Biosensor for tissue engineering: Bio-analytical tool in Therapeutics

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Abstract:

In recent years biosensors have gained attention in medicine, nanotechnology and tissue engineering (TE) in addition to other fields that include food, beverages, environmental and agricultural applications. TE and regenerative medicine have presented tremendous potential for the development of engineered tissue constructs that help restore the damaged functions of diseased or damaged tissue and organs. Biosensors have become an integral part of TE systems specially microfluidic tissue engineering models as they can sense specific biological molecules within the miniaturized tissue constructs in real-time, at very low concentration levels, through ultrasensitive optical, electrochemical, or acoustic sensing systems. In recent protocols for TE we face the challenges of incapability to control/ monitor cellular functions and various properties (biological, mechanical, electrochemical and others) which has increased the demand and led to the development of more sensitive nano and carbon based biosensors. Based on recent advances in this domain the chapter aims to explain the basic biosensor models that are being used in tissue engineering. Concepts of nano-biosensor, bringing a new boon to this tool by combining biological signal and therapeutic delivery systems for *in-vivo* screening and treatment. Through this chapter we focus on the new trends which have been developed for increasing specificity and sensitivity of biosensors including carbon-nanotubes (CNTs), quantum dots (QDo), graphene oxide biosensor, Micro and Nano electromechanical system MEMS/NEMS. Carbon nanotubes (CNTs) provide support for immobilization of biological molecules at their superficial surface and combine electrochemical characteristics properties which make them suited for the transduction of biological signals associated with the recognition of biomolecules in disorder. The binding, conducting properties of gold, metallic nanoparticles (GNPs, SNPs) and metal oxides provide antimicrobial properties. The fluorescence properties of quantum dots have made them very useful in TE applications.

Key words: Tissue engineering, Nanobiosensor, carbonnantubes, quantum dots.

1. Introduction to Biosensor and Tissue Engineering

Tissue engineering is a highly interesting and multipurpose field having a vital role in combining the ideologies of alternative materials to replace the injured tissue or promote endogenous rejuvenation. The tissue engineering approach became major technology in the medical era to overcome the limitations of organ transplantation, graft rejection, complexities in the repair of functional tissue and the specificities in the site of regeneration. 1962, Clark and Lyons assembled the world's first biosensor which was specific and sensitive. Biosensor developed by Clark and Lyon was a glucose sensor which measures the glucose level via utilizing glucose oxidase enzyme as recognition element and detect the oxygen reduction levels which further correlated with the glucose level in the body. Biosensors are the analytical devices that enable the sensing of molecular interactions and convert it into a detectable electrical signal. Biosensors involves the combination [1], [2]event of the biological elements such as enzyme, DNA, RNA, metabolites, cells, oligonucleotides, and transducers like electrochemical, optical, piezoelectric, acoustics and calorimetric. Medically, biosensors are designed for the accurate and precise detection of tumors, pathogens, toxins, and biomarkers to identify the onset of various disorders at an initial stage. Biosensors have shown the tremendous potential in the field of tissue engineering. Concentrations of biomolecules such as glucose, adenosines, and hydrogen peroxide levels play major roles in the maintenance and development of three-dimensional cell cultures and organs. Here, biosensors can apply their potential to detect such biological analytes in real time which further provide the insight into cellular activities.

2. Basic Approach to Nano-Biosensor: background and concepts

Nanotechnology offers a unique solution to the detection of biomolecules in biosensors. The nanofiber sensors have a much higher surface-area-to-volume ratio than their macro-scale counterparts and can be easily integrated into other devices such as lab-on-a-chip systems.[1] Nanobiosensor is a source for the analysis of biological agents such as antibodies, nucleic acids, bacteria, and metabolites. Its purpose is to bind biomolecules of interest to bioreceptors, and to regulate the physiochemical signal associated with binding. Later, the transducer captures and converts the physiochemical signal into an electrical signal. By definition "Nanobiosensors are devices that measure a biochemical or biological event using any electronic, optical, or magnetic technology through a compact probe"(fig 1).[3] They also possess electrochemical properties that allow them to manifest biological signaling and transduction mechanisms. These properties make them ideal for use in biosensing.[4] The nanobiosensor may be homogeneous or heterogeneous, ranging from 1-100nm making them exclusively sensitive in diagnosis.[5] Most electronic and mechanical properties of some nanomaterials including nanotubes, nanorods, nanowires, nanoparticles, and thin films consisting of crystalline matter are well known and utilized in improving transduction mechanisms and biological signaling.[2]. In the biomedical, nanoparticles are used for controlled drug delivery, imaging of specific sites, probing of DNA structures, identifying biomolecular, gene/drug delivery, photothermal ablation of cells, and, most recently, TE.[6]. It has improved the mechanical and biochemical performance for instance gold nanoparticles have properties of conjugation and conductance which have been extensively used, the antimicrobial properties of silver and other metallic/metal oxides nanoparticles, the

