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Review Article

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Biosensors in Tissue Engineering: An Exhaustive Review with Future Therapeutic Potentials -With Further Updates in Electrochemical Sensors in Medical Practice

Kulvinder Kochar Kaur[1]*, Gautam Allahbadia[2] and Mandeep Singh[3]

[1*]*Kulvinder Kaur Centre for Human Reproduction, India.*

[2]*Rotunda-A Centre for Human Reproduction, India.*

[3]*Consultant Neurologist, Swami Satyanand Hospital, India.*

***Corresponding Author**
Kulvinder Kochar Kaur, Kulvinder Kaur Centre for Human Reproduction, India.

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Abstract

Biosensor research is fast picking up pace with umpteen publications along with industry having become very rich worth billions of dollars.Various industries like food and beverage s,environmental,medical diagnostics along with pharmaceutical industries utilize these applications.For detection of peptides,proteins,enzymes along with various bimolecules for diverse applications have been developed.In recent years their use in tissue engineering has remained limited.Although interest is being shown in development of novel biosensors in cell culture and tissue engineering ,e.g. detection of small molecules in real-time like lactose,glucose besides serum proteins with large molecular size like α feto protein,along with inflammatory cytokines e.g TNFα and IFNγ.This review provides various advancements in biosensor research regarding applications for tissue engineeringwith further latest updates in electrochemical sensors.

Keywords: Carbon Nanotubes; Quantum Dots; Graphene-Based Biosensors; Microfluidic Systems; Tissue Engineering

1. Introduction

Regarding applications in tissue Engineering as well as regenerative medicine, biosensors have shown good potential [1, 2]. Gradually these biosensors are becoming a part of these systems related to tissue engineering and more so with the microfluidic models for the same with their ability to sense biological molecules in minute tissue constructs which are in real time scenario and in very low levels. Blood glucose monitoring composes the biggest use of biosensors till now [3, 4]. Also, the most commonly used biological elements for biosensing are enzymes, receptors along with antibodies [5]. Their use has extended to 'in vivo' sensing of disease as disease specific biomarkers [6].

Real –time biological signals in the form of like antibodies/ proteins which are released as a result of tissue injury, muscle dystrophy, coronary artery stenosis, inflammation or bacterial or viral infections can be detected in vitro [7]. This makes them important tools regarding early-stage disease detection [7]. To get precise signaling in cellular microenvironments, probes with micro or nano dimensions are required. Thus, biosensors have very small dimensions e. g in nanotube or nanowires have been constructed. pH can be measured or they can be used to detect minute quantities of biochemical and biological species utilizing specific capture molecules [7]. Like nano cantilevers have been used to study serum protein marker levels, as well as content of specific DNA moieties [8, 9]. Highly sensitive fluorescent nanocrystals, the quantum dots can be used as well for making out specific protein or DNA [10].

Further research is going on to combine nanobiosensors, signaling along with devices which deliver medicines therapeutically and both for screening as well management [11-13]. Both biosensors having different micro and nanostructures have been used for short as well as long term in vivo studies [14]. They showed increased biointegration, differentiation and signaling potentials, besides being biocompatible. Still till date the biosensors applications are limited in biomedical engineering, in being in early stage of development. Combination of these two multidisciplines shows great promise and finally may get translated from bench to bedside applications. This review comprehensively reviews various principles of biosensors fabrication, design and operative mechanisms and include the longtime feasibility in coming times in the field of bioengineering mainly where tissue engineering is concerned.

1.1. Definition

A biosensor can be defined as a self-contained analytical device

that combines a biological component with a physico chemical component for the detection of the analyte of biological importance. It has 3 principal components i) A Detector for detecting the stimulus ii) A Transducer to convert the stimulus to output signals subsequently iii) Asignal processing system which then processes the output and presents it in an appropriate form.

Based on biosensing components biosensors can be divided onto:
• **Catalytic Type:** which include enzymes, microbes, organelles, cells or tissues or
• **Affinity Type:** including antibodies, receptors and nucleic acids.

Enzymes: Enzymes used are usually proteins of oxidase type which can selectively react with specific analytes ,consume dissolved 02and produce H2O2,an easily detectable compound .ii)Other mechanisms include detection of enzyme activation or inhibition by the analyte and modification of the enzyme properties by the analyte .The enzyme molecules can be directly immobilized on the transducer surfaces using entrapment in gels,attachment through covalent bonding,physical adsorption on the surfaces ,or other available techniques [15, 16]. The advantages of enzyme based biosensing includes the commercial availability, of enzymes, at high purity levels, the high specificity of their binding capabilities ,suitability with various transduction techniques,and the ability to detect a wide range of analytes .Besides, where mechanism of action of enzyme is of catalytic in nature leading to the enzyme remaining unaltered ,at the end of the reaction these sensors can be reused continuously ;disadvantages of enzyme based sensors include the limited stability of the enzymes and the dependency of their activities on various factors e.g., pH, ionic strength, chemical inhibition and temperature.

Microbes: Advantages-They are present allover and have great capacity to acclimitize to undesirable conditions and to develop the ability to metabolize new molecules with time.They are cheaper than enzymes or antibodies.They can carry out several complex reactions while maintaining their stability .Whole cells can be used either in a viable or nonviable form .Viable cells have gained importance in the manufacture of biosensors and these cells metabolize various organic compounds either anaerobically or aerobically leading to various end products like ammonia,carbon dioxide,acids and so forth that can be monitored using a variety of transducers.Use of microbiosensors is common in environmental fields which include detection of harmful bacteria or pesticides, in air water ,or food and biological oxygen demand[17-19].

Organelles: Organelle are compartments located inside the cell e. g lysosome, chloroplast and mitochondria. Mitochondria control the calcium metabolism and calcium depending pathways within the cell. High concentrations of calcium stimulate the mitochondria to open calcium channels This biosensor strategy can be used to measure calcium concentration in medium. Mitochondria for water pollution detection is another application of organelles in biosensors [20].

Cells and Tissues: Cells have high sensitivity to adjacent environment. They attach on surfaces, which is the main characteristic because of which they can be easily immobilized. They are often used to detect global parameters like stress condition, toxicity and organic derivatives, and to monitor the treatment effect of drugs. Cells are also used in ion selective transducers [21, 22]. Tissues are used as biosensors, as they contain a lot of enzymes. Advantages over organelles is easier immobilization, higher activity and stability, low price and experience of necessary cofactors to function [23]. Their disadvantages being lack of specificity because of presence of undesirable enzymes which leads to the reaction becoming complicated and result being ambiguous and thus less reliable outputs.

Antibodies: Antibodies are proteins produced by B lymphocytes as a result of antigenic stimulation. Antibody phase sensors are also known as immunosensors. They are usually used in Surface Plasmon Resonance (SPR). Biosensors to design target specific sensors for detection of specific molecules. This works through antigen antibody interaction process. Antibodies are usually linked to the surface of trasducers by covalent bonds like amides, ester or thiol bonds. The transducer surface needs to be modified by polymers or monomers to introduce functional groups like carboxyl, monoaldehyde sulfhydryl groups to facilitate conjugation between the antibody and transducer. Many antibodies available today for immunoassays, they being more accurate and faster as compared to the traditional assays [24]. Limitations of antibody-based assays include irreversible reaction and the strength of binding affinity. This is dependent on pH and temperature, which makes the results highly variable due to the measurement conditions [25, 26].

Nucleic Acids: Oligonucleotides-integrate in nucleic acid biosensors with a signal transducer. Oligonucleotide probe is immobilized on the trasducer to detect DNA/RNA fragments. For detection it is the code of complementary nucleotide base pairing Adenine(A); Thymine(T) and Cytosine (C); Guanine(G) in DNA. The hybridization probes in the sensor can then base pair with the target sequences and create an optical signal [25].

1.2. Biotransducer Components

Electrochemical Biosensors: Used for hybridized DNA detection, glucose concentration etc. Their classification is based on electrical parameters e.g. a) Conductometric b) Amperometric c) Affinniometric types. Electrochemical biosensors contain 3 electrodes usually i) a reference electrode, ii) A working electrode and a iii) counter electrode. The reaction for target analysis takes place on the activity electrode surface. The reaction causes either electron transfer across the double layer or can contribute to the double layer potential. Three kinds of biosensors often made by screen printing, the electrode pattern for a plastic substrate coated with a conducting polymer and then some protein is attached to all biosensors which usually involve minimal sample preparation as the biological component is highly selective and the signal is produced by electrochemical and physical changes in the conductance polymer layer.

Other Sensors: Optical sensors are usually based on optical diffraction. These sensors can detect microscopic changes when cells bind to receptors, immobilized on the transducer surface. They use changes in mass, concentration, or number of molecules to dissect changes characteristic of light. Researchers have used optical techniques such as SPR and Estodesmometry for the detection of bacterial pathogens [27, 28].

Others

Acoustic Transducers: used in biosensors are based on either the bulk acoustic wave or the surface acoustic wave. The transduction is through detection of changes, on their physicochemical properties e.g. mass densitometry, elasticity, viscoelasticity or electrical conductivity [29].

Calorimetric Transducers: on the other hand, depend on change in temperature of the sensing site due to biochemical reaction [30]. The thermometric, magnetic and piezoelectric transducers have failed so far to have any practical impact on tissue engineering applications [31].

1.3. Clinical Applications of Biosensors in Tissue Engineering

Glucose: For glucose biosensors biorecognition event into a measurable signal ii) a signal processing system which converts it into readable form [32-34]. iii) the molecular recognition events include receptors, enzymes, antibodies, nucleic acids, microorganisms and lectins [35, 36], iv) the five principal transducer classes are electrochemical, optical, thermoelectric, piezoelectric, and magnetic [37]. Majority of the current glucose biosensors are of the electrochemical type because of their better sensitivity, reproducibility, and easy maintainance as well as their low cost. Electrochemical sensors can further be i) potentiometric, ii) Amperometric iii) conductomtric types [38- 40]. The most common glucose biosensoer is the enzymatic amperometric glucose biosensor, commercially available, studied over decades most extensively. Amperometric sensors monitor current generated when electrons are exchanged either directly or indirectly between a biological system and an electrode [41, 42].

General glucose measurements are based on interactions between one of the 3 enzymes namely; hexokinase, glucose oxidase (GOx) OR Glutathione dehydrogenase (GDH) [43, 44]. Hexokinase method is the reference method used in spectrophotometry in many clinical laboratories [45]. Glucose biosensors for SMBG are usually based on two enzyme families ie GOx and GDH. These enzymes differ in redox potential, cofactors, turnover rate and selectivity for glucose [46]. The GDH family includes GDH pyrroquino line quinine (PQQ) [47] and GDH-nicotinamide adenine dinucleotide (NAD) [48]. reviewed in [49]. Several studies have reported optical biosensors using inactive apozymes for glucose detection, binding proteins and receptors. It includes alternative strategies and approaches for detecting spent or reversible implantable, and/or in line sensing systems [50-52], investigations are also focused on discovering techniques to measure the glucose content noninvasively despite some improvements in recent years, there is still no noninvasive tool, which is in use in clinical practice [53]. On the contrary polarimetry [54, 55], absorption transmission spectroscopy [56, 57]. diffuse reflection spectroscopy [58, 59], emission spectroscopy a [60, 61], photoacoustic spectroscopy [62,] and near infrared spectroscopy [63-65] have demonstrated promising success in measuring glucose levels with high accuracy. But the responsiveness of these methods is considerably slow due to the weak glucose absorption bands (combination bands and the presence of various undesired bands from other constituents of the system. On the other hand, the mid infra-red (MIR) region involves a prominent glucose absorption band and it gives isolated band in human blood [66-69]. The limitations of this method remain, strong after absorption and background fluctuation which frequently hamper the results. Photoacoustic and thermal radiation methods [57, 61] also demonstrate variable results due to water accumulation. A noninvasive and noncontracting technique recently, the wavelength modulated differentiation laser photothermal radiometry (WM-DPTR) has been developed for continuous or intermittent glucose monitoring in the MIR range. This can be applied to measure serum glucose levels in human skin in vitro [70,71]. These advances in nanobiosensors technologies in monitoring glucose concentrations are primarily targeted towards the measurement of blood glucose levels in diabetic patients [72]. These techniques can also be applied to monitor the cellular metabolism in engineered tissue constructs in real time during their fabrication, proliferation, and growth given that consumption of glucose by cells is the best indicator of cell metabolism [73].

