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Chemistry

Application of carbon nanotubes in the construction of biosensors: review

Aplicação de nanotubos de carbono na construção de biossensores: revisão

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ABSTRACT

Biosensors are devices capable of sensing a variety of compounds and can be used for medicine, food quality control and environmental safety. Carbon nanotubes (CNTs) are materials that show promising properties when acting as components for biosensors, offering superior electrical performance in field-effect transistors used in chemical and biological devices. Thus, this study aims to elaborate a bibliographic review of the use of biosensors based on CNTs to detect a variety of important analytes, such as tumor biomarkers, neurotransmitters, viruses, glucose and hydrogen peroxide. For that, searches were made in the PubMed (US National Library of Medicine), Web of Science and Scopus databases, with the following descriptors: “carbon nanotube”, “composite” and “biosensor”. Inclusion criteria were defined as original articles written in English and published in the last 5 years, leading to 24 articles in total. Results showed that CNT-based biosensors have a low detection limit, high sensitivity and reproducibility, while the CNTs offer adsorption control, reactivity, thermal stability, flexibility and electronic conductivity. Therefore, CNT biosensors can provide fast and highly sensitive detection for a wide range of applications, with some advantages over current standard methods, such as lower costs and greater accessibility. However, even with their important capabilities, biosensors still have some challenges before being applied to daily life, as studies should focus on enhancing the functionality of these devices at physiological pH and room and body temperatures, while maintaining their sensitivity and stability for longer periods.

Keywords: Nanocomposites; Nanotechnology; Sensors

RESUMO

Os biossensores são dispositivos capazes de detectar uma variedade de compostos e podem ser usados para medicina, controle de qualidade de alimentos e segurança ambiental. Os nanotubos de carbono

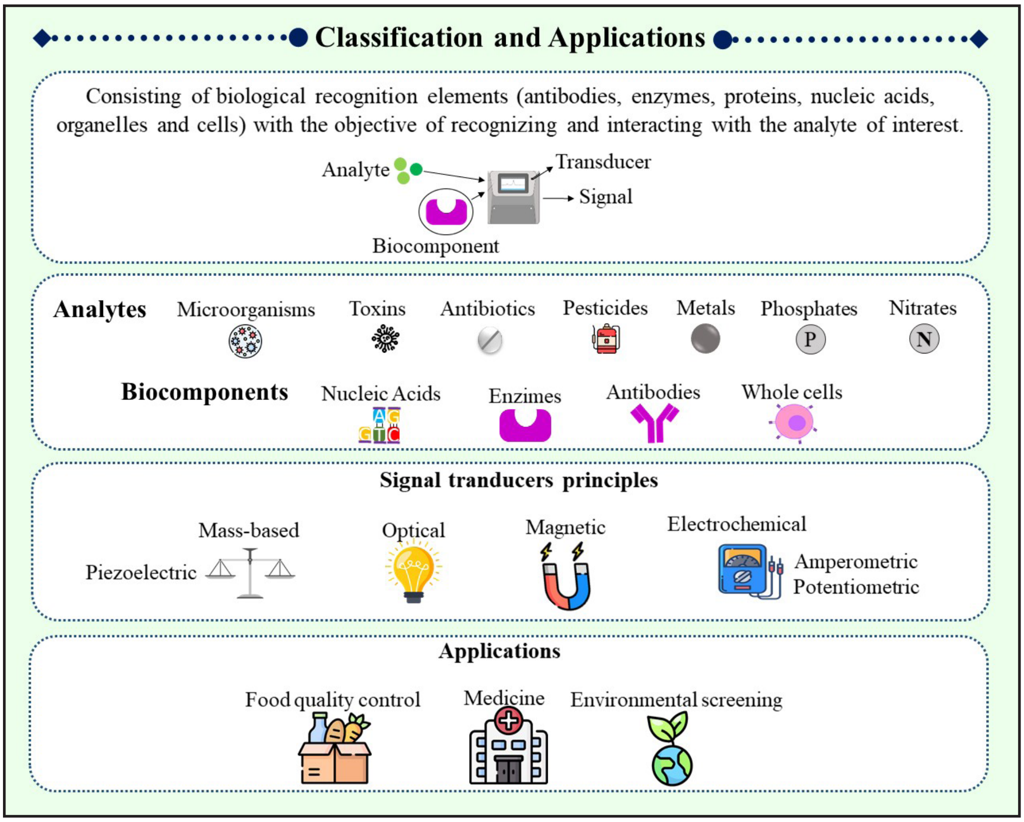
(CNT) são materiais que apresentam propriedades promissoras ao atuar como componentes para biossensores, oferecendo desempenho elétrico superior como transistores de efeito em dispositivos químicos e biológicos. Portanto, o objetivo deste trabalho foi elaborar uma revisão bibliográfica sobre o uso de biossensores baseados em CNT para detectar uma variedade de analitos importantes, como biomarcadores tumorais, neurotransmissores, vírus, glicose e peróxido de hidrogênio. Desta forma, foram realizadas buscas nas bases de dados PubMed (US National Library of Medicine), Web of Science e Scopus, com os seguintes descritores: “carbon nanotube”, “composite” e “biosensor”. Os critérios de inclusão foram definidos como artigos originais escritos em língua inglesa e publicados nos últimos 5 anos, totalizando 24 artigos. Os resultados mostraram que os biossensores baseados em CNT têm baixo limite de detecção, alta sensibilidade e reprodutibilidade, enquanto os CNT oferecem controle de adsorção, reatividade, estabilidade térmica, flexibilidade e condutividade eletrônica. Portanto, os biossensores baseados em CNT podem fornecer detecção rápida e altamente sensível para uma ampla gama de aplicações, com algumas vantagens sobre os métodos padrão atuais, como custos mais baixos e maior acessibilidade. No entanto, mesmo com suas importantes capacidades, os biossensores ainda apresentam alguns desafios antes de serem aplicados na vida cotidiana, e os estudos devem focar em aprimorar a funcionalidade desses dispositivos em pH fisiológico e temperatura ambiente e corporal, mantendo sua sensibilidade e estabilidade por períodos mais longos.

Palavras-chave: Nanocompósitos; Nanotecnologia; Sensores

1 INTRODUCTION

Biosensors are analytical devices capable of detecting a variety of chemical and biological compounds, with applications in drug detection, clinical diagnosis, biomedicine, food safety, environmental monitoring, and security (Vigneshvar et al., 2016). These devices operate through specific biochemical reactions mediated by isolated enzymes, immune components, tissues, organelles, or whole cells, generating electrical, thermal, or optical signals that enable the identification of target analytes (Fracchiolla, Artuso & Cortelezzi, 2013). Structurally, biosensors consist of three essential elements: a biorecognition component responsible for selectively interacting with the analyte, a transducer that converts this interaction into a measurable signal, and an amplifier or processor that interprets the signal, as illustrated in Figure 1 (Purohit et al., 2020).

Figure 1 – Application and principle of a biosensor, where the analyte binds specifically to a biocomponent, leading to a signal generation (electrochemical, optical, magnetic or piezoelectric) that can be amplified and read by data processing. It can be used for clinical, environmental and food analysis purposes



Source: Construction by the authors

The performance of biosensors is determined mainly by sensitivity, selectivity, limit of detection, detection range, and reusability. However, their efficiency can be compromised by signal interferences caused by the sensing matrix or co-existing molecules (Rocchitta et al., 2016). To overcome these limitations, nanomaterials have been incorporated into biosensor design, improving sensitivity and enabling point-of-care diagnostic platforms (Nagraik et al., 2021). Metallic, polymeric, and carbon-based nanomaterials increase the active surface area, enhance catalytic activity, and promote efficient electron transfer, enabling miniaturization and cost reduction while maintaining high analytical performance (De Luna et al., 2017).

2 METHODOLOGY

The present study is characterized as an exploratory and qualitative literature review. The search for articles was performed using the following descriptors: "carbon nanotube", "composite" and "biosensor", through PubMed (US National Library of Medicine), Web of Science and Scopus databases. Inclusion criteria were defined as original articles written in English and published in the last 5 years. The review articles published in a language other than the English language or repeated were excluded. The search resulted in 10,064 articles, of which 24 are within the defined inclusion criteria, and therefore selected to compose this review study.

3 RESULTS AND DISCUSSION

Table 1 shows the articles used in this review, described according authorship and year of publication, biosensor used and main objective of the study.

Table 1 – Presentation of the studies included in this review

(To be continued...)

Reference	Biosensor (Nanocomposite)	Detection
Makableh, Y, et al., 2023.	Gold nanoparticles and CNT	Detection of HER2.
Sharifi, J, & Fayazfar, H, 2021.	Glassy carbon electrode modified with MWCNT decorated with gold nanoparticles	Detection of doxorubicin hydrochloride.
Fazri, L, et al. 2019.	MWCNT/L-histidine functionalized with reduced graphene oxide (His-rGO)	Detection of Prostate Specific Antigen.
Dong, M, et al., 2023.	MWCNT-chitosan-based biosensor (MWCNTs-CS)	Detection of circulating tumor cells based on a highly efficient enzymatic cascade reaction.
Chauhan, N, Balayan, S, & Jain, U, 2020.	MWCNT-manganese oxide (MnO_2)-reduced graphene oxide (rGO) biosensor	Detection of acetylcholine.
Anshori, I, et al., 2021.	Functionalized CNT/silver nanoparticle (f-MWCNT/AgNP) biosensor	Detection of dopamine.
Shu, Y, et al., 2020.	Carbon nanotube biosensor coated with Ni-MOF/Au nanoparticles	Detection of dopamine.
Li, J, et al., 2021.	Composed of CNT and DNA aptamer	Detection of serotonin.

Table 1 – Presentation of the studies included in this review

(To be continued...)

Reference	Biosensor (Nanocomposite)	Detection
Rajeshwari, V, Vedhi, C, & Fernando, J, 2022.	Based on coreshell poly paraphenylene diamine/ titanium dioxide/ MWCNT	Detection of dopamine.
Hossain, MF, & Slaughter, G, 2020.	Based on glucose oxidase immobilized on platinum nanoparticles (PtNPs) decorated chemically derived graphene (CG) and CNT electrode	Detection of glucose.
Choi, Y, et al., 2019.	Based on the chitosan-CNT hybrid.	Detection of glucose.
Sriwichai, S, & Phanichphant, S, 2022.	Based on poly(3-aminobenzylamine) composite (PABA)/functionalized CNT.	Detection of glucose.
Comba, FN, et al., 2018.	Mucin and carbon nanotube-based biosensor.	Detection of glucose.
Zou, L, Wang, S, & Qiu, J, 2020.	Based on an ionic liquid-functionalized graphene/CNT composite.	Detection of glucose.
Sun, Z, Liu, H, & Wang, X, 2022.	Based on phase change microcapsules decorated with CNT	Detection of glucose.
Zamzami, MA, et al., 2022.	CNT transistor (CNT-FET) based biosensor	Detection of SARS-CoV-2.
Thanihaichelvan, M, et al., 2021.	CNT field-effect transistor (FET)-based biosensor	Detection of SARS-CoV-2.
Kalinke, C, et al., 2023.	Lactic acid (rPLA), polyethylene succinate (PES), carbon black (CB) and carboxylated multi-walled carbon nanotubes (COOH-MWCNT).	Detection of yellow fever virus.
Palomar, Q, et al., 2020.	Based on MWCNT composites decorated with Au nanoparticles.	Detection of dengue virus.
Guerrero, LA, et al., 2020.	Based on Nanocomposite of TiO2 Nanoparticle/MWCNT Modified Glassy Carbon Electrode.	Detection of hydrogen peroxide.
Alvarez-Paguay, J, et al., 2022.	Electrochemical biosensor based on CNT, hydroxyapatite and horseradish peroxidase.	Detection of hydrogen peroxide.
Murphy, M, et al., 2019.	Cytochrome C covalently immobilized on carboxyl functionalized ionic Liquid/MWCNT hybrid	Detection of hydrogen peroxide.

Table 1 – Presentation of the studies included in this review

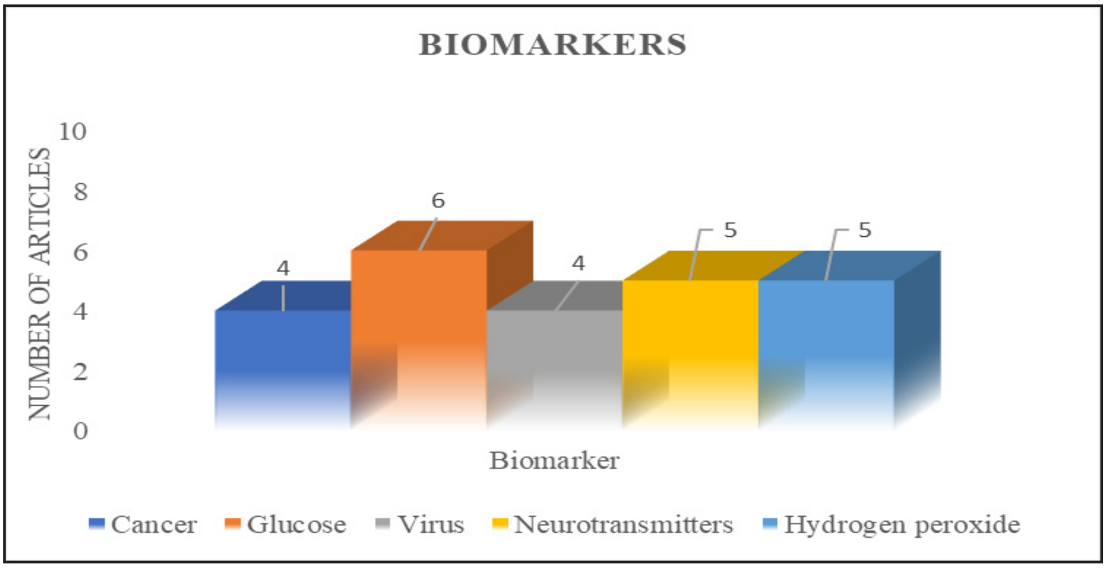
(Conclusion)

Reference	Biosensor (Nanocomposite)	Detection
Huang, X, et al., 2020.	Screen-Printed Carbon Electrodes Modified with Polymeric Nanoparticle-CNT Composites	Detection of hydrogen peroxide.
Jiang, T, et al., 2020.	Multi-walled carbon nanotubes and glassy carbon electrode and the hybrid of metal organic framework [NH2-MIL-53(Fe)] and horseradish peroxidase	Detection of hydrogen peroxide.

Source: Construction by the authors

As can be seen in Table 1, 24 studies were selected for this review, which encompassed all predetermined aspects with the descriptors. Among the analyzed studies, several biomarkers were focused on the development of CNT-biosensors, as shown in Figure 2.

Figure 2 – Target biomarkers



Source: Construction by the authors

As can be seen in Figure 2, the biomarkers most investigated by the authors were those that used biosensors for glucose detection, with 6 articles, biomarkers related to neurotransmitters, such as acetylcholine, dopamine and serotonin were also widely explored. Other biomarkers, such as cancer biomarkers, viruses and hydrogen peroxide were also targets of the authors in the development of CNT-biosensors.

3.1 Cancer

Cancer remains one of the major healthcare challenges and early detection is the best factor for a positive prognosis (Hubbell et al., 2020). Cancer biomarkers are a fundamental aspect of diagnosis, and those include genes, gene products, DNA, RNA, proteins, enzymes, hormones and specific cells resulting from genetic alterations (Chang et al., 2019). Precise measurement of these biomarkers in human body fluids such as blood, urine, serum, and plasma can potentially ensure the early detection of several cancer types, thus providing better chances of treatment and remission for the patients (Siegel et al., 2019).

Western blotting, polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) are common existing techniques used for biomarker screening, but these methods are usually expensive and/or inaccessible for many. Moreover, oncological imaging and biopsy-cytology techniques are invasive methods with important drawbacks such as being uncomfortable and costly. Thus, the development of fast, precise and economically feasible strategies for cancer detection is very important. Different types of biosensors, such as electrochemical, optical, and mass sensitive, are increasingly being reported for detecting cancer biomarkers. For that purpose, biosensors utilize various biorecognition elements such as antibodies, enzymes, DNA, RNA, cancer cells, nucleic acid probes or other specific biomolecules, which are immobilized on the transducer surface (Yang et al., 2019). The superior properties of these devices include their ease of use, high sensitivity, low detection limit, specificity and multiplexing capability (Hasan et al., 2021).

Dong et al. (2023) developed an ultrasensitive electrochemical biosensor to detect circulating tumor cells (CTCs) based on a highly efficient enzymatic cascade reaction. This biosensor was developed from a composite of MWCNT-chitosan

(MWCNTs-CS). A high electrochemical effective surface area was obtained for a glassy carbon electrode modified by MWCNTs-CS (GCE) for glucose oxidase (GOD) charging, as well as a high charging rate and high electrical activity of the enzyme. As an energy source, the MWCNTs-CS composite provided a strong driving power for horseradish peroxidase (HRP) onto the surface of polystyrene (PS) microspheres, which acted as probes to capture CTCs and allowed the reaction to proceed with greater facilitation of electronic transfer. In this study, the authors found a highly efficient cascade enzymatic reaction for biosensor electrochemical detection of CTCs that exhibited a wide linear concentration range (10 cells mL^{-1} to 10^6 cells mL^{-1}) with high sensitivity, selectivity, reproducibility, and limit of detection (LD) (3 cells mL^{-1} for CTCs in 3σ), standing out as a potential clinical electrochemical biosensor for detecting CTCs.

Breast cancer is the leading tumor-related cause of disease burden for women and prevention offers the most cost-effective strategy for control (Britt, Cuzick, & Phillips, 2020). Makableh et al. (2023) reported a screen-printed carbon electrode modified with gold nanoparticles (GNP) and MWCNTs to detect human epidermal growth factor 2 (HER2), a biomarker for early breast cancer detection. As shown by the Differential Pulse Voltammetry (DPV), the biosensor took only 5 min to bind to the target protein HER2, which was detected with a linear range of 1 pg mL^{-1} to 25 ng mL^{-1} and a LD of 4.4P g mL^{-1}. In addition, the selectivity test demonstrated no binding to interfering compounds, such as human serum albumin (HSA) and prostate specific antigen (PSA). Thus, GNP-CNT biosensors could be very effective to detect an important breast cancer biomarker in a fast and precise manner, with the potential to be a good alternative method for current techniques such as fluorescence in situ hybridization (FISH) or immunohistochemistry screening (IHC).

Prostate cancer is the second commonest male cancer worldwide and, although a generally slow growing tumor, it can metastasize to the bones, lungs, and brain if not detected early (Harrison et al., 2020). The PSA is the most used biomarker, as it is a blood-based biomarker used in all the main phases of prostate cancer detection and patient management (Duffy, 2019). Fazri et al. (2019) developed a voltametric biosensor for the detection of PSA, utilizing MWCNT/L-histidine functionalized with reduced graphene oxide (His-rGO) for covalently attaching thionine redox indicator and anti-PSA antibody (Ab). Notably, the MWCNT enhanced electrical conductivity and facilitated the electron transfer between thionine and the glassy carbon electrode. The linear calibration curve for PSA determination was 10 fg mL^{-1} – 20 ng mL^{-1} and, under optimized conditions, the sensor was able to selectively detect PSA with a LD of 2.8 fg mL^{-1}. The relative standard deviations (RSDs) for single-electrode repeatability and electrode-to-electrode reproducibility were less than 2.9% and 5.7% (n = 5), respectively. In addition, the sensor detection capability was compared with an ELISA kit, showing no significant statistical difference (12.3 ± 0.9 ng mL^{-1} and 12.6 ± 1.0 ng mL^{-1}, respectively). Thus, the proposed device can be successfully applied to PSA measurement in human serum samples with high sensitivity and accuracy, comparable to a reference method, but with important advantages, such as low cost, elimination of labeled antibodies and no sophisticated equipment and specialists' necessity.

Besides tumoral biomarkers, antitumor agents detection is another important factor for cancer treatment, considering drugs such as Doxorubicin hydrochloride (DOX) are highly dose-dependent and can show severe side effects, such as tissue necrosis, myocardial toxicity, cardiotoxicity, liver failure, myelodysplasia, and myelosuppression, making its detection valuable for therapeutic control (Abdullah et al., 2019; Octavia et al., 2012). For that reason, Sharifi & Fayazfar (2021) developed a glassy carbon electrode modified with gold nanoparticles and MWCNTs biosensor for the detection of DOX. The cyclic and linear sweep voltammetry electrochemical techniques showed that the use of MWCNTs significantly increased DOX detection in relation to the bare glassy carbon plate sensor, demonstrating an efficient catalytic activity for DOX reduction, increasing the peak current density while decreasing the reduction over-potential. In addition, under optimal conditions, a linear DOX concentration range from $1x10^{-11}$ to $1x10^{-6}$ mol L^{-1} with a very low LD of 6.5 pmol L^{-1} was observed, indicating that the functionalized sensor had an appropriate selectivity, reproducibility, repeatability and long-term stability.

3.2 Neurotransmitters

Neurotransmitters (NTs) are endogenous chemicals involved in the signal transmission between neurons across chemical synapses, exchanging information throughout the brain and body (Rizo, 2018). NTs play a key role in brain functioning and control various behavioral and physiological conditions that affect daily life, such as learning, memorizing, sleeping, mood and regulation of muscle tone, heart rate and blood pressure (Moon et al., 2018). Consequently, variations in the production, secretion, uptake and/or metabolism of NTs may lead to serious and hard to treat neuro and physical disorders, such as Alzheimer's and Parkinson's diseases, schizophrenia, epilepsy, depression, cancers and many others (Mobed et al., 2020). Therefore, fast and accurate determination of NTs levels in physiological samples is imperative for effective disease diagnosis and monitoring, therapeutic interventions and even understanding the role of these chemicals in brain functions (Moon et al., 2018). Various analytical tools have been reported for the quantification of NTs in biological samples, including mass spectroscopy, fluorimetry, chromatography and capillary electrophoresis (Banerjee et al., 2020). However, most of them are complex, expensive and suffer from poor sensitivity and selectivity, in contrast to electrochemical methods that are known to provide a low-cost, simple, sensitive, fast and selective determination of various biological species, such as NTs (Tavakolian-Ardakani et al., 2019).

Dopamine (DA) is a monoamine that acts in reward, cognition, movement, and learning (Channer et al., 2023). Considering the vital functions that are dependent on DA and its related pathways, malfunctions of dopaminergic signaling are implicated in the progression of various disorders, such as Parkinson's disease, depression, schizophrenia and even food and drug addiction (Klein et al., 2018). Shu et al. (2020) developed a stretchable electrochemical sensor based on nickel metal-organic framework composite and Au nanoparticle-coated CNTs for the detection of DA released in C6 cell line. The hybrid device showed excellent electrochemical performance with a wide linear range from 50 nmol L^{-1} to 15 µmol L^{-1} and a high sensitivity of 1250 mA/[(cm^2 mol/L)], with good stability against mechanical deformation. Furthermore, the stretchable electrode displayed good cellular compatibility with strong adhesion and no significant toxicity in C6 cell culture. Similarly, Anshori et al. (2021) synthesized MWCNTs with silver nanoparticle (f-MWCNTs/AgNPs) nanocomposite as biosensing material to detect DA. According to the DPV measurements, the sensor's LD found was 0.2778 mmol L^{-1} in the linear range of 0–8 mmol L^{-1}, which is lower than the required concentration value for DA detection in human urine (0.3–3 mmol L^{-1}). In addition, the device showed a high selectivity for DA even in the presence of other human-related compounds acting as interferents, such as glucose, urea and uric acid. Rajeshwari et al. (2022) developed a DA sensor (nanocomposite)_ based on core-shell poly paraphenylene diamine, titanium dioxide and MWCNTs. The nanocomposites had a stronger reaction to detect lower and higher concentrations of DA in solution, being able to bind to DA by stacking aromatic rings and hydrogen bonds between DA amino groups and dioxide oxygen-containing groups of titanium. The sensor based on the nanocomposite showed a high current response, low DL and strong selectivity.

Moreover, serotonin (5-HT) is a highly investigated monoamine that is related to appetite, sleep, mood, libido, motor control, learning, neuronal degeneration, gastrointestinal motility and vasoconstriction. 5-HT dysfunction is involved in a series of neuropsychiatric diseases and most psychotropic therapy drugs interfere with the 5-HT system (Marazziti, 2017). Li et al. (2021) proposed a voltametric biosensor composed of poly(diallyldimethylammonium)-wrapped oxidized SWCNT (oSWCNTs), where 5-HT-specific aptamer and tyrosinase on Au nanoparticles deposited screen printed carbon electrode for 5-HT measurement. The effects of 5-HT-specific aptamer and oSWCNTs on the detection of 5-HT were investigated by differential pulse voltammetry (DPV). The peak current at the potential of 0.29 V (vs. Ag/AgCl) for 5-HT concentration resulting in two dynamic ranges from 0.05 to 0.5 and 1 to 20 mM, with a 2 nM LD from the biosensor in buffer solution, which were better than those without the aptamer and oSWCNTs. The biosensor was then applied to determine 5-HT concentrations in healthy control and Internet Gaming Disorder (IGD) serum samples, with results showing that the 5-HT levels were higher in IGD than in HCs, demonstrating the positive relation of 5-HT and IGD and that the proposed device could be a good alternative for 5-HT measurement.

Another neurotransmitter that plays a crucial role in neuromodulating both central and peripheral nervous systems is acetylcholine (ACh), which is involved in memory regulation, behavioral activities and muscle contraction. The cholinergic system is highly associated with Alzheimer's disease and ACh detection can contribute to the diagnosis of various neurodegenerative disorders (Singh et al., 2013). Chauhan, Balayan & Jain (2020) proposed a biosensor based on the co-immobilization of acetylcholinesterase (AChE) and choline oxidase (ChO) over the Au-electrode coated with nanocomposite layer of MWCNT-manganese oxide (MnO_2)- reduced graphene oxide (rGO). The current observed in proportion to various ACh concentrations demonstrated a linear relation ranging between 0.1–100 μmol L^{-1}, with a 0.1 μmol L^{-1} LD in the cyclic voltammetry (CV) technique. In addition, the sensor exhibited very high reproducibility and good stability over 3 months at 4 °C storage condition.

3.3 Glucose

Glucose is one of the most life-critical biological compounds, being the primary fuel for glycolysis and the downstream pathways of aerobic and anaerobic respiration, thus responsible for generating much of the energy required for body functions and life itself (Galant, Kaufman, & Wilson, 2015). For that reason, a disorder in glucose metabolism is closely related to serious health problems, the main one being diabetes mellitus, a disease with increasing incidence that occurs due to insulin deficiency or resistance by the organism (Nichols et al., 2013). Type I diabetes is caused by the lack of insulin production in pancreatic cells, while type II has resulted from the body's underutilization of insulin produced in the pancreas. Although it is a chronic disease, diabetes can lead to complications and severe symptoms, such as heart attack, kidney failure, high blood pressure, nerve damage, blindness and death (Dong, Ryu, & Lei, 2021). As a clinical indicator of diabetes, the accurate determination of glucose is essential for monitoring and managing the disease, and extensive research on diabetes treatment shows that blood glucose monitoring can delay the onset and progression of complications and associated mortality significantly (Danne et al., 2017). Consequently, there is a high demand for the development of affordable and accessible medical care instruments and protocols for diabetes patients to monitor and self-manage their blood glucose levels (Dong, Ryu, & Lei, 2021).

The amount of glucose in healthy individuals is known to be between 3.9 and 6.1 mM and 3.9 and 6.9 mmol L^{-1} for whole blood and plasma, respectively, while in diabetic patients it is more than 6.1 m mol L^{-1} and 7 m mol L^{-1}. Although glucose levