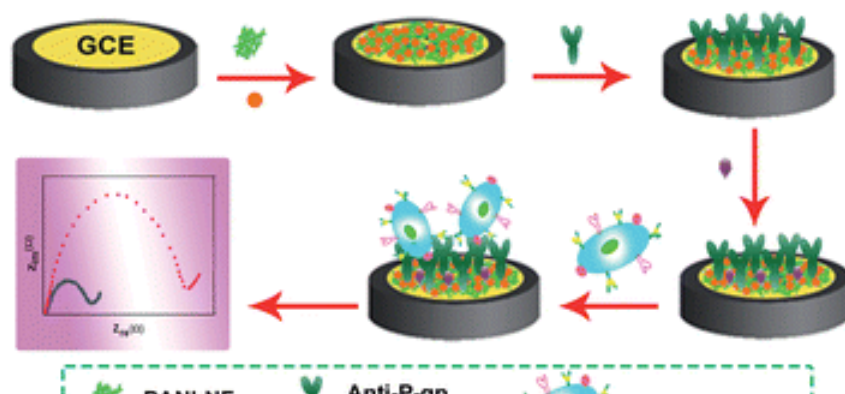

Figure 19. The nanocomposite interface of the gold nanoparticles/polyaniline nanofibers (AuNPs/PANI-NF) was chosen to plan the biosensor for electrochemical discovery of drug-resistant leukemia K562/ADM cells based on the P-glycoprotein (P-gp) expression level on a cell film.

The detection of rare circulating tumor cells (CTCs) in patients' blood is pivotal for theearly determination of

ancer, exceedingly exact cancer treatment and observing restorative results in real time (Li et al., 2018). In this consider created a productive technique to capture and identify CTCs from the blood of cancer patients employing a benzoboric acid adjusted gold-plated polymeric substrate with a normal 3D surface cluster (Fig. 23) (Li et al., 2018). Compared with the smooth substrate, the substrate with the surface 3D microarrays shown the following capture efficiency, i.e. 3.8 times that overseen by the smooth substrate (Li et al., 2018). Moreover, due to the reversible reaction between the benzoboric corrosive on the 3D microarray and the sialic corrosive on CTCs, their technique allowed for basic division of the captured CTCs from the substrate without causing essential hurt to the cells (Li et al., 2018). The proposed procedure gives a few points of interest, including enhanced capture efficiency, high sensitivity, low cost and recovery of isolated CTCs, and could become a promising platform for early-stage diagnosis of cancer (Li et al., 2018).
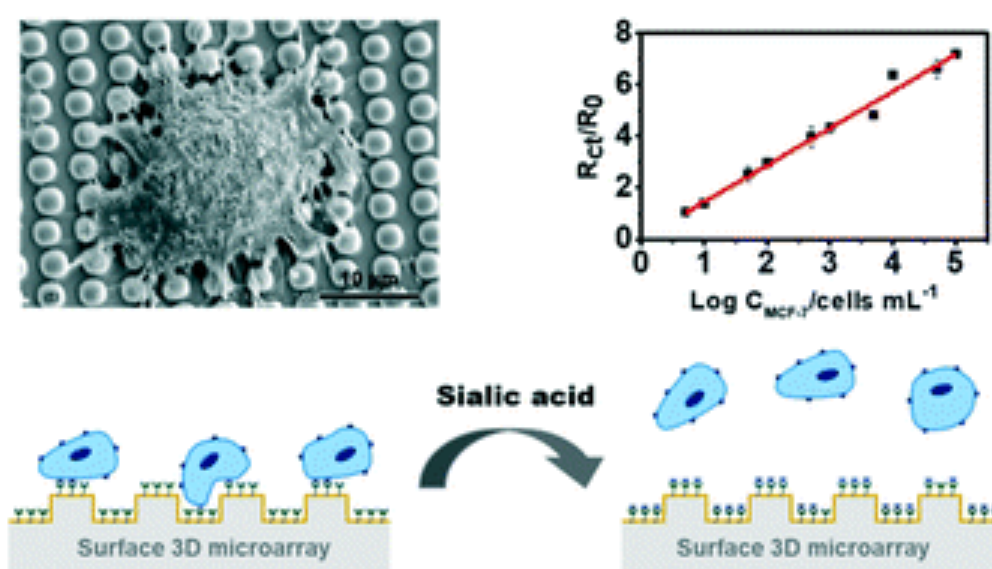

Figure 20. 3D microarrays technique to capture and identify CTCs from the blood of cancer patients employing a benzoboric acid adjusted gold-plated polymeric substrate with a normal 3D surface cluste. (An et al., 2018)