fluorescence properties of quantum dots, and the unique electromechanical properties of carbon nanotubes (CNTs) have made them very useful in abundant TE applications.[1]

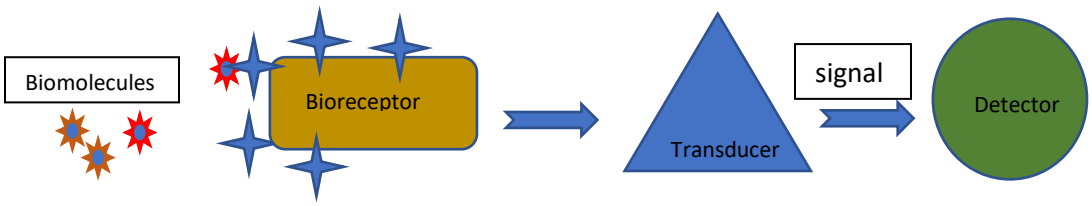

Fig 1: General representation of Nanobiosensor

2.1. Carbon Nanotube Biosensor:

Carbon Nanotubes (CNTs) are also known as Buckytubes, Japanese scientist named Sumio Iijima in 1991 marked the evidence of multi-walled (MCNTs) followed by the single-walled CNTs [7]. The hollow cavity of CNTs provides a chemically inert environment, and it is also a potential site of magnetic/electromagnetic response for novel nanobiosensor technologies and nanoreactors through electromagnetic or electric impulses.[8], [9] They parade a well- designed carbon atoms linked via sp^2 bonds, making them steady and rigid fibers. They provide both exohedral and endohedral functionalization properties by giving a base to conjugate other compounds at their surfaces and shells without losing their abilities. After conjugation these CNTs have capacities to penetrate through the cell membrane and enter the cell, hence making them a very suitable candidate as biosensors.[7] Generally categorized into single-walled carbon nanotubes (SWNTs), consisting of a single graphene sheet “rolled” into a tube, or multi-walled carbon nanotubes (MWNTs), containing several concentric tubes sharing a common axis. MWNTs occur in various morphologies such as “hollow tube”, “bamboo” and “herringbone”, depending on their mode of preparation. The sidewalls of these tubes are finished up of a hexagonal lattice of carbon atoms, comparable to the atomic planes of graphene, and are typically overlaid at both ends by one-half of a fullerene-like molecule. SWNTs are classified by the geometric arrangement of the carbon atoms at the seam of the cylinders. While most SWNTs are chiral (m 6= n), majority present armchair (m = n) or zigzag (m = 0) conformation[10]–[12] (fig 2)

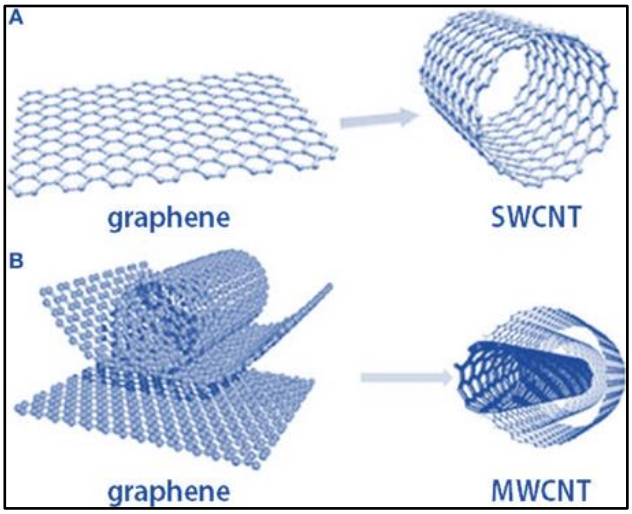

Figure 2: Graphene and carbon nanotubes as (A) single-wall carbon nanotube (SWCNT) and (B) multi-wall carbon nanotube (MWCNT) structures[13] The Rise of Carbon Nanotube Electronics - Embedded Computing Design

With the demand for CNTs in the nanobiosensor, there are three methods proposed to synthesize them :1 Arc discharge, 2: Laser ablation 3: Chemical vapor deposition (CVD, of which synthesizing CNTs by CVD is much cost-effective as it requires low temperature and pressure yielding a highly efficient and purified form. Functionalization of CNTs is required as they have a large capacity and stable structure of inner tubes making them insoluble in many solvents. The scientist has involved three approaches to achieve this viz Covalently linking chemical groups to the skeleton of CNTs,2. Adsorption of various functional molecules to CNTs, 3. Endohedral filling of the internal cavity of CNTs [14][15]. Hence for the application as a biosensor, the surface is modified by covalent and non-covalent immobilization of gold, silver, platinum, graphene, glass, and silica particles thus increasing the solubility and electron rate transfer.[16], [17] The chemical modification of the surface of the CNTs has great properties on the formation of subtle functional groups that prevent accumulation, improve host compatibility and boost solubility in different solvents. Electrochemical CNTs have been developed to detect ions, metabolites, and protein biomarkers. For instance, several CNT-glucose biosensors based on the conjugation of glucose oxidase have been engineered, Patolsky et al. reported on the structural alignment of glucose oxidase (GOx) on electrodes using SWNTs as electrical connectors between the enzyme redox centers and the electrode[7] Electrochemical biosensors based on functionalized CNTs have further been developed for detection of nitric oxide, epinephrine sensing, and dopamine monitoring in rat striatum[7], [16] In current discoveries, a large variety of amperometric biosensors based on CNT-modified electrodes have been engineered. Fei and co-workers carried out the detection of cysteine on Pt/CNT electrodes by cyclic voltammetry. Antiochia et al. reported an amperometric CNT-biosensor developed by coating CNT with a polymer of dihydroxybenzaldehyde and Fayazfar et al. reported a new platform based on electrochemical growth of gold nanoparticles on aligned MWNTs for sensitive label-free DNA detection of the TP53 gene mutation. Over the more current years, new peers of nano-immunosensors have been engineered by immobilizing recombinant antibodies or antibody fragments onto CNTs, nanowires,

nanoparticles, and quantum dots, thereby enhancing binding capacity and sensitivity thresholds compared to more traditional biosensors.[7]

Fig 3: carbon Nanotube: Cancer detection, HIV virus diagnosis, DNA sensing, Glucose monitoring and Enzyme detection

2.2. Quantum Dots Based Biosensor:

"A semiconductor crystal of nanometer dimensions with distinctive conductive properties determined by its size". As they have exceptional properties of optical and fluorescence they are used extensively in biosensing since the 1980s. It was discovered by Ekimov. QDs have high quantum yields, narrow band emission with a wide-ranging excitation wavelength, resistance to photobleaching, relatively lengthy luminescence lifetime (>10 ns), and a high surface-area-to-volume ratio that allows efficient functionalization with biomolecules, (fig 4).[18]

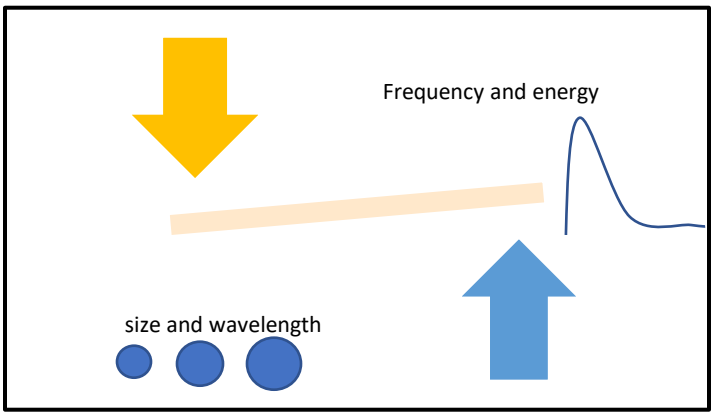

Fig 4: Relation of quantum dot size and wavelength excitation to the frequency

They are semiconductors composed of CdTe, CdSe, GaAs, PbSe, InP, etc.) with a diameter range of 2–10 nm. Since the heavy metals are involved in the synthesis QDs are considered toxic and they are less biocompatible. Several different approaches are taken to make it biocompatible, for example, there are core-shell assemblies in which a semiconductor shell, naturally zinc sulfide (ZnS), stabilizes the core; furthermore, an additional capping or coating using biocompatible materials or polymeric layers (such as PEG) to the QD core-shell helps to diminish toxicity. These smart dots have application in immunofluorescence assays, as a very small amount is required to produce the signal. Li et. Al used lateral flow strips which had copper carrying protein to step quantitate the nitrate ceruloplasmin in blood. It has shown a very important application in the detection of pathogens i.e *E.coli* by combining the immunomagnetic separation (IMS) and the QD fluorescence.[2][18] The iron oxide core-gold shell (Fe_3O_4@Au) magnetic nanoparticles modified

with biotinylated antibodies captured the E. coli in solution, and then chitosan-coated CdTe quantum dots (CdTe QDs) modified with a secondary antibody were added in solution. The bacteria were removed from the matrix by employing the IMS technique for fluorescence analysis. Xu et al. displayed that identification and specific labeling of targeted DNA sequences can be skillful by employing green, blue, and red QDs in different combinations. Peculiar spectral barcodes were obtained with the mixture of these QDs that allowed a high degree of multiplexing, which is necessary for complex genetic analyses.[18] Graphene quantum dots (GDots) have unique properties of quantum internment and zig-zag edge effects. The ultra-nanosized GDots with a wide range of excitation/emission spectrum are promising aspirants for applications in electronic, photoluminescence, electrochemical, and electrochemiluminescence sensors creation for various chemical and biological analyses. The colors of Gdots are related to the basic factors such as size, shape, excitation, pH, band gap, degree of oxidation, surface functionalization, and doping of S and N. The synthesized Gdots are very convenient for detecting any positively charged ions (cationic) such as Ag2+ and Fe3+ through charge-to-charge interactions. A Gdot-based electrochemiluminescence sensor was investigated for detecting Cd2+, cysteine, and ATP. Low cytotoxicity, low cost, excellent solubility, and ease of labeling of Gdots are also attractive for application in the development of novel electrochemical biosensors [2][19]

2.3. MEM/NEM Biosensor:

The striking developments in micro / nano-electromechanical systems (MEMS; NEMS) have directed to a modern standard of chemical and biological sensors created on micro and nano cantilevers. These sensors encompass natural phenomenon of mechanical energy which is exploited as a platform for ultrasensitive chemical and biological sensors. These nano system offer more precise, specific, sensitive, lucrative devices with high reproducible results. NEMS/MEMS cantilever-based biosensors has three main parts: 1) Cantilever transducer element, 2) Sensing biolayer and 3) Readout system. Micro and nano cantilevers can be made-up either by bulk or surface micromachining, utilizing a wide range of materials, including silicon oxide, silicon nitride, polycrystalline silicon (polysilicon), SU-8 and metal films.

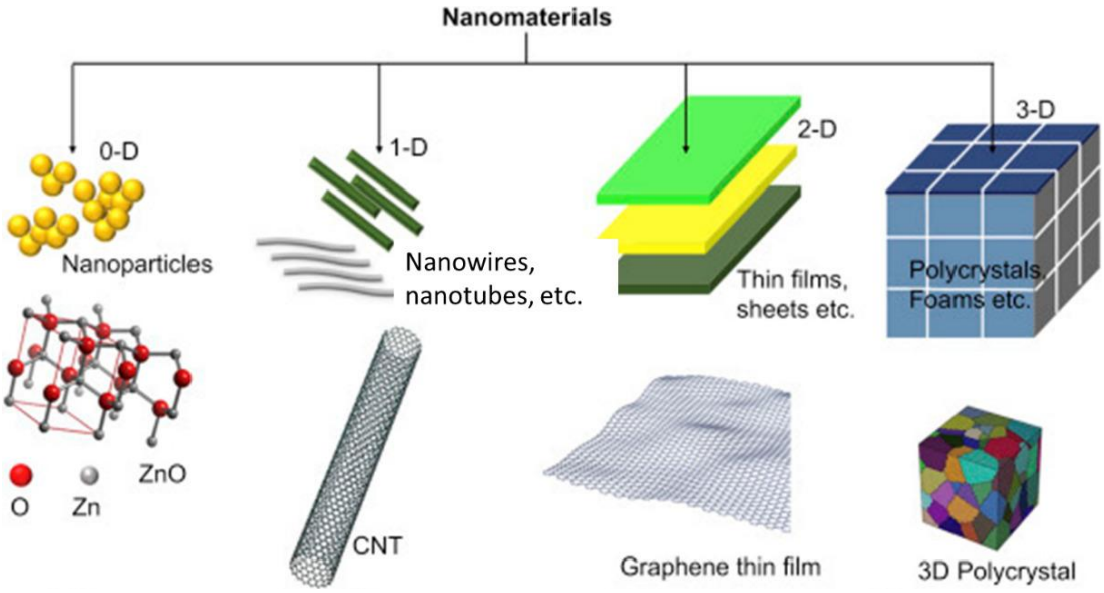

Fig 5:Classification of nanomaterial according to their dimensions (Malhotra & Ali, 2018).Figure adapted with permission from Elsevier

3. Epitomes of Nano-Biosensor

There is a growing need for cost-effective and highly sensitive methods in high-throughput format to measure drug-target interactions. A nanobiosensor is a means of detecting biological agents such as antibodies, nucleic acids, pathogens, and metabolites. Nanosensors work on the nanoscale which measures 10-9 m (single molecule level), such a device can transfer data and information about the behavior and properties of nanoscale particles to the macroscopic level. Nanosensors can be used to detect chemical or mechanical information such as the presence of chemical species and nanoparticles, or to monitor physical parameters such as temperature at the nanoscale. Nanosensors find wide ranging applications viz for virus detection and hygiene/disease control in livestock, active transport tracking devices and other diverse applications such as: (i) nanochips for identity (radio frequency identification), (ii) food inspection, (iii) intelligent food packaging and (iv) intelligent storage.The working principle consists of binding bioanalytes of interest onto bioreceptors, which in turn modulate the physiochemical signal associated with the binding.

Different types of nanosensors follow different detection principles: optical nanosensors measure the change in light intensity; electrochemical nanosensors measure the change in electrical distribution; piezoelectric nanosensors measure the change in mass; calorimetric nanosensors measure the change in heat. When an ammonia molecule is present in carbon nanotube-based sensors, it reacts with water vapor and makes the carbon nanotube more conductive by donating an electron. In contrast, if a molecule of nitrogen dioxide is present, it will make the carbon nanotube less conductive by stripping an electron from the nanotube.

Recent advances in experimental and analytical techniques have enabled researchers to explore the limits within which nanostructures can operate. These studies help one establish whether these devices provide consistent performance throughout their operational lifetime thus defining their suitability for future applications. Cantilever biosensors are ultra-high sensitivity

electromechanical sensors that have been successfully used for the label-free detection of large numbers of biological entities. They are emerging as a technology that appears attractive for high-throughput drug discovery applications.

3.1. Nanowire nanosensors

Nanowire-based nanosensors have emerged as a powerful tool due to their ultrasensitivity, ultraselectivity, and direct detection of chemical and biological species. These nanosensors can detect proteins, small organic molecules that bind to proteins, viruses and DNA, and are used in drug discovery, disease diagnosis and genetic testing.

Carbon nanotubes (CNTs) are one-dimensional (1-D) nanoscale structures based on graphene sheets and exhibit exotic material properties ranging from mechanical to electronic device properties [20,21]. The graphene-based structure of CNTs gives them unique attributes such as very high mechanical strength, ballistic charge transport, and many other electronic device properties. Since the CNT structure is derived from graphene [22], all carbon atoms make up the entire surface, making the CNTs more unique for sensor studies. Any change in surface structure during interaction with reactant molecules results in a change in their electronic properties, enabling detection of the analyte molecules under study. In fact, the CNTs offer very good detection sensitivity for a range of analytes such as gaseous molecules, organic charge-transfer complexes, proteins, DNA and antibodies. CNT is an excellent electrical and thermal conductor. In addition, its small size leaves room for the development of an ultra-small sensor, which is in the micron or even nano range. CNT arrays are hierarchical structures with unique advantages over isolated structures. CNT-array-based nanoelectrodes, called nanoelectrode arrays (NEAs) have been investigated for their unique applications such as chemical and biological sensors [23-26].

3.2. Cantilever Sensors

Microcantilever-based sensors offer many applications for a wide range of novel sensors in the detection of various analytes in a liquid, gaseous, or vacuum medium. These sensors offer high sensitivity, low cost, fast response, and high specificity without the need for pre-analysis labeling. Derived from atomic force microscopy (AFM),[27] which is capable of imaging a surface with nanoscale resolution by measuring the tiny force between a sharp tip of a suspending cantilever and the surface, microcantilever sensors do not require a sharp tip or a sample surface; instead, it is used to sense a biochemical reaction taking place on the cantilever surface by measuring its nanomechanical response.[28]

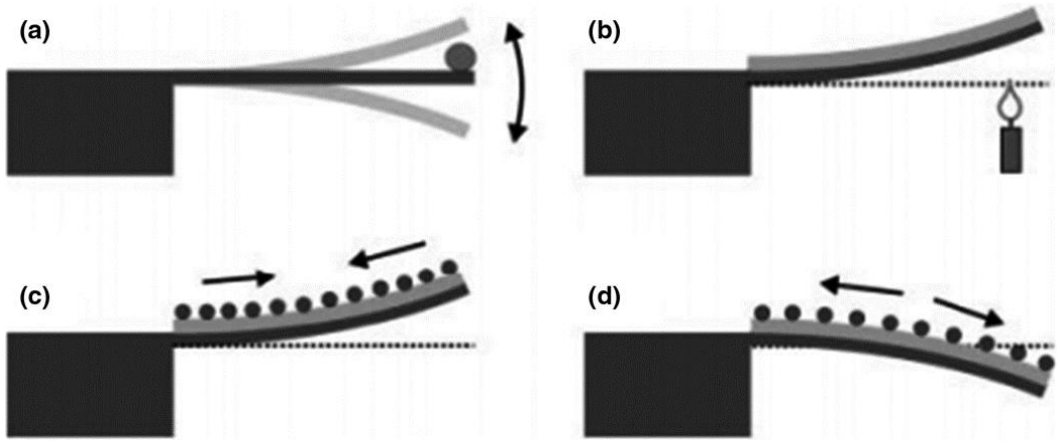

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Fig 6: Cantilever sensor operation mode: (a) detecting mass variations on the cantilever by deviations in resonance frequency; (b) bimetallic mode detecting temperature variations by a static bending; and (c, d) surface stress mode, where asymmetric molecular binding to the cantilever's top or bottom surface leads to an overall cantilever bending. For example, adsorption on the top surface can either cause tensile stress (c), bending the cantilever upwards, or compressive stress (d), bending the cantilever downwards (Fritz, 2008). Figure reproduced with permission from The Royal Society of Chemistry

The major advantages of microcantilevers sensors include label-free detection, small size, rapid response, high sensitivity, and the ability for high-throughput and multiplexed detection of various substances. Microcantilever sensors detect molecular binding on the cantilever surface through its nanomechanical motion, without the need for fluorescent or radioactive labeling. The signal transduction of microcantilever sensors is rapid because the small-scale devices have relatively high mechanical self-resonance frequencies in solution. Hence, the microcantilever platform is well suited to real-time monitoring of biomolecular interaction events on a sub-millisecond timescale. [29] One of the major challenges in microcantilever biosensing research is to reliably and efficiently functionalize each suspended microcantilever beam on an array with different bio/chemical molecules. This can be done either with microcapillaries. [30,31] Microcapillaries are relatively easy to handle and suitable for functionalizing cantilevers in small amounts.

3.3. Optical sensing

There have been many attempts at glucose monitoring using different signal transduction mechanisms as well as different glucose recognition elements. The methods used to determine the concentration of glucose in blood and body fluids can be divided into two broad groups, namely (a) spot sampling and (b) continuous glucose monitoring (CGM). Commercially available finger-prick glucometers and urine test strips demonstrate the spot sampling method of glucose estimation. Based on the positioning of the sensor as well as its design and detection mechanism, the CGM sensors are commonly classified into categories such as invasive (subcutaneous sensors, microdialysis modules, intravenously implantable sensors), minimally invasive (micropore, microneedle) and noninvasive (transdermal and optical) methods

Most of the commercially available glucose sensors are designed and developed with the intention of detecting blood glucose concentration, i. H. to target extracellular glucose. Nevertheless, the monitoring or visualization of intracellular glucose in living cells has recently received much attention due to the need to elucidate mechanisms underlying the development of insulin resistance syndrome in diabetics. Intracellular glucose, unlike glucose-6-phosphate, has not often been considered as a signaling molecule for glucose repression and other glucose-triggered regulatory events, probably because of their allegedly low concentration, but mainly because of the lack of suitable methods to measure intracellular glucose concentration to be followed in living cells.

One of the most widely reported FRET designs for glucose sensing employs Concanavalin A (ConA).[32] Concanavalin A is a plant lectin protein originally extracted from the jack-bean, Canavalia ensiformis. It selectively binds to α-mannopyranosyl and α-glucopyranosyl residues found in various sugars, glycoproteins, and glycolipids. ConA binds reversibly with glucose (and mannose).

4. Application of Nano-biosensor in Tissue engineering

Biosensor-based systems contribute a convenient strategy to detect various signals from tissue, implying that biosensor-based systems possess potential applications for diagnosis of diseases. Traditional diagnosis of neurological diseases is time-consuming and inconvenient because it needs a doctor checking the symptoms from patient overcomes and exists a risk that around 40% of people can't be estimated in some diseases such as Parkinson's disease (PD) at early stage. Neurological research is another field of investigation where cell-based biosensors have proven to be significant, and the MEA technology is also the primary mode of determine neuronal circuits, physiology and abnormalities. Recently, Li et al. developed reversible electrode for rapidly diagnosis of Alzheimer's disease by using magnetic graphene nanomaterials. They conjugated the antibody of Alzheimer's disease biomarker, Amyloid-beta peptide 1–42 (Aβ42), on a magnetic nitrogen-doped graphene (MNG). Then, an Alzheimer's disease biosensor could be rapidly constructed by dropping the magnetic MNG immunocarriers on an Au electrode surface which has a tapping permeant magnet at the underside of electrode. Afterward, the used MNG biosensors could be removed, and the Au electrode could be reproduced by switching of the tapped permeant magnet.[2], [20]

bioelectrical activity is also an important myocardial function which can respond to the health of heart tissues. In general, the bioelectrical activity could be generated from the cardiomyocytes which are induced the change of action potential of cellular membrane; through the change of action potential, heart could be induced a synchronized pumping behavior via this organized electrical propagation. Therefore, a continuous electrocardiogram (ECG) monitoring technique provides a clinical standard method to detect the cardiac rhythm signal for diagnosing cardiac related diseases. To easily monitor cardiac rhythm signals, Lee et al. developed a small wearable flexible cardiac sensor which integrates an electrode, a near-field-communication chip, and a battery in polyurethane substrates. Feiner and co-worker developed a degradable electronic scaffold as a cardiac patch. The gold electrodes are deposited on an electrospun albumin-fiber

scaffold as passivation layer. Through this design, the flexible cardiac patch enables to sense the spontaneous contraction signal of cardiac cells and further provide an external electrical stimulation for regulate the contraction of cardiac cells.

mitochondrial dysfunction involves in the development of chemical or pharmaceutical toxicity. Balvi et al. used HepG2 cell-based liver organoids as a tissue model and cultured the organoids in a microfluidic device. By real-time monitoring the metabolic function of liver organoids, it endows the microdevice as a biosensor with a feature to track the dynamic of mitochondrial dysfunction. Via sensing the oxidative phosphorylation of glycolysis or glutaminolysis, the liver organoid-based system permits to evaluate the safety and effect of drug concentration on mitochondrial damage.[5], [21], [22]

Cancer research has been studies from last past several decades. Traditionally, cancer research focuses the therapy of cancer diseases so that many new efficient therapeutic methods are widely developed. But, in some case, patients with cancer diseases are often discovered at last stage, causing that patients missed the best time of therapy. Kamei and co-worker combined a microfluidic chip, heart, and liver cancer cells as a cancer-on-a-chip model. Pan et al. developed a dual biomarkers-label chip (i.e., VEGF- and PSA-labeled) for prostate cancers and their circulating tumor cells . In addition, Hu and co-workers also developed another type visible signal-amplifiable biosensor for detection. They used a nanoparticle-based chip to capture extracellular vesicle (EV)-associated RNA for overcoming the challenge that low expression EV-associated RNA in cancers at early stage are difficult for detection.[2], [19], [20]

Biosensors contribute to selective, sensitive, and rapid tools for the disease diagnosis in tissue engineering applications. Compared to standard ELISA analytical technique, biosensors provide a strategy to real-time and on-site monitor micro biophysiological signals via the combination of biological, chemical, and physical technologies. Although biosensors have well developed in the past decade, there are still some challenges needed to be improved. The major challenges of biosensors are the scale-up process and the long-term stability of commercial products. The current biosensors are usually shown as a prototype in a research laboratory or academic departments.

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