Hydrogen Peroxide(H2O2): It is of paramount importance to accurately measure H2O2 in both tissue engineering and clinical applications. By measuring H2O2 one can determine the oxidative stressor hypoxic conditions in the cell and tissue culture. Currently available analytical methods of H2O2 measurements include techniques such as electrochemistry, photometry and titration [74]. High(>50μM) levels of H2O2 are cytotoxic to human and a wide range of animals, plants and bacterial cells. Abnormal levels are highly detrimental, to the biological systems. In tissue engineering applications; fluoresecence based and electrochemical methods have been used for H2O2 detection. The limitations of these are poor H2O2 specificity, low sensitivity, difficulty in applying to the biological environments, and invasiveness of measurement e.g. electrode-based method, amperometric enzymes-based biosensors have gained much attention due to their relative expediency, selectivity and sensitivity [75, 76]. Development of steady and sensitive sensors stems from the efficient binding of enzyme to solid electrode surface [77]. Various strategies applied to efficiently immobilize enzymes on the electrode surface for H2O2 detection, which include but are not limited to polymers quantum dots, and various nano materials [78-81]. Of these the nanomaterials-based methods have been the most widely explored ones for this purpose e. g electrochemical biosensors based on silver (AG)nanoparticles AGNPS) can be used as an important component for the electrode. Xu et aldeveloped a H2O2 biosensor based on the direct electrochemistry of hemoglobin (Hb)in Hb AGS on glassy carbon (GC) electrode, Hb showed a pair of distinct

redox peaks on GC electrode and exhibited a high sensibility, good reproducibility and long-term stability. Nanoprobes are a part of nano biosensors, are able to detect H2O2 using detection principles, chemiluminiscence, fluorescence, localized surface Plasmon resonance, near infrared absorption and electrochemical methods) materials of nanoparticles matrix and dependent on enzymes [82-85]. Use of nanoprobes/ nanosensors in general nanoprobes are in H2O2 detection has certain advantages in both ex vivo and in vivo tissue engineering. In general, nanoprobes are in the size range of 10-500nM, which is much smaller than the size of biological cells and this minimizes physical disturbance to the cells or tissues while performing measurements besides nonoprobes offer multifunctionalities and they can be made tissue or cell specific by conjugated targeted specific ligand moieties onto the nanoparticles decorated electrode surface.

Adenosines: ADP and ATP which are extracellular are vital molecules which are multifunctional, present in blood, heart and liver. ATP has an important role in cellular metabolism. Besides that, it is now recognized to be having an important extracellular signaling agent which can modulate a number of physiological pathways by activating specific plasma membrane receptors, luciferase-based methods have been used for measuring adenoamines for a long time. But they have a low sensitivity and resolution which limits their in vivo application. A need for alternative, more convenient methods for ATP measurement is there. Hence Biosensors can offer an option for in situ extracellular ATP measurement and sensitive in vivo applications [86, 87]. ATP, ADP and uridine triphosphate (UTP) are known to be involved in umpteen biological processes like apoptosis, cell proliferation, migration and differentiation, cytokine release as well as necrosis [88]. There is a role of nucleotide signaling in various physiological /pathological events like immune system, maturation, neurodegeneration, inflammation and cancer [89]. In vivo extracellular ATP concentrations can reach hundred micromolar levels in diseases like hypoxia, trauma, ischemia, cancer or inflammation [90]. Extracellular ATP is measured in the cell supernatant by using the standard bioluminescence luciferin/luciferase assay. But this method does not permit real time measurement of the extracellular ATP concentration. A microelectrode for recording system for in vivo measurements of ATP levels was developed by Llaudet and coworkers. This method requires electrode to be placed inside the tissue which may affect the ATP measurement. A microeletrode biosensor was used by another group to measure the purine in guanine primary cell culture for neuronal degeneration [91]. A scanning tip was engineered by Schneider and coworkers, coated with the ATPase containing SI Myosin fragment, to identify the sources of ATP release and to measure ATP concentrations [92]. This method is complicated for clinical applications. A biosensor was developed by Hayashi and coworkers which can be placed near the ATP releasing target cells [93]. A novel localized surface plasma resonance (LSPR) array chip or pacile was developed recently by Xie and coworkers. Later free and high throughput detection of ATP using a normal microplate read MHC NEE report suggested that the developed LSPR sensor chip can be used for miniaturized and high throughput detection of biological samples in tissue engineering applications [94].

Detection of Functional Proteins: Measurement of functional protein molecules like bioenzymes released from cells under different microenvironmental conditions is important to understand the fundamentals of cell biology for therapeutic, diagnostic and tissue engineering applications. Matrix metalloproteinase (MMPS)a member of proteinases family is released by cells as a biological response to their natural tissue modeling progressively [95]. Even in pathological conditions like cancer MMPS are released to various extents [96]. Thus, in different diseased states MMPS may be used as biosensors, including in cancer, which can be detected and quantified with the help of biosensors at present by colorimetric, methods with commercially available proteinase. Assay kits are used to measure proteinase activity [97]. Besides that, enzyme responsive polymers are also popular in sensing elements in biological devices [98-100]. In these cases, fluorescent molecules are connected to a quencher through peptide sequences and cleavage of the peptide sequences by proteinase enzymes gives a fluorescence signal which can be quantified to monitor the target protein concentration and activities [101]. In detection of suboptimal biomolecules levels in 3D in vitro tissue culture conditions, the Labelling method is not suitable sometimes. Label free biosensors have been developed, recently based on sensitive optical biosensing methods [102, 103]. Such biosensors exhibit real time monitoring of abnormalities of extracellular proteins, e.g autophagy and proteostasis [104, 105], as well as in vitro cell culture conditions [106]. Biophotonic based sensors can also be used to monitor level of extracellular protein, hormones and soluble molecules.

They have also been used as biomarkers for detection of cancer early from blood samples in a noninvasive method. Both surface Plasmon resonance (SPR)and electrochemical biosensors can detect carcinoembryonic antigen efficiently for diagnosis of lung cancers in serum [107-109]. Epidermal growth factor receptor (EGFR) can be detected by Lab on chip and optical biosensors; which is a biomarker for early detection of cancer [110, 111]. Flourescence based biosensors are useful tools in engineered tumour models for the early detection of clinical diagnostics for monitoring disease progression as well as response to therapeutics/ treatment [112-114]. In cell signaling pathways and disease progression, protein kinases are major proteins and can act as real-time biomarkers in response to different therapeutics and this can also be detected using biosensors [115-117]. Since many biomarkers are at nanogram and picogram levels, these trace amounts get detected only by highly sensitive biosensing systems having proper surface chemistries, nanomaterial functionalization and amplification methodologies. Many hurdles are still present in detecting disease markers using biosensors e.g. the reflection between the sensing molecule and the target, noninvasive binding in the case of serum or real patient samples, the small size of the target and the effect of microfluidic systems of the sensors on the measurement process. These issues need more investigations since

they pose major challenges.

Detection of Other Analytes: Biosensors have been developed to detect pathogenic microbes. For indirect detection of E. Coli and direct detection of Salmonella, amperometric biosensors have been developed [118, 119]. A light addressable potentiometric sensor has been developed to detect Neisseria Meningitides and Brucella Neltensis [120]. All gram-negative bacteria have endotoxins, which are complex lipopolysaccharides (LPS) of outer cell wall, which causes fever, multiorgan failure, septic shock, meningococcemia along with severe morbidities like neurological disability and hearing loss. Hence it is important to detect endotoxins by quality control in biological products, food and water security [27, 121]. Fluorescence technique detected E. coli endotoxin which had a lower detection limit of 10ng/ml and detection time of 30sec [122]. While that of Salmonella Minnesota was isolated at 0.1ng/ml with an amperometric biosensor and 0.1pg/ml by a piezoelectric biosensor [123, 124]. Viruses' detection is essential for a wide variety of applications from sanitation and food production to diagnostics and therapeutics [125, 126]. Optical biosensor detects dengue virus effectively [127], while human deficiency virus by SPR EIS biosensors [128, 129], hepatitis C virus which causes liver inflammation by optical and quartz crystal microbalance (QCM) Biosensor [130, 131].

1.4. Recent Trends in Biosensors

Quantum Dots Based Optical Biosensors: One of the most promising optical imaging agents are semiconductor quantum dots (QDots), which can be used in vitro as biosensors and chemical sensors or in vivo for noninvasive imaging of deep tissues. They can be used for diagnosis of disease because of their ultrastability as well as good quantum confinement effects [5, 132-134]. They have a narrow size (5-10nm in diameter) and broad excitation, tunable emission spectrum with narrow emission width. Because of these properties QDots can be used in a wide field like Biology, Biosensor, Electronics and Solar cells [3-5] With the decoration and surface modification, novel ultimodal probes-based biosensors have been inspired with linking them with peptides, nucleic acids, or targeting ligands. Fluorescent intensity of QDots being highly stable and sensitive, fluorescence transduction based on chemical or physical interaction occurs on the surface either through direct photoluminescent activation or through quenching [7, 8]. They have been investigated for testing pH, Ions, organic compounds and biomolecules (nucleic acids, protein and enzymes) as well as other molecules of biological interest [9, 10, 13, 14]. Although the toxic effects of QDots have been of concern [12], the recent advancements in applications of QDots in tissue engineering to detect enzymes and biomolecules are significant achievements of biosensor research.

Carbon Nanotube-Based Biosensors: Many new and improved sensing devices have been made based on the unique chemical and physical properties of carbon nanotubes. A breakthrough invention from carbon nanotube-based biosensors is the early detection of cancer in "In vitro systems" [135, 136]. Detection of proteins and viruses of interest can be done utilizing the specific antibody coated surface of carbon nanotubes. The Invention main insights are not able changes in the electrical conductivity of the nanotubes when the distance be wide the antibody and protein changes. The distance change can be made out by an electrical meter. These nanotubules have been investigated for applications in dehyrogenase, peroxidase and catalase, DNA, glucose and enzyme sonsors. Significant movements of the activities of amperometric enzyme electrodes, immunosensors and nucleic acid sensing biosensors is demonstrated in carbon nanotube – based electrochemical transduction [137]. These properties with enhanced performance and properties on carbon nanotubes can prove important to overcome the limitations like improving elasticity, flexibility, cell growth and cell patterning. Reviewed in [138].

Nems/Mems Based Biosensors: With increasing interest in miniaturizing biosensors has led to increased development of interest in microelectrochemechanical (MEMS) [139, 140]. Nano electromechanical systems (NEMS) and microfluidic on lab on chip system-based biosensors [11, 141]. These miniaturized systems are more accurate, specific, sensitive, cost effective, besides being high performance biosensor devices [142]. Various methods used in MEMS based biosensors are optical, mechanical, magnetic, and electrochemical detections. Probes used are organ dyes, semiconductor quantum dots and other optical florescence probes for the optical detection method [143]. For magnetic MEMS Biosensors conjugation of magnetic, paramagnetic, ferromagnetic nanoparticles have been used [144]. For designing mechanical MEMS Biosensors, two factors are seen be it changes in surface stress and changes in mass. Adsorption of analytes and biochemical reactions can't ever result in changes of surface stress. Amperometric, potentiometric or conductometric detection is used by the electrochemical based MEMS Biosensors.

Graphene Based Biosensors: Since grapheme has important characteristics like low production cost, large specific surface area, good biocompatibility, high electrical conductivity with excellent electrochemical stability, these biosensors have attracted scientific and technological interest [145-147]. Since it has 2D structure, it favours electro conjugation, making its surface available for other chemical species. Hence graphene is emerging as the preferred choice regarding fabrication of various biosensor devices in tissue engineering [145, 148, 149].

Graphene Quantum Dot (Godts) Based Biosensors: They are photoluminescent after it is derived from graphene or carbon fibers [150-152]. GDOTS, have very unique optical properties in combination of quantum confinement and zigzag edge effects. Wide range of excitation/emission spectrum make GDOTS important candidates for applications in electronic, photoluminescence sensor fabrication for various biological and chemical analysis. They are superior to other optical imaging agents like organic dyes and cadmium based Qdots because of their high photostability against photobleaching, blinking, biocompatilility and low toxicity [153,

154]. These qualities make them to be used in electronic sensors, electro chemiluminiscence sensors, electrochemical sensors and photoluminescence sensors [155]. Unlike the extensive application of graphene in field effect transistors, GDOTS are mainly in single electron transistor-based charge sensors. Various methods have been used to synthesize blue, green yellow and red GDOTS from graphene or carbon fiber [152]. Basic factors like size, shape, excitation pH, band gap, degree of oxidation, surface functionalization, and doping of S and N relate to the colors of GDOTS. These are very convenient for detecting positively charged ion(cationic) like Ag2+and Fe2+through charge-to-charge interactions [156]. The tunable size of GDOTS can be used for ssDNA detection, enzyme immobilization and avian leukosis virus subgroup J(ALVs-J) detection, decoration of gold (Au)on the planer surface of GDOTS offers a wide range and low level of detection for detection of H2O2.An electro chemiluscence sensor which was GDOT based was investigated for detecting Cu2+, cysteine and ATP. Advantages of GDOTS are low cytotoxicity, low cost, excellent stability, and case of labeling to use them for applications in development of novel ECL biosensors.

Graphene Based Glucose Biosensor: In tissue engineering glucose-based biosensors can be used for continuous measurements of metabolic activities of cells. Graphene oxide (GO) is the precursor of graphene and its use has been taken as a novel highly efficient enzyme electrode for the detection of glucose in phosphate buffer saline solution (PBS) [157, 158] Covalently the amine functional groups of glucose oxidase (GOD) get attached to the carboxyl functional groups of GO. On investigating the electrochemistry, it was found that the GOD immobilized electrode retains its native structure and catalytic activity with effective direct electron transfer rate being constant. The chemically derived graphene sheet's electrocatalytic activity exhibited enhanced electrocatalytic activity towards the detection of glucose in PBS. Excellent sensitivity, selectivity and reproducibility was shown by the designed electrodes, which suggests a possible use in fabricating low-cost glucose sensor devices. Graphene based nanocomposite materials have been used in fabrication of glucose biosensor in a large scale [159-161]. Graphene/gold nanoparticle (AuNP/ Nation nanocomposite biosensor displayed typical catalytic oxidation response to glucose which was very fast upon the addition of glucose [160]. This sensing efficiency along with detection limits of the graphene-based glucose biosensor got enhanced on the addition of silver nanoparticle (AgNP/AuNP) hybrid to catalyze electrochemical reactions of GOD [161]. On examination of the long-term stability of the developed biosensor over 30days it was found to be stable after the immobilization of the electrode with GOD. Further demonstration by Wu et al regarding platinum nanoparticles (PtNP) decorating these graphene chitosan nanocomposite films could be utilized for glucose detection [162]. A wide linear range along with fast response and high sensitivity was demonstrated by this biosensor. But Kang et al reported that the standard deviation and detection limit of the graphene/chitosan nanocomposite biosensor decreased in absence of PtNP [163]. Excellent response time<5s, with good sensitivity was shown to be exhibited by ionic liquid modified graphene electrodes. These graphene-based glucose sensors were functionalized by ionic liquid and they retained their sensitivity and selectivity after immersion in PBS at low temperature for a few weeks. Similarly, Liang et a confirmed the glucose sensing efficiency of the electrochemically reduced carboxyl graphene [159]. Alinea response to glucose at moderate concentrations with a detection limit of 0.02nmM was shown by the designed biosensor. Thus, a graphene-based biosensor is highlyselctive, sensitive as well as reproducible in nature. It can detect glucose both in presence and absence usually interfering species like ascorbic acid (AA), uric acid (UA) and dopamine.

Graphene Based Cholesterol Biosensor: Detection of cholesterol becomes important as its excess over limits of 1.0-2.2Mm M results in excessive accumulation in blood and further various vessels leading to fatal diseases. A cholesterol biosensor using graphene, ionic liquid modified glass carbon electrode was prepared by Ghlivandi and Khodadadi an [164]. To develop a highly sensitive amprometric biosensor, cholesterol oxidase (ChOx) and catalase (CAT) were immobilized. It showed good reproducibility with minimal interference from AA and UA with RSD being<5%. On investigating the effect of PtNP on the biosensing efficiency of cholesterol, the detection limit was found to be 0.5Mm M in the absence of ChOx or cholesterol esterase. Excellent sensitivity, and linear response for the detection of cholesterol in physiological solutions was exhibited by these types of biosensors. Similarly, they held great promise for in vivo measurement of free cholesterol.

Graphene Based Hydrogen Peroxide Biosensor: Using graphene Fe3O4-Au NP and graphene AuNP Nanocomposites, a novel H2O2 Biosensor was developed [165, 166]. Excellent performance with a linear response in the range of detection limit of 2x 10-5 moles lit-1 with a detection limit of 1.2x10-5 mol lit-1 was highly sensitive, low-cost biosensor having trong ant interference, which suggests it being a reliable device for H2O2 detection. Although the sensitivity, selsctivity and detection limit of the enzymatic electrodes are impressive, their limit lies in high cost, reproducibility and complex immobilization procedure. They are also very sensitive to changes in pHof the solution, temperature as well as toxic chemicals. Hence to overcome these nonenzymatic biosensors have been introduced for the detection of biomolecules.

Non-Enzymatic Biosensors: Nonenzymatic detection of H2O2 using GO/MnO nanocomposite was shown by Li et al [167]. It had a range 5-600µM. Common interfering species like SO4, Cl, NO3, CO3-2and citric acid did not affect this biosensor. Chemically derived graphene could detect dopamine with a linear range from 5-200µM in a large excess of AA as shown by Wang etal [168]. Chemicals derived from graphene/chitosan/AuNP nanocomosite showed increased sensitivity towards the detection of AA and UA [169]. The effect of AgNP on the graphene thin films for the nonenzymatic biosensor for H2O2 detection was shown by Zhang et al [170]. Fast amperometric response time of<2s along with a good linear range and a detection limit of 3x 10-6M was exhibited

by this biosensor. For the selective detection of dopamine, a new type of chemically modified graphene was used [171]. Salinization of graphene with N- (tri methoxy silyl propyl) ethylene diamine triacetin acid (EDTA-silane) was used to prepare the surface of graphene. Detection of NADH based on graphene modified glassy carbon (GC)electrode by electrochemical detection was studied by Zhu et al [172]. Very high sensitivity was exhibited by the sensor in view of large graphitic edges as well as porous structure of graphene sheets. It had a detection limit of 0.23μM along with an RSD of 3.4%.

Using graphene /PANI Nanocomposite films a DNA sensor was constructed [173]. The additive effect of graphene and PANI improved the response of the electrode due to fast electron transfer at the electrode surfaces. A highly selective and sensitive DNA biosensor using GO/AuNP nanocomposites [174]. Elect rochemical impedance spectroscopy (EIS) analysis confirmed the immobilization of ssDNA. Increasing the concentration of DNA, the charge transfer resistance was found to increase. As compared to the graphene /PANI biosensor the detection limit as well as linear range were significantly improved in the GO/Au NP biosensor [175]. Anovel electrochemical biosensor for selective as well as sensitive detection of DNA using polydopamine graphene composite [176], was developed by Huang et al. The detection limit was 3.2x 10-15M and the linear range was 1.0x10-13M to 1.0x 10-8M, besides high selectivity for differentiating one-mismatched DNA. Thus, graphene-based sensing system is low cost, reproducible, facile, rapid and has high selectivity for detection of DNA.

Microfluidics and Biosensors: Need for reliable as well as sensitive tools for assessing the artificial tissue environments is required for the development of tissue engineered constructs. Constant monitoring is required in terms of various physiologically relevant parameters for evaluating the functionality of these platforms. Direct cell fate to create specific organ constructs is mimicked by these microfluidic systems by precise control of the chemical as well as mechanical stimuli at microscale [177]. Tiny volumes of fluid are handled (in μl to nanoliters) in a high throughput manner besides integrating several functions like multiplexing capability or having possibility of carrying numerous reactions is there with these microfluidic platforms [178, 179]. Organs on chip applications or for in vitro tissue models these platforms have become important in tissue engineering because of these advantages. Further this research has extended to point of care (POC) devices which are analytical tools routinely used in clinical laboratory along with at patient bedside, are intended to give a reliable response in a short time [180]. Thus, combining POC abilities along with microfluidic platforms remains a big challenge, since many biomarkers need to be monitored for assessing functionality of any tissue engineered construct in vitro.

A multiparametric microphysiometry platform to monitor metabolism in T98G human brain cancer cells which were cultured in dynamic flow conditions was made by Weltin et al [181]. For facilitating optical imaging, a glass made microfluidic device was used, with integrating several microfabricated biosensors in the cell chamber as well as in upstream and downstream compartments. Monitoring of cell metabolites (lactate and glucose), Ph, oxygen consumption monitoring was done using external equipment like potentiostat. A light addressable potentiometric sensor (LAPS) in a microfluidic system was developed by Hu et al, to monitor the metabolism of human breast cancer cells in real time [182]. For controlling the temperature and handling various fluids, Micro binders and micropumps were integrated as well to these systems [183]. Impedance analysis was used to study the adherent cells in tissue engineered constructs. On the surface of of a microfabricated electrode, cells were cultures and exposed to low –magnitude AC voltage. Various cell parameters like numbed, type, state and migration of cells were measured by electrical impedence. In lab made microfluidic systems [184-186], this technique has been used successfully, besides in commercially available tissue culture plates like ECTS culture ware Disposable electrode Arrays from Applied Biophysics Inc. Several challenges are being faced by researchers to translate these biosensing technologies to tissue engineered platforms and integrate it in microfluidic systems [187]. A very compex mixture of molecules (smallones as well as a complex molecule like proteins and nucleic acids) extremely different in size and concentrations, constitute the cell culture media. Number of cells are extremely low as compared to static conventional standard culture; analytes can be prepared at very low levels. Other disadvantages include surface bifouiing at the biosensor level, besides no specific adsorption of target biomolecules in compartments which are different from the biosensor surface, which leads to false response errors, with decreased sensitivity. Use of PEG bovine serum albumin for passivating the surface of the microfluidic circuit can affect the system meaning the tissue engineered construct in some unpredictable way and should not be done.

POC systems can be used for several functions using a single device like handling fluid ,sample preparation like concentration, washing along with chance of performing different reactions for biochemical assays .The problem lies with miniaturization along with integrating in microfluidic cell culture systems from the angle of designing and operating these systems although various attempts at these have been demonstrated[188, 189].Usually these systems have been used as an endpoint detection tool after samples have been discarded following the test. So, challenge lies in real-time use to do tests with very minute volumes without wasting any sample volumes.

1.5. Update on Electrochemical Biosensors

In Olfaction and Taste Assessment: Biosensors are robust evaluation gadgets used to isolate as well as determine target molecules. Electrochemical biosensors, which combine biosensing with electrochemical assessment strategies, are effective analytical instruments that translate quantity signals into electrical signals, aiding the quantitative in addition to qualitative assessment of target molecules. Electrochemical biosensors have been widely used in

variable fields of estimation along with evaluation in view of their greater sensitivity, better selectivity, fast response time, as well as cheap. Nevertheless, the signal alteration caused by crosstalk amongst a biological probe in addition to a target molecule are substantially weak along with tough to capture directly by using estimation instruments. Therefore, variable signal amplification techniques have been posited in addition to generate for escalating the accuracy along with sensitivity of estimation systems. This review by Wang etal.[190], works in the form of a reference for biosensor detector research, as it introduces the research progress of electrochemical signal amplification strategies in olfactory as well as taste assessment. It further details the most recent signal amplification strategies presently being utilized in electrochemical biosensors for nanomaterial generation, enzyme labeling, in addition to nucleic acid amplification techniques, along with emphasizes the most recent work in using cell tissues in the form of sensitive elements (see Fig 1-5) [rev in ref 190].

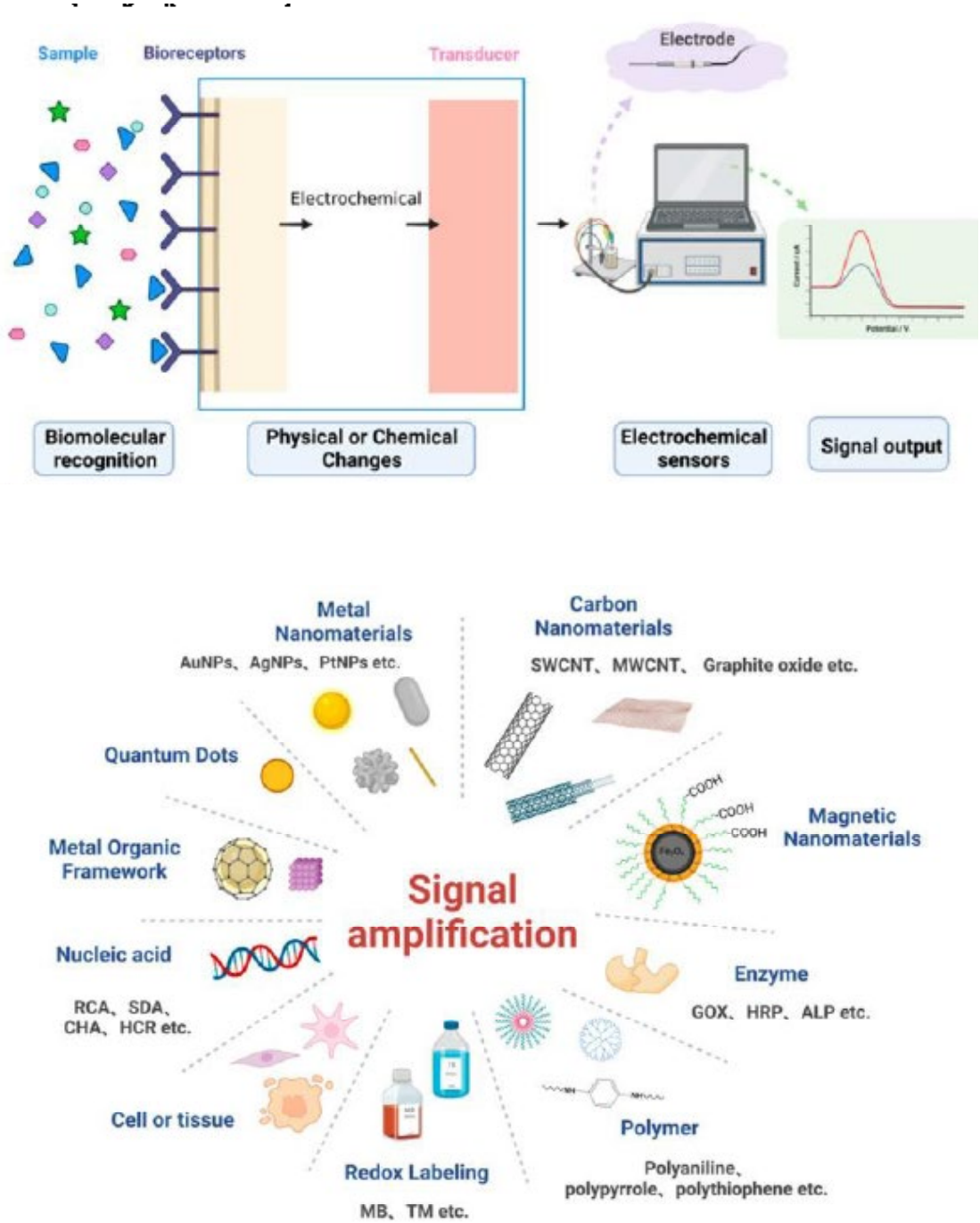


Figure 2: Courtesy ref no-190-Signal amplification strategies commonly used in electrochemical biosensors.

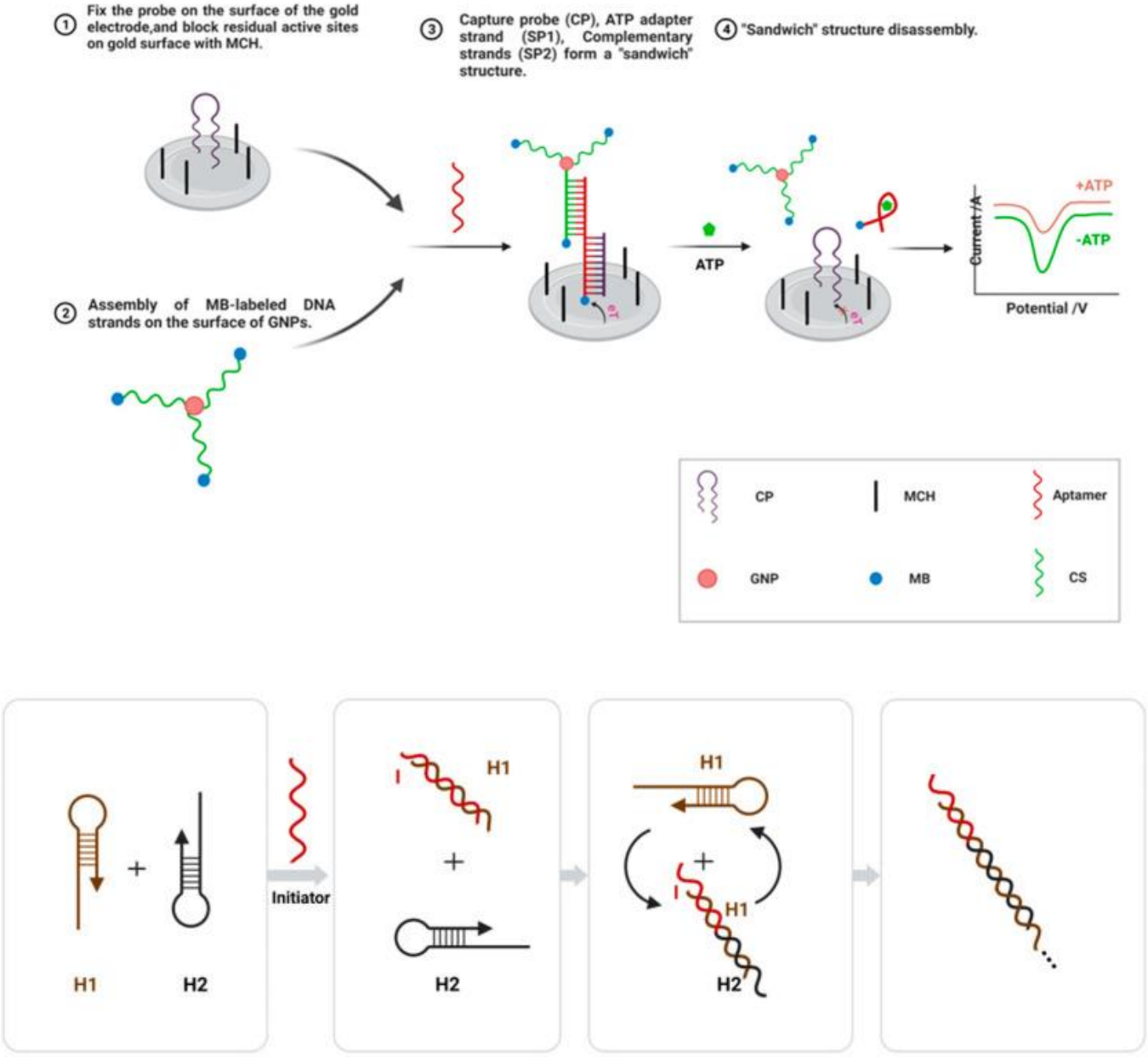


Figure 4: Courtesy ref no-190-Principal diagram of the hybridization chain reaction (HCR) amplification.

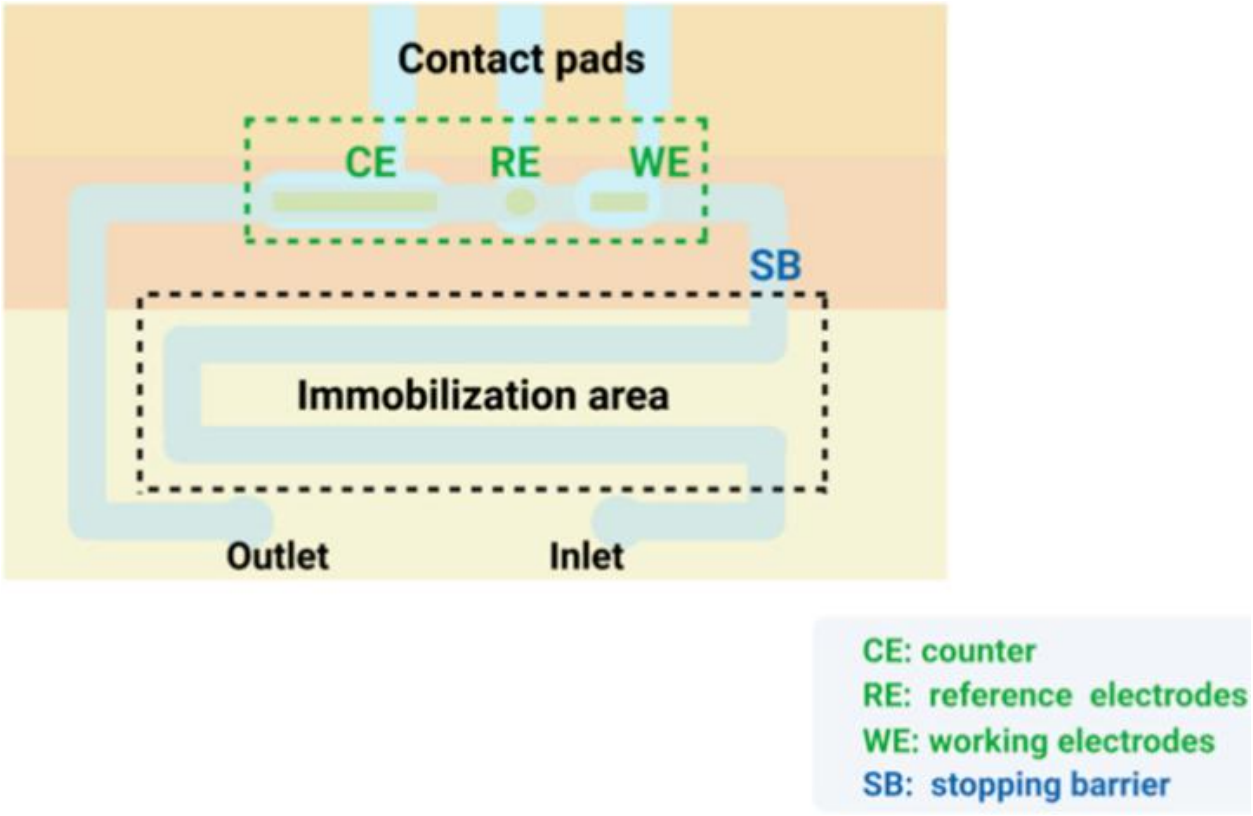


Figure 5: Courtesy ref no-190-The structure of microfluidic chip.

Neuro Biosensors in Neurotransmitters Determination: Neurotransmitters portray chemical substances, liberated by nerve cells, inclusive of neurons, astrocytes, in addition to and oligodendrocytes, which possess a necessary part in the spreading of the signals in the living organisms, specifically in the central nervous system, as well as they further conduct part in realizing the working in addition to sustenance of the state of every organ in the body. The decontrolling of neurotransmitters might result in neurological conditions. This emphasizes the importance of appropriate neurotransmitter monitoring to aid in early diagnosis as well as treatment. Choi etal. [191], detailed a full multidisciplinary evaluation of electrochemical biosensors incorporating nanomaterials as well as nanotechnologies with the idea of attainment of the precise determination in addition to monitoring of neurotransmitters. They provided substantially researched neurotransmitters along with and their corresponding working in biological beings. Following that, classification of electrochemical biosensors is done dependent on strategies utilized for direct determination, covering the recently revealed cell- dependent electrochemical monitoring systems. These approaches implicated in the determination of neurotransmitters n neuronal cells in vitro, the of neurotransmitters emitted by stem cells, as well as the invivo monitoring of neurotransmitters. The integration of nanomaterials along with nanotechnologies into electrochemical biosensors has the potential to assist in the timely determination along with management of neurological conditions. This study yields remarkably important understanding for researchers in addition to clinicians with regards to appropriate neurotransmitter monitoring as well as and its repercussion with regards to plethora of biological applications.

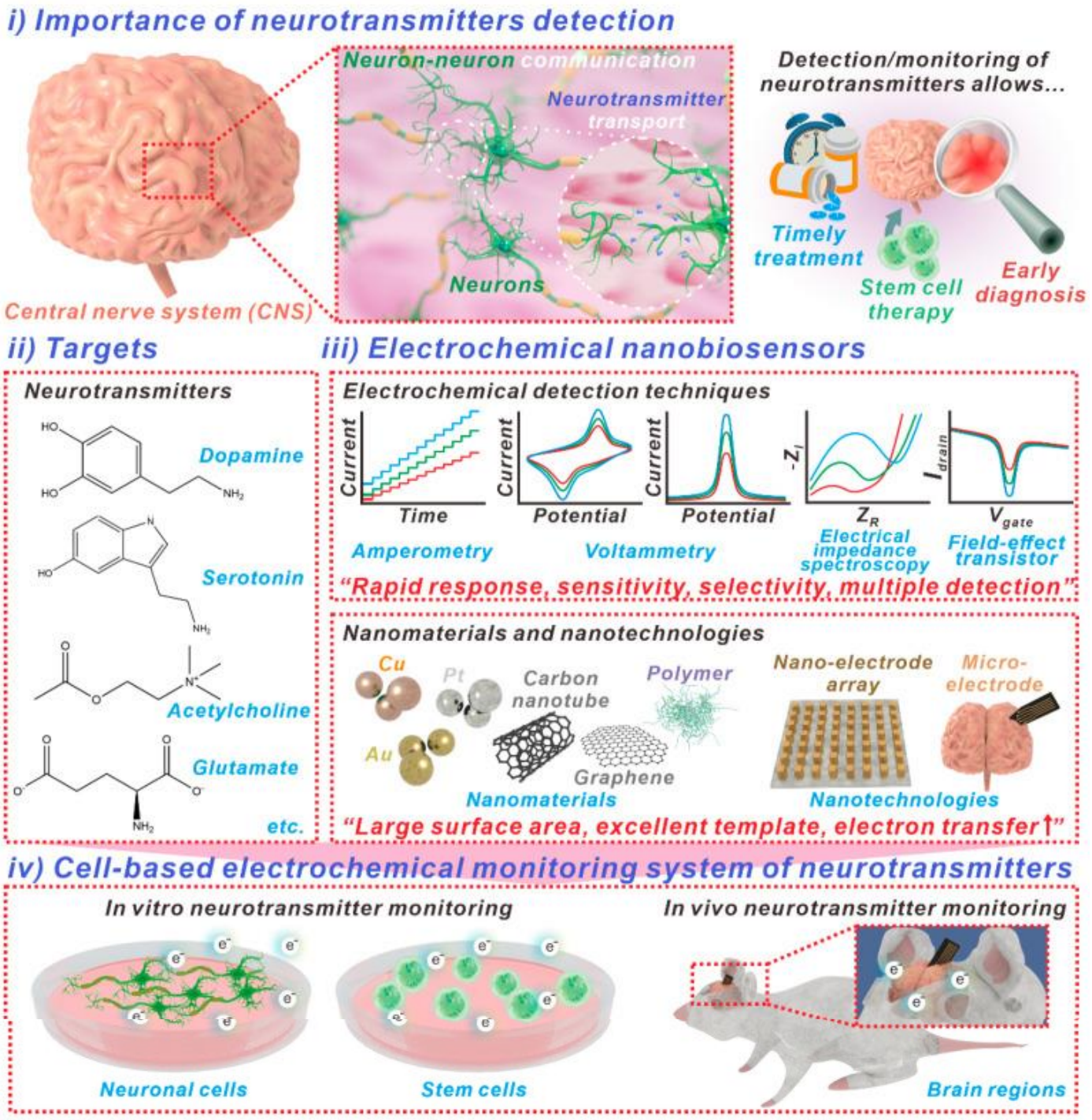


Figure 6: Courtesy ref no-191-Schematic of electrochemical nanobiosensors used for neurotransmitter detection. (i) Detection of neurotransmitters is important, since a problem in the production and transmission of neurotransmitters could potentially have fatal consequences in the signal transmission process in nerves and may cause cranial nerve-related diseases. To achieve monitoring of (ii) the neurotransmitters sensitively and selectively, (iii) various nanomaterials and nanotechnologies have been applied in the development of electrochemical biosensors. Additionally, utilizing fabricated biosensors, the neurotransmitters in (iv) neuronal cells, stem cells, and animal models can be monitored.

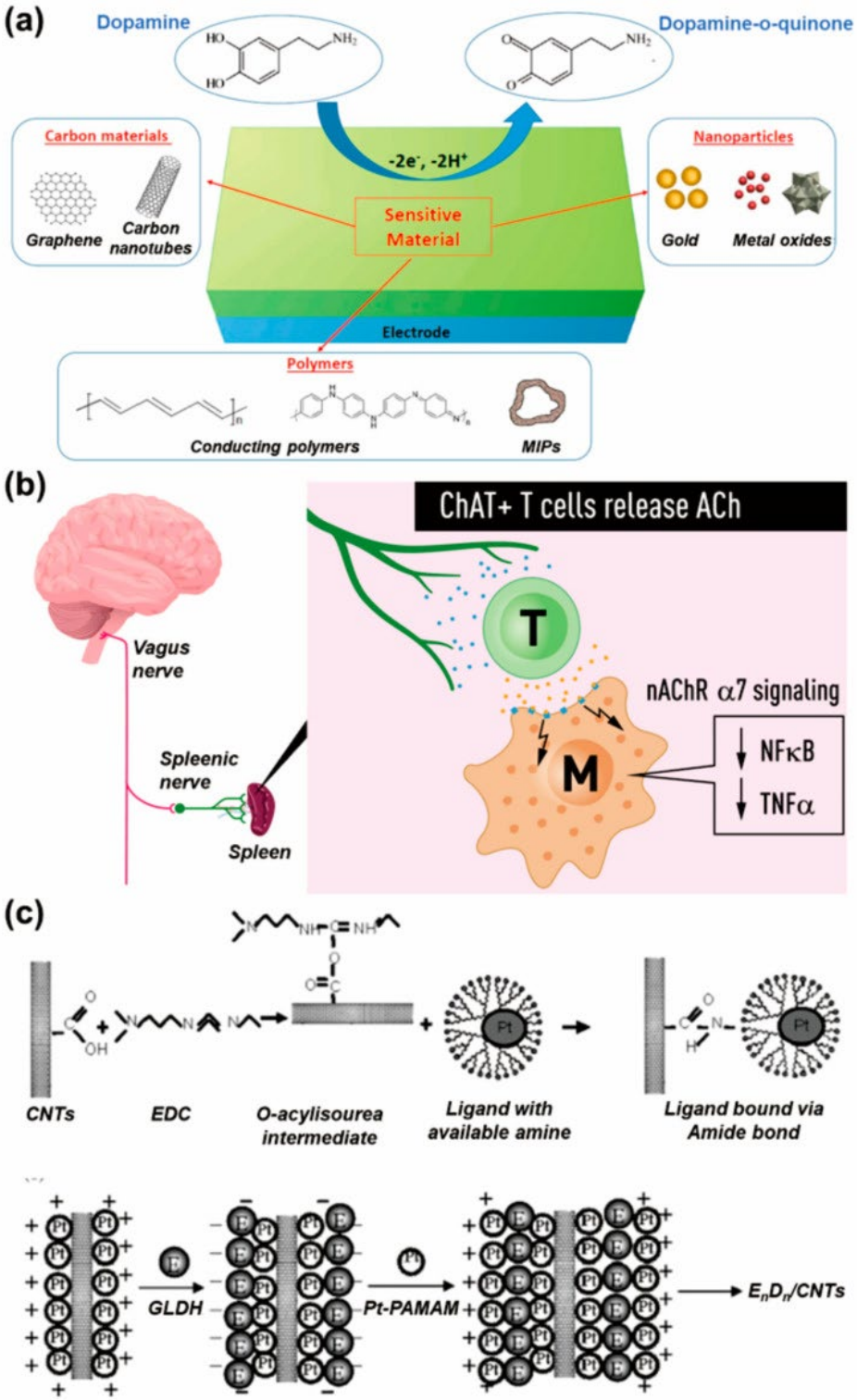


Figure 7: Courtesy ref no-191-Representative neurotransmitters and their detection for early diagnosis and pathophysiological monitoring. (a) Electrochemical dopamine detection achieved via the conversion between dopamine and dopamine-o-quinone (reprinted with permission from [26]; Copyright © 2021 by the authors. Licensee: MDPI). (b) Role of acetylcholine in the immunomodulation by T cells (reprinted with permission from [30]; Copyright © 2019, The Association for the Publication of the Journal of Internal Medicine). (c) Electrochemical glutamate biosensor composed of glutamate dehydrogenase (GLDH), poly(amidoamine) dendrimer-encapsulated platinum nanoparticles (Pt-PAMAM) and carbon nanotubes (CNTs) (reprinted with permission from [37]; Copyright © 2007, Elsevier B.V.).

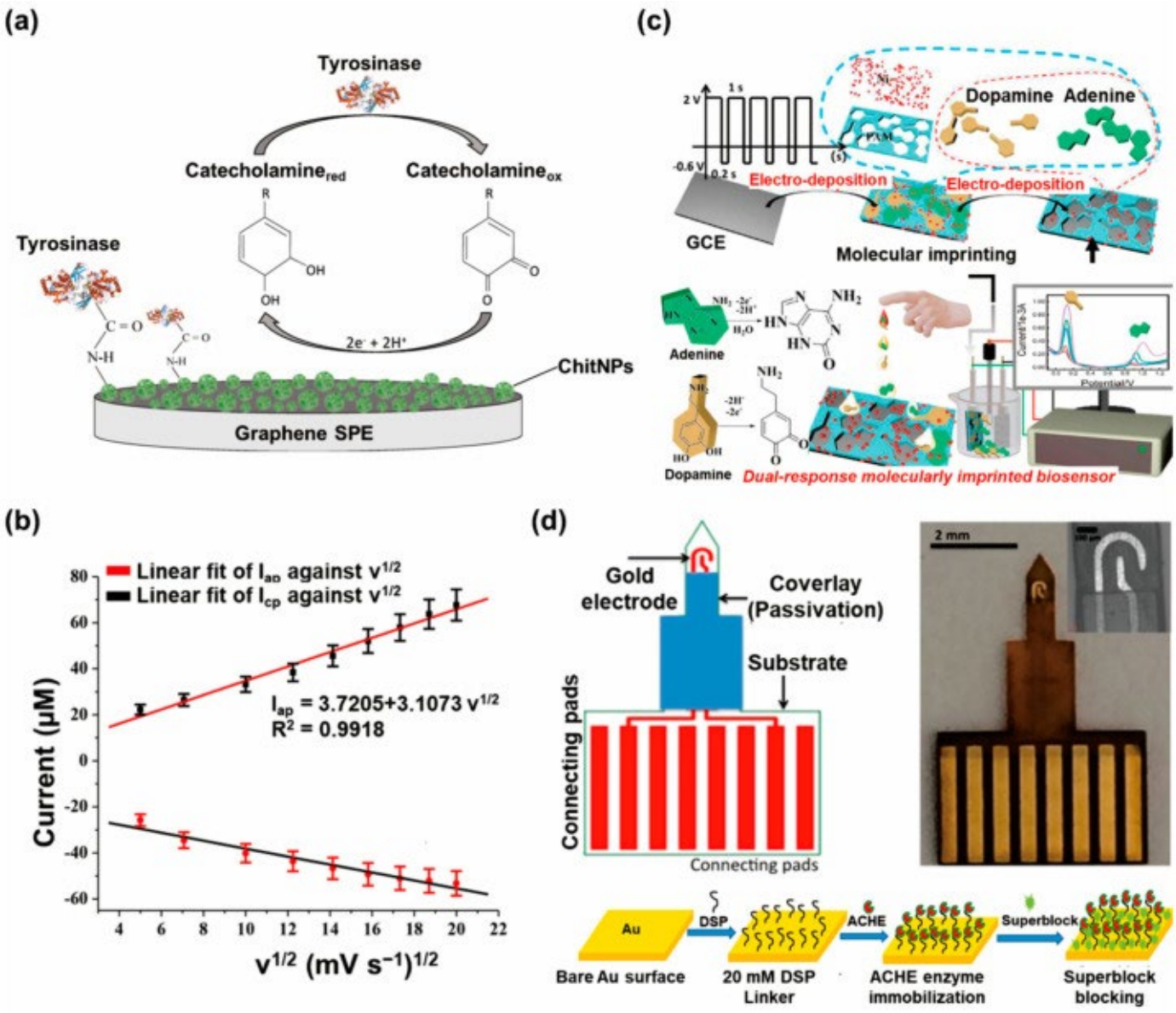


Figure 8: Courtesy ref no-191-Recent types of electrochemical nanobiosensors used for neurotransmitter detection. (a) Structure of the amperometric biosensor for the detection of various catecholamines (reprinted with permission from [54]; Copyright © 2022 by the authors. Licensee: MDPI). (b) Plot of the peak current of the cyclic voltammogram of carbon quantum dot/copper oxide nanocomplex-modified glassy carbon electrode in 0.4 mM epinephrine solution as a function of the square root of scan rate (reprinted with permission from [64]; Copyright © 2022, Elsevier B.V.). (c) Schematic of the assembly process of the dual-response MIP-sensing membrane and electrochemical detection of dopamine and adenine (reprinted with permission from [73]; Copyright © 2022, Elsevier B.V.). (d) Schematic of the assembly process of the dual-response MIP-sensing membrane and electrochemical detection of dopamine and adenine (reprinted with permission from [78]; Copyright © 2023, Elsevier B.V.).

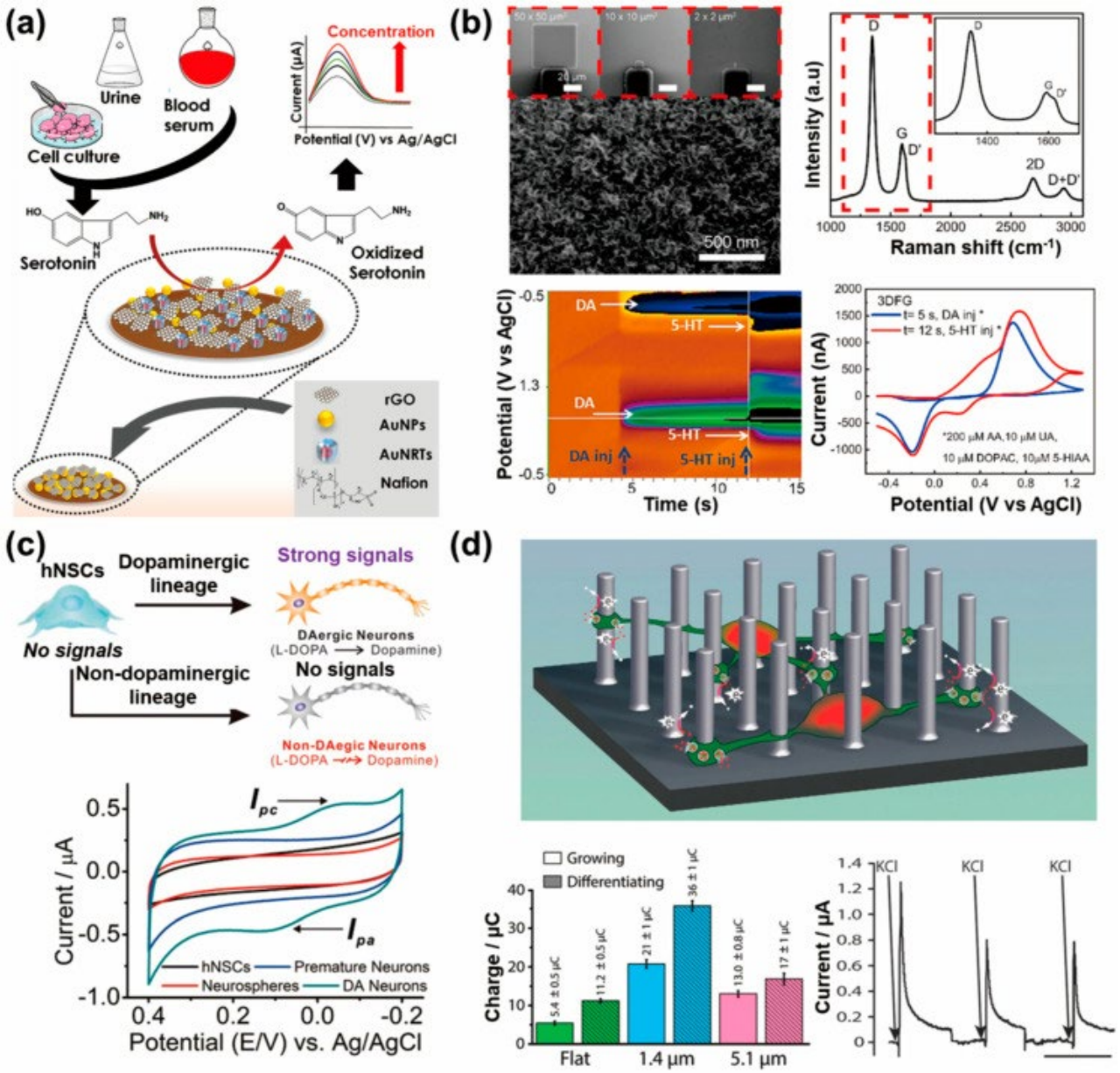


Figure 9: Courtesy ref no-191-In vitro neurotransmitter detection system from neuronal cells and stem cells using electrochemical sensors: (a) Schematic of the sensor probe developed using Au-nanorattles (AuNRTs) and reduced graphene oxide (rGO) (AuNRTs-rGO) nanocomposite based on the serotonin detection mechanism (reprinted with permission from [90]; Copyright © 2022, Elsevier B.V.). (b) Scanning electron microscopy image and Raman spectra of three-dimensional fuzzy graphene microelectrode (top) and color plot (bottom left) and background-subtracted cyclic voltammogram showing reduction and oxidation peaks of injected dopamine and serotonin (reprinted with permission from [93]; Copyright © 2022 Elsevier B.V.). (c) Schematic representation of the conversion of hNSCs into dopaminergic (DAergic) and non-DAergic neurons (top) and cyclic voltammogram of cells undergoing differentiation into DAergic neurons (DA neurons) (bottom) (reprinted with permission from [17]; Copyright © 2015 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim). (d) Schematic of p3D-carbon where the cells differentiate at the bottom or between pillars (top), calculated average charge related to the amount of detected dopamine released by human neural stem cells (hNSCs) (bottom left), and characteristic current–time trace recorded during amperometric detection of dopamine (bottom right) (reprinted with permission from [102]; Copyright © 2014 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim).

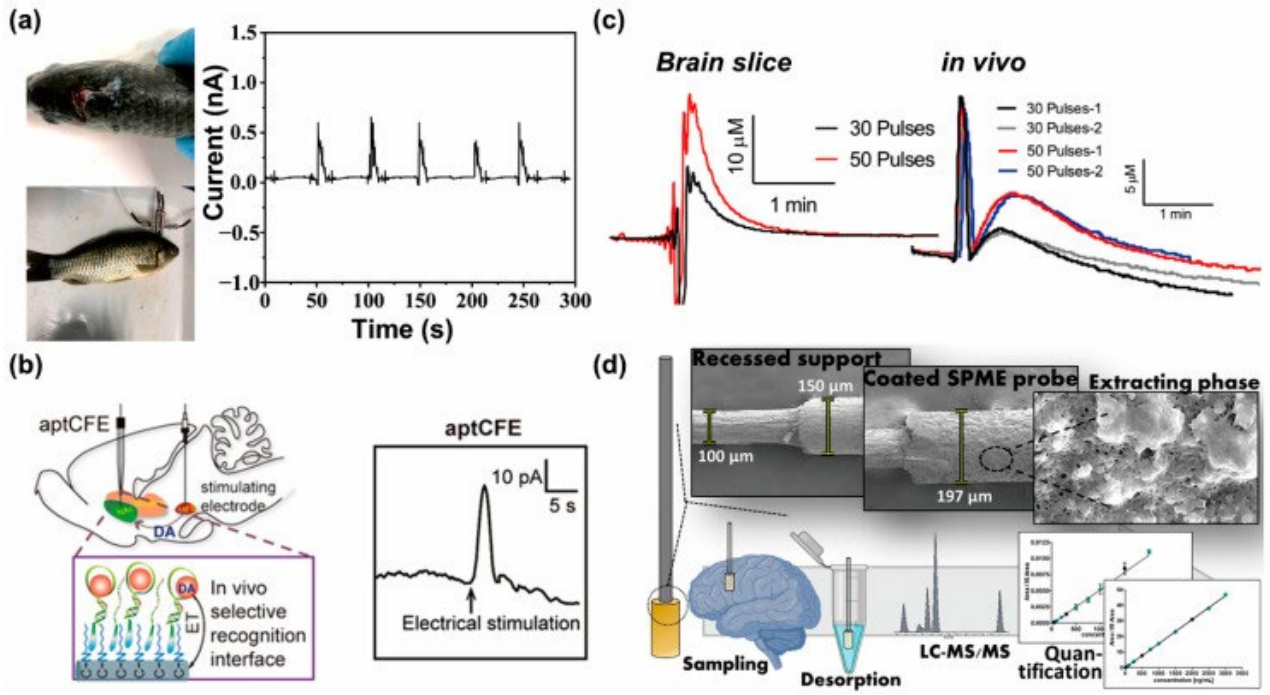


Figure 10: Courtesy ref no-191-In vivo neurotransmitter monitoring system using electrochemical sensors: (a) photograph of the crucian fish brain, the field-effect transistor biosensor setup on the fish brain (left), and the Ids output signal of the crucian fish brain monitored by the biosensor (right) Copyright © 2021, Elsevier B.V.). (b) Schematic of aptCA-functionalized carbon fiber electrodes (CFEs) for in vivo dopamine sensing (left), and the current responses of aptCA-functionalized CFEs in the rat nucleus accumbens upon electrical stimulation of the rat's medial forebrain bundle (right) (reprinted with permission from [109]; Copyright © 2020, Wiley-VCH GmbH). (c) Amperogram of stimulated glutamate release in the subthalamic nucleus of a rat brain slice (left) and stimulated rat brain in vivo (right)]; (d) Schematic of the solid-phase microextraction-based approach used for quantitative measurements of multiple neurotransmitters.

In Estimation of Animal viruses: Animal viruses portray a considerable important threat with regards to animal health in addition to are transmitted with ease worldwide with enhanced globalization. The restrictions in diagnosis getting established as well as treatment of viral infections ensured have made the spreading of diseases and demise of animals unanticipated. Thereby, it is imperative for getting an early diagnosis of animal viral infections made for the avoidance of transmission of diseases as well as having the cost incurred diminished. Regarding tackling these issues &fast diagnosis electrochemical sensors have surfaced in the form of attractive gadgets. Electrochemical approaches present myriad of advantages inclusive of enhancement of sensitivity in addition to and selectivity, cost effectiveness, easy to use, transportation, along with fast assessment enabling them ideal for real-time virus estimation. He etal.[192], concentrated on the generation of electrochemical biosensors, in addition to attractive biosensor models, along with expounds its benefits in virus estimation, that is an attractive research trajectory

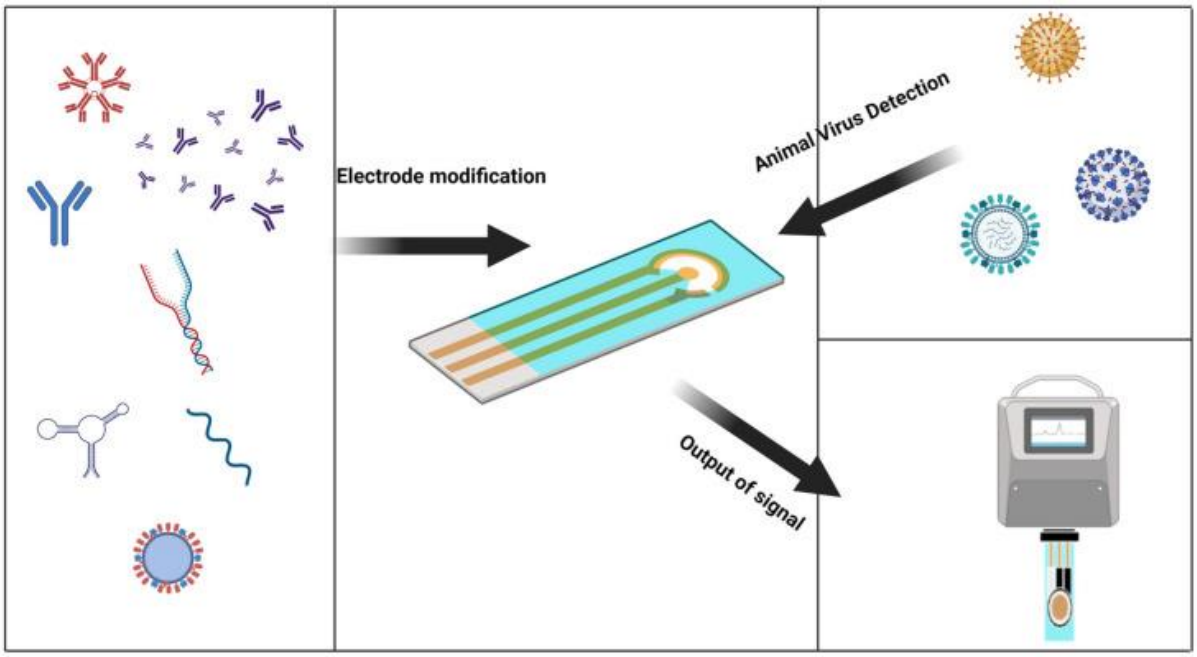
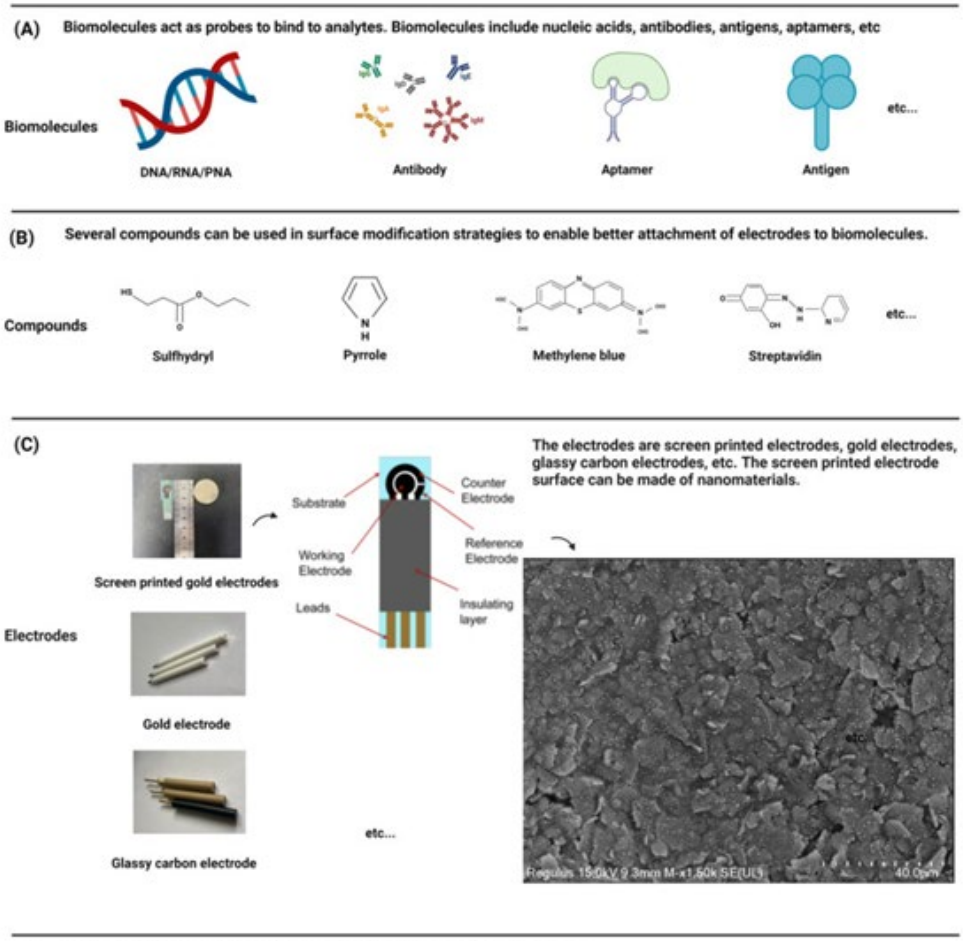


Figure 11: Courtesy ref no-192-A general introduction to the electrochemical sensor detection process



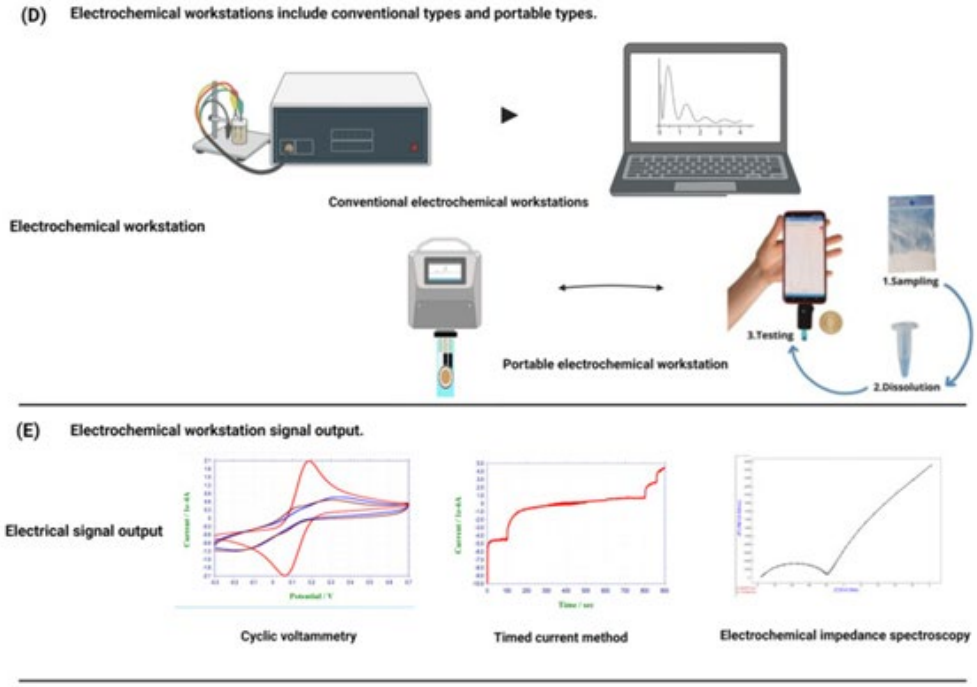


Figure 12: Courtesy ref no-192-A schematic diagram of a standard electrochemical workstation and portable electrochemical workstation is presented. The components of the detection system, including electrodes, biomolecules, compounds, etc., and the conventional signal output are introduced. (Created with BioRender.com, accessed on 17 September 2023).

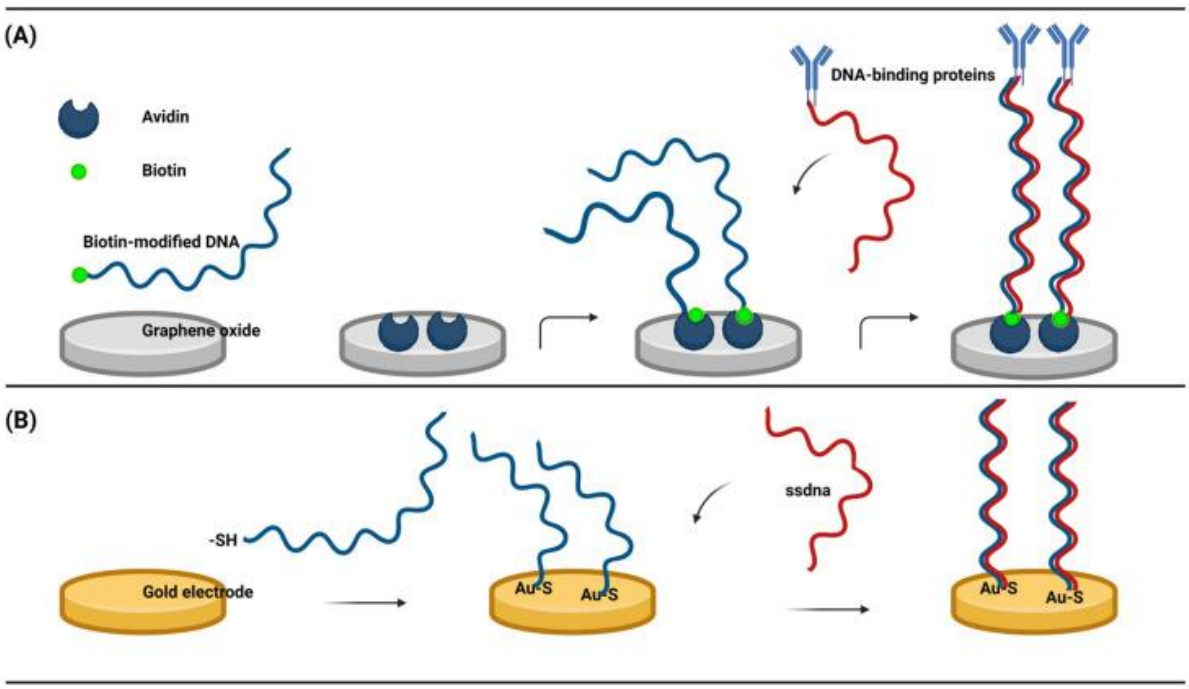


Figure 13: Courtesy ref no-192- (A) Graphene and avidin formed polymers by a hydrophobic force to link biotin-modified ssDNAs and detect DNA assembly proteins. (B) Gold electrodes were ligated with sulfhydryl modified DNA to detect complementary ssDNAs. (Created with BioRender.com, accessed on 17 September 2023).

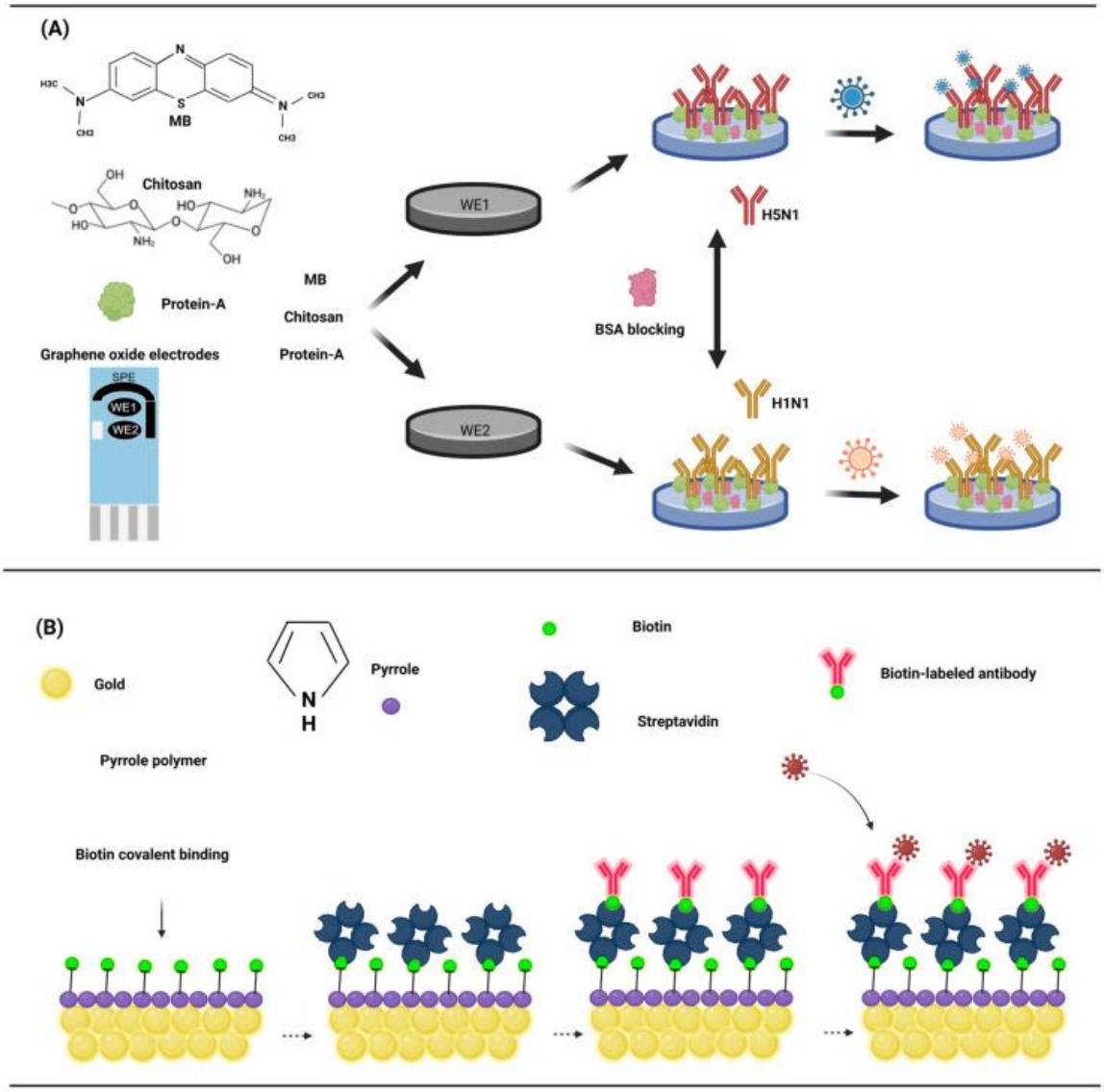


Figure 14: Courtesy ref no-192- (A) The GO surface is rich in a large number of oxygen-containing functional groups that adsorb methylene blue, and protein A was adsorbed by chitosan. The influenza virus was detected by a protein A-linked influenza virus antibody. (B) After the electro polymerization of pyrrole on the surface of the gold electrode, biotin was covalently bound, and streptavidin was used as a bond to adsorb the biotin-labeled antibody. (Created with BioRender.com, accessed on 17 September 2023).

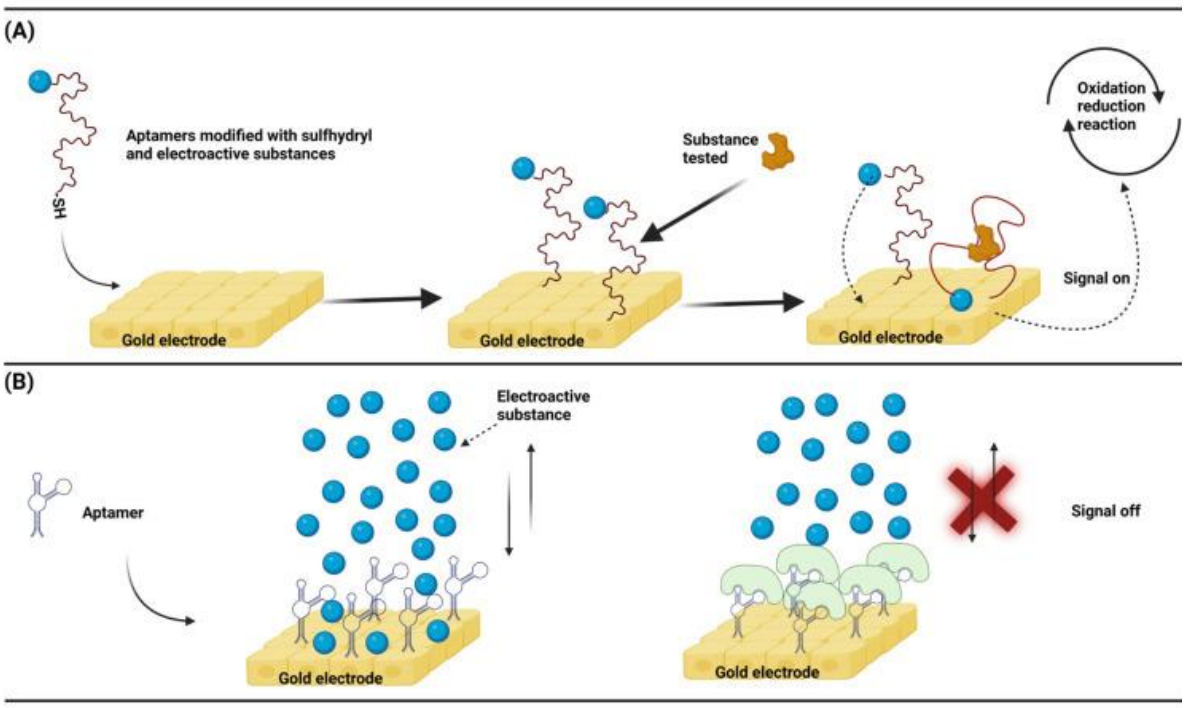


Figure 15: Courtesy ref no-192- (A) The spatial structure of the aptamer changes after binding to the analyte, and the analyte detection generates an electrical signal by the proximity of the electroactive substance to the electrode. (B) Upon binding of the analyte to the aptamer, the electroactive species free in the solution cannot approach the electrode and the impedance increases. (Created with BioRender.com, accessed on 17 September 2023).

2. Conclusions

Though various electrochemical, magnetic, acoustic, optical thermometric along with piezoelectric sensors have been introduced both in literature as well as are available in market with great sensitivity along with sensibility, biggest success has been achieved with the electrochemical and optical ones in tissue engineering applications. The magnetic and thermometric transductions have failed to impact in practicality. The biggest challenge lies in diminiaturization along with integrating with microfluidic systems for these biosensors to be used on large scale in tissue engineering. Using in real time continuously in tissue engineering is in a budding stage, although can get various possibilities in the tissue engineering field. Building such microfluidic platforms for tissue engineering, having systems which are automated, sensitive and have capabilities of real time monitoring will really help translating these systems in real life assessment in hospital setup and getting used clinically. Moreover, these systems will need to be standardized specially regarding use in tissue engineering microfliuidic platforms.

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