7. Advanced electrochemical sensors for virus detection
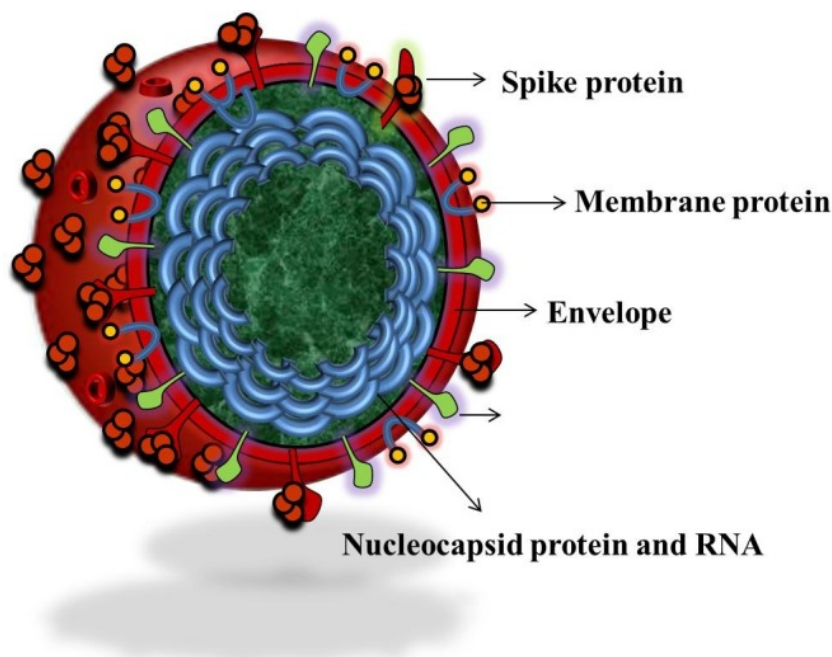
7.1 Coronavirus detection

Figure 21. Structure of SARS-CoV-2. (Bukkitgar et al., 2021)

Infections can be caused by a large number of pathogenic microorganisms, in most cases by pathogenic bacteria or viruses, as well as by other types of pathogens. Organisms that receive the uninvited "visit" of pathogens react immediately with their "defense" system, their own immune system. Inflammations are caused by the innate immune system, followed by additional reactions of the body's immune system. These intercellular operators cannot duplicate exterior the host cell, but stay as a crystalline structure for longer period until they come in contact with a host. (Kaya et al., 2020) The hereditary material, either DNA or RNA, is encompassed by a sheet of protein, called capsid. (D. P. Clark, N. J. Pazdernik, Viruses. Molecular Biology, 2nd edition, Academic Cell 2013, e517–e522) Once the viral genome enters the host cell, the replication and protein amalgamation apparatus is captured to create more infection particles, called Virions that have the capacity to contaminate unused cells after discharging from the host cell. Infections get effectively adjusted to unused conditions due to mutation, thereby expanding the hereditary differences. Since infection don't have any chemical system, antibodies may not influence them.

These infections comprise of as   it were digestive protein supportive to dissolve the have cell layer and infections have a place to a wide assortment of families such as DNA infection and RNA infection. Ordinary examples are Adenoviridae, Parvoviridae, *Herpesviridae, Retroviridae, Rhabdoviridae,* etc.

The structure of corona takes after generally a circular shape with projections showing up like a crown (Fig. 24) (Bukkitgar et al., 2020). The infection has roughly 125 nm estimate comprising of an envelope of 85 nm dia, whereas the spikes are 20 nm long. It may be a single stranded RNA with the measure extending from 26,000 to 37,000 bases and is the biggest known genome among the RNA infections. (Weiss et al., 2005) Envelope within the infection is made of lipid bilayer, which is anchored with membrane proteins, envelope proteins and spike structural proteins within the proportion of 1:20:300 (Bukkitgar et al., 2020). The spike protein in corona infection may be a class I fusion protein. With two subunits S1 and S2, the S1 subunit is characterised by two subdomains, viz., C-terminal and N-terminals, which are competent of authoritative receptors angiotensin-converting protein 2 (ACE2). (Bukkitgar et al., 2020) The spike protein is intensely glycosylated and encourages the connection to have receptors by getting to ER with the assistance of N-terminal flag arrangement (Bukkitgar et al., 2020). The nucleocapsid protein is intensely phosphorylated those ties to RNA in vitro (Bukkitgar et al., 2020). This protein is supportive in bundling the typified genome into the viral particles by restricting the viral genome to replicase-transcriptase complex (Bukkitgar et al., 2020). The foremost plenteous basic protein in infection is the layer protein, which has two diverse conformations that can advance the authoritative to nucleocapsid. In little amount, the envelope protein acts like a Trans film protein for particle channel action. (Lu et al., 2020)

Yakoh et al., 2020 designed an electrochemical sensor, an immunosensor, that detects antibodies of the SARS – CoV – 2, i.e. immunoglobulins against SARS – CoV – 2. The presence of antibodies interrupts the redox conversion of the indicator and consequently the flow of current. The immunosensor was found to be effective in patient sera and extended to further antigen detection of SARS – CoV – 2, the spike protein. A schematic representation of the immunosensor is shown in the figure below.

Each immunosensor (ePAD) consists of three folding levels of paper: a working ePAD, a counter ePAD and a closing ePAD (Abdulhadee et al., 2021). In their center the ePADs are hydrophilic and are surrounded by wax so that the solution can remain in the center of the ePADs (Abdulhadee et al., 2021). At their center the ePADs contain a hole through which the solution will pass when the redox solution is added (Abdulhadee et al., 2021).
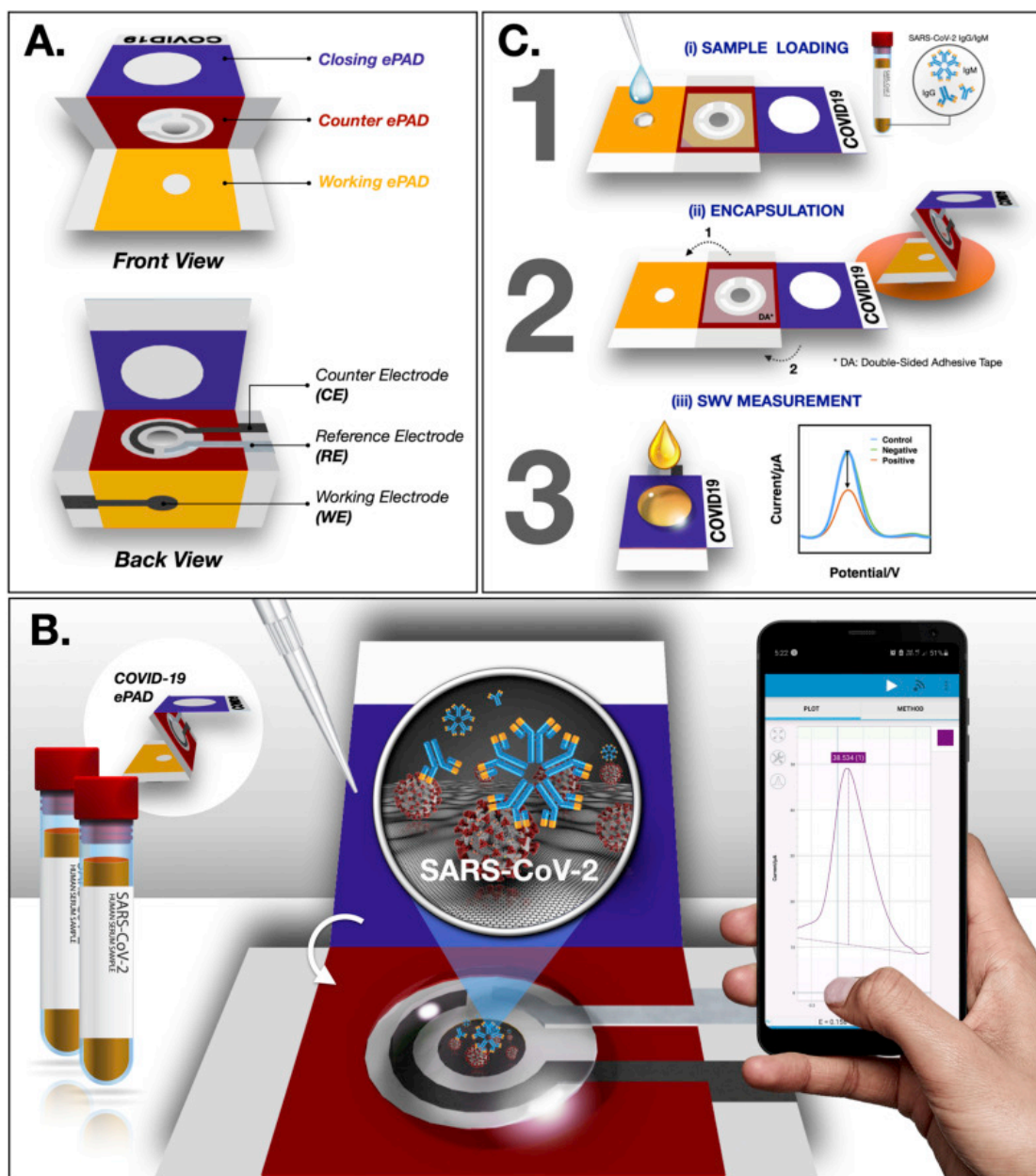

Figure 22. Schematic illustration of the (A) device components, (B) detection principle and (C) detection procedure of the COVID-19 ePAD. (Yakoh et al., 2020)

7.2 DENV detection

Navakul et al. 2017, illustrated electrochemical biosensor based on impedance spectroscopy utilizing gold anode stored with graphene oxide to identify within the constrain of 0.12 pfu/ml (Bukkitgar et al., 2020). Schematic representation of the testimony of graphene oxide polymer onto the surface of gold anode is appeared (Fig. 26) (Bukkitgar et al., 2020). The specificity of manufactured anode was evaluated since the copolymer was electrically conductive due to the nearness of graphene oxide (Bukkitgar et al., 2020). The negative charge of oxygen iota in graphene draws in the dengue infection particles with a positive potential. Joshi et al. 2020, illustrated a strategy utilizing thermally diminished graphene oxide stored onto indium tin oxide/glass anodes for quantitative assurance of flu infection H1N1 as a name free electrochemical immuno-sensor utilizing impedance spectroscopy (Bukkitgar et al., 2020). The location constrain detailed by this strategy was 26 and 33 pfu/mL for saline and weakened spit, separately (Fig. 27) (Bukkitgar et al., 2020).
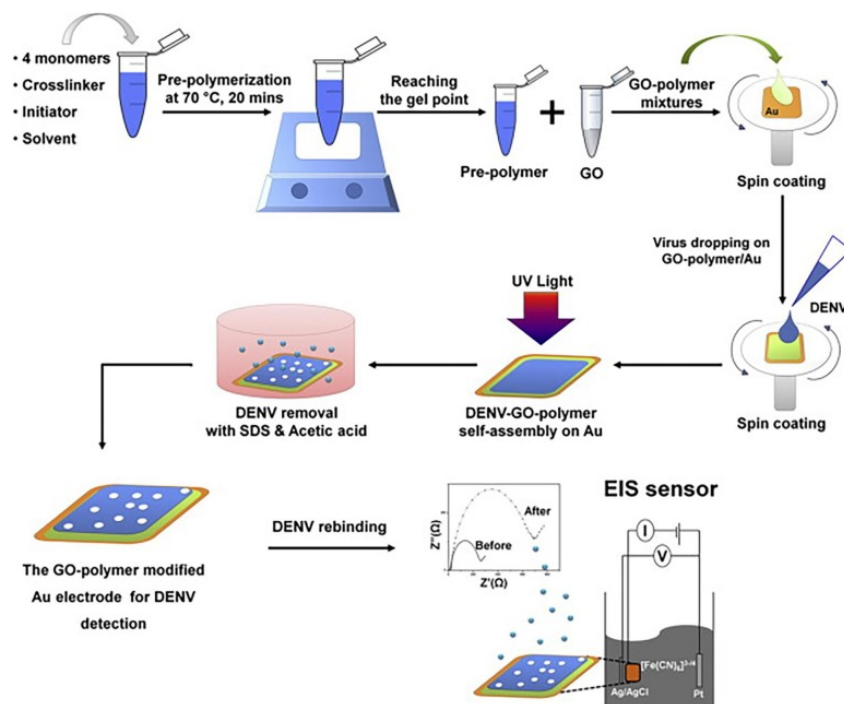
Figure 23. Schematic representation of the preparation of GO-polymer on gold electrode for DENV detection (Navakul et al. 2017).
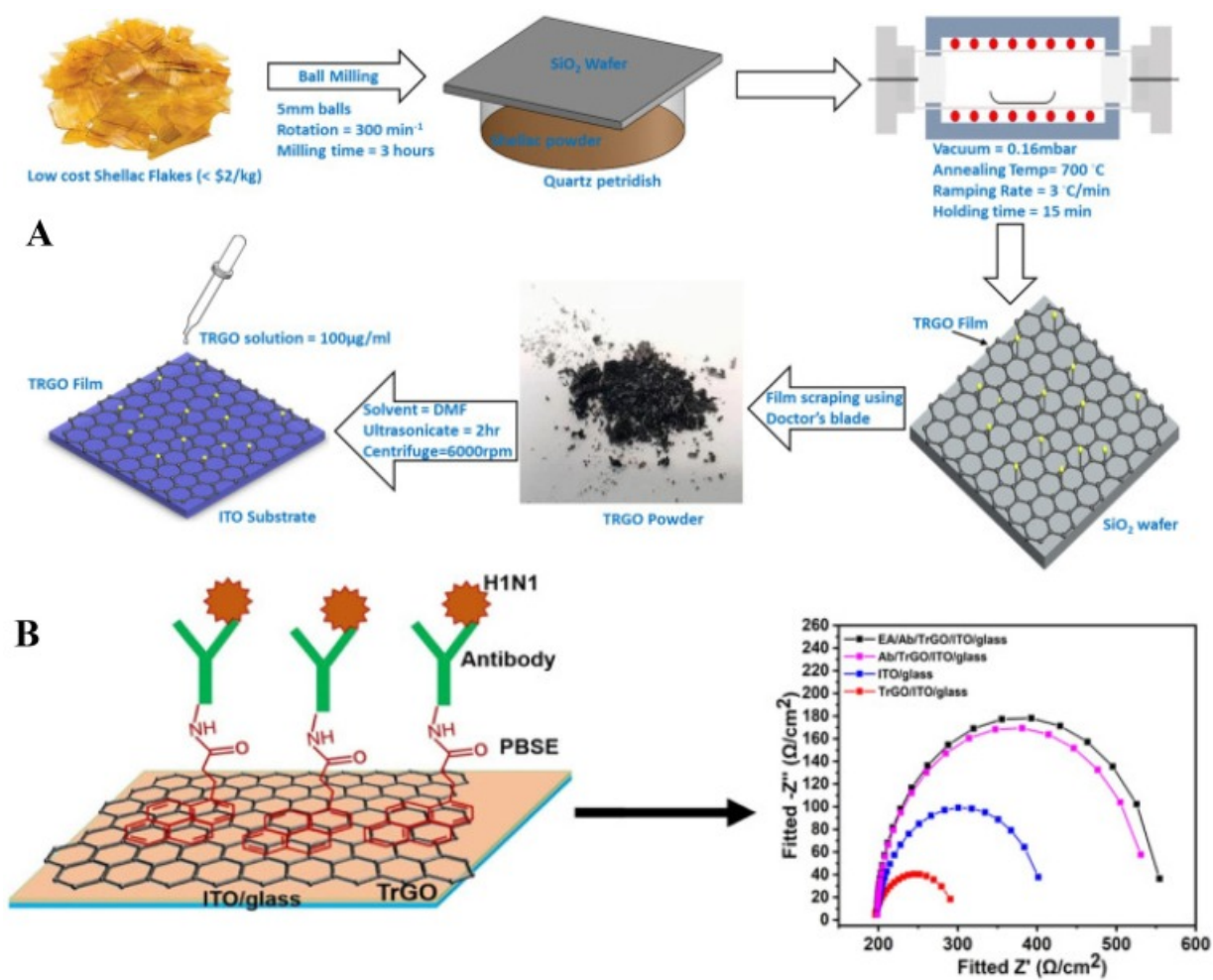
Figure 24. (A) Schematic illustration to display the synthesis route of TrGO using Shellac biopolymer; (B) Schematics of the proposed thermally – decomposed reduced graphene oxide (Joshi et al. 2020).

Conclusion

Effective presentation of different techniques based on DNA collection and protein promotion enhances the explanatory reports. Indeed, although huge sums of works have been detailed, there's still a huge and significant crevice between scholastic inquire about and clinical interpretation. For case, the broadly utilized biorecognition components (antibodies and aptamers) may endure from fast debasement in complex clinical tests that contains nucleases, proteases, and another potential interferer (Zhi-Fang et al., 2021). Indeed, although pre-concentration can decrease the impedances, it'll increment the complexity of the location method and may diminish the practicality of cells (Zhi-Fang et al., 2021).

Unused frameworks and devices that allow cells to be analyzed in situ and recouped for advance characterization are advantage to way better extricate more data approximately their phenotype and clinical pertinence (Zhi-Fang et al., 2021). Coordinates counterfeit insights and rising nanotechnology and effective explanatory strategies may give a promising approach. Moreover, the soundness and work of nanomaterials beneath physiological conditions ought to be considered (Zhi-Fang et al., 2021). Appropriate surface adjustments may resolve these challenges, but they may bring unused issues (Zhi-Fang et al., 2021). For occasion, the dynamic locales of nanozymes and nanoelectrocatalysts would be blocked due to the surface alteration (Zhi-Fang et al., 2021). In the interim, appropriate alteration strategies ought to be investigated to affirm the aptamer arrangement and counter acting agent introduction on the surface of nanomaterials and nanostructures since they can impact the liking between the biorecognition components and CTCs (Zhi-Fang et al., 2021).

Electrochemical biosensors have come to the constrain of location as moo as colony-forming units and single plaque-forming units (Bukkitgar et al., 2020). The use of different nanostructured materials for cathode manufacture has been comprised of metal/metal oxides, carbon materials, polymers, and composites (Bukkitgar et al., 2020). Indeed, although writing proposes fruitful applications of the electrochemical sensors in pathogen location, still a wide crevice exists in such gadget accessibility at the point-of care (Bukkitgar et al., 2020). Within the close future, there may be still a few chances of such pandemics to happen and subsequently, there may be a more noteworthy require of keen gadgets (Bukkitgar et al., 2020). Certainly, combining

electrochemical sensors with nanotechnology would offer a prepared road to illuminate the issues related to dangerous pandemics (Bukkitgar et al., 2020).

References

1. **Argyriadis V., Nanotubes: Types, Manufacturing Methods, Properties, Applications, Diploma Thesis, Athens, 2012.**

2. **Afreen S, He Z, Xiao Y, Zhu JJ. Nanoscale metal-organic frameworks in detecting cancer biomarkers. J Mater Chem B. 2020 Feb 21; 8(7):1338-1349.**

3. **An L, Wang G, Han Y, Li T, Jin P and Liu S. Electrochemical biosensor for cancer cell detection based on a surface 3D micro-array. Lab On a Chip. 2018. Issue 2.**

4. **Bukkitgar SD, Shetti NP and Aminabhavib TM. Electrochemical investigations for COVID-19 detection-A comparison with other viral detection methods. Chem Eng J. 2021 Sep 15; 420: 127575.**

5. **Bhushan, (2010), Springer Handbook of Nanotechnology**

6. **Clark D,P N. J. Pazdernik, Viruses. Molecular Biology, 2nd edition, Academic Cell 2013, e517–e522.**

7. **Cathcart N, Chen JIL. Sensing Biomarkers with Plasmonics. Anal Chem. 2020 Jun 2; 92(11):7373-7381**

8. **Chen X.J., Wang Y.Z., Zhang Y.Y., Chen Z.H., Liu Y., Li Z.L., Li J.H. Sensitive electrochemical aptamer biosensor for dynamic cell surface N-glycan evaluation featuring multivalent recognition and signal amplification on a dendrimer-graphene electrode interface. Anal. Chem. 2014;86:4278–4286.**

9. **Chen D.B., Sun D.P., Wang Z.R., Qin W.W., Chen L., Zhou L.D., Zhang Y.Q. A DNA nanostructured aptasensor for the sensitive electrochemical detection of HepG2 cells based on multibranched hybridization chain reaction amplification strategy. Biosens. Bioelectron. 2018;117:416–421**

10. **Ding L., Ji Q., Qian R., Cheng W., Ju H. Lectin-based nanoprobes functionalized with enzyme for highly sensitive electrochemical monitoring of dynamic carbohydrate expression on living cells. Anal. Chem. 2010;82:1292–1298.**

11. **Dou B, Xu L, Jiang B, Yuan R, Xiang Y. Ultrasensitive electrochemical detection of breast cancer cells based on DNA-rolling-circle- Aptamer-Functionalized and Gold Nanoparticle Array-Decorated Magnetic Graphene Nanosheets Enable Multiplexed and Sensitive Electrochemical Detection of Rare Circulating Tumor Cells in Whole Blood. Anal Chem. 2019 Aug 20; 91(16):10792-10799.**

12. **El Aamri M, Yammouri G, Mohammadi H, Amine A, Korri-Youssoufi H. Electrochemical Biosensors for Detection of MicroRNA as a Cancer Biomarker: Pros and Cons. Biosensors (Basel). 2020 Nov 20; 10(11)**

13. **Fattahi Z, Khosroushahi AY, Hasanzadeh M. Recent progress on developing of plasmon biosensing of tumor biomarkers: Efficient method towards early stage recognition of cancer. Biomed Pharmacother. 2020 Dec; 132():110850.**

14. Grieshaber, D., MacKenzie, R., Vörös, J., & Reimhult, E. (2008). Electrochemical Biosensors - Sensor Principles and Architectures. Sensors, 8(3), 1400–1458.

15. He, X., Zhou, L., He, D., Wang, K., & Qin, D. (2011). Biosensing technologies for mycobacterium tuberculosis detection: Status and new developments. Clinical and Developmental Immunology, Vol. 2011.

16. Joshi S.R., Sharma A., Kim G.-H., Jang J. Low cost synthesis of reduced graphene oxide using biopolymer for influenza virus sensor. Mater. Sci. Eng: C Mater Biol Appl. 2020;108

17. Kaya S.I., Karadurmus L., Ozcelikay G., Bakirhan N.K., Ozkan S.A. Electrochemical virus detections with nanobiosensors, Nanosensors for Smart Cities. Micro and Nano Technologies. 2020:303–326. doi: 10.1016/B978-0-12-819870-4.00017-7.

18. Koromilas N., Modification of carbon nanotubes in multilayers that present biostatic properties, Postgraduate Thesis, Patras, 2012.

19. Keyes TE, Venkatanarayanan A, Forster RJ. Label-free impedance detection of cancer cells. Anal Chem. 2013 Feb 19; 85(4):2216-22.

20. Koyun, A., Ahlatcolu, E., & Koca, Y. (2012). Biosensors and Their Principles. In A Roadmap of Biomedical Engineers and Milestones.

21. Liu Y., Zhu F., Dan W., Fu Y., Liu S. Construction of carbon nanotube based nanoarchitectures for selective impedimetric detection of cancer cells in whole blood. Analyst. 2014;139:5086–5092.

22. Lu R., Zhao X., Li J. Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet. 2020;395:565–574.

23. Liu Y, Kwa T, and Revzin A. Simultaneous detection of cell-secreted TNF-α and IFN-γ using micropatterned aptamer-modified electrodes. Biomaterials. Author manuscript; available in PMC 2013 Dec 16.

24. Navakul K., Warakulwit C., Yenchitsomanus P.-T., Panya A., Lieberzeit P.A., Sangma C. A novel method for DENV detection and antibody screening using a graphene-polymer based electrochemical biosensor, Nanomedicine: Nanotechnology. Biology, and Medicine. 2017;13:549–557.

25. Nguyen, H. H., Lee, S. H., Lee, U. J., Fermin, C. D., & Kim, M. (2019, January 2). Immobilized enzymes in biosensor applications. Materials, Vol. 12.

26. Ou D., Sun D., Liang Z., Chen B., Lin X., Chen Z. A novel cytosensor for capture, detection and release of breast cancer cells based on metal organic framework PCN-224 and DNA tetrahedron linked dual-aptamer. Sens. Actuators B Chem. 2019;285:398–404.

27. Pantel K, Alix-Panabières C. Liquid biopsy and minimal residual disease - latest advances and implications for cure. Nat Rev Clin Oncol. 2019 Jul; 16(7):409-424

28. Paniel, N., Baudart, J., Hayat, A., & Barthelmebs, L. (2013, December 15). Aptasensor and genosensor methods for detection of microbes in real world samples. Methods, Vol. 64, pp. 229–240.

29. Perumal, V., & Hashim, U. (2014). Advances in biosensors: Principle, architecture and applications. Journal of Applied Biomedicine, Vol. 12, pp. 1–15.

30. Ruiyi L., Fangchao C., Haiyan Z., Xiulan S., Zaijun L. Electrochemical sensor for detection of cancer cell based on folic acid and octadecylamine-functionalized graphene aerogel microspheres. Biosens. Bioelectron. 2018;119:156–162.

31. Rocchitta, G., Spanu, A., Babudieri, S., Latte, G., Madeddu, G., Galleri, G., … Serra, P. A. (2016, June 1). Enzyme biosensors for biomedical applications: Strategies for safeguarding analytical performances in biological fluids. Sensors (Switzerland), Vol. 16.

32. Shao N, Wickstrom E, Panchapakesan B. Nanotube-antibody biosensor arrays for the detection of circulating breast cancer cells. Nanotechnology. 2008 Nov 19; 19(46):465101.

33. Shen H, Yang J, Chen Z, Chen X, Wang L, Hu J, Ji F, Xie G, Feng W. A novel label-free and reusable electrochemical cytosensor for highly sensitive detection and specific collection of CTCs. Biosens Bioelectron. 2016 Jul 15; 81():495-502.

34. Shabaninejad, Z., Yousefi, F., Movahedpour, A., Ghasemi, Y., Dokanehiifard, S., Rezaei, S., … Mirzaei, H. (2019, September 15). Electrochemical-based biosensors for microRNA detection: Nanotechnology comes into view. Analytical Biochemistry, Vol. 581.

35. Shen Z, Wu A, Chen X. Current detection technologies for circulating tumor cells. Chem Soc Rev. 2017 Apr 18; 46(8):2038-2056.

36. Sheng Q, Cheng N, Bai W, Zheng J. Amplification-directed enzyme-catalyzed polymerization. Chem Commun (Camb). 2015 Feb 7; 51(11):2114-7.

37. Truong, J., Hsieh, Y. F., Truong, L., Jia, G., & Hammond, M. C. (2018). Designing fluorescent biosensors using circular permutations of riboswitches. Methods.

38. Weiss S.R., Navas-Martin S. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol. Mol. Biol. Rev. 2005;69:635–664.

39. Wang Q., Zou L., Yang X., Liu X., Nie W., Zheng Y., Cheng Q., Wang K. Direct quantification of cancerous exosomes via surface plasmon resonance with dual gold nanoparticle-assisted signal amplification. Biosens. Bioelectron. 2019B;135:129–136.

40. Wang SS, Zhao XP, Liu FF, Younis MR, Xia XH, Wang C. Direct Plasmon-Enhanced Electrochemistry for Enabling Ultrasensitive and Label-Free Detection of Circulating Tumor Cells in Blood. Anal Chem. 2019 Apr 2; 91(7):4413-4420.

41. **Xu Y, Ding L, Lei J, Ju H.A simple electrochemical lectin-probe for in situ homogeneous cytosensing and facile evaluation of cell surface glycan. Biosens Bioelectron. 2010 Sep 15; 26(1):169-74.**

42. **Xu Y.N., Wu H., Huang C.S., Hao C.Q., Wu B.N., Miao C.C., Chen S., Jia N.Q. Sensitive detection of tumor cells by a new cytosensor with 3D-MWCNTs array based on vicinal-dithiol-containing proteins (VDPs) Biosens. Bioelectron. 2015;66:321–326.**

43. **Yu T., Zhang H., Huang Z., Luo Z., Huang N., Ding S., Feng W. A simple electrochemical aptamer cytosensor for direct detection of chronic myelogenous leukemia K562 cells. Electroanalysis. 2017;29:828–834. doi: 10.1002/elan.201600505.**

44. **Zhao J, Jin J, Wu C, Jiang H, Zhou Y, Zuo J, Wang X. Highly sensitive identification of cancer cells by combining the new tetrathiafulvalene derivative with a β-cyclodextrin/multi-walled carbon nanotubes modified GCE. Analyst. 2010 Nov; 135(11):2965-9.**

45. **Zheng T.T., Zhang R., Zou L.F., Zhu J.J. A label-free cytosensor for the enhanced electrochemical detection of cancer cells using polydopamine-coated carbon nanotubes. Analyst. 2012;137:1316–1318.**

46. **Zhang R, Rejeeth C, Xu W, Zhu C, Liu X, Wan J, Jiang M, Qian K. Label-Free Electrochemical Sensor for CD44 by Ligand-Protein Interaction. Anal Chem. 2019 Jun 4; 91(11):7078-7085.**

47. **Zhang X, Teng Y, Fu Y, Xu L, Zhang S, He B, Wang C, Zhang W. Lectin-based biosensor strategy for electrochemical assay of glycan expression on living cancer cells. Anal Chem. 2010 Nov 15; 82(22):9455-60.**

48. **Zhi-Fang Sun, Yong Chang, and Ning Xia. Recent Development of Nanomaterials-Based Cytosensors for the Detection of Circulating Tumor Cells. Biosensors (Basel). 2021 Aug; 11(8): 281.**

49. **Zhang, L., Huang, J., Yang, N., Greshock, J., Megraw, M. S., Giannakakis, A., … Coukos, G. (2006). microRNAs exhibit high frequency genomic alterations in human cancer. Proceedings of the National Academy of Sciences of the United States of America, 103(24), 9136–9141.**

50. **Zhu, Y.-C., Mei, L.-P., Ruan, Y.-F., Zhang, N., Zhao, W.-W., Xu, J.-J., & Chen, H.-Y. (2019). Enzyme-Based Biosensors and Their Applications. In Advances in Enzyme Technology (pp. 201–223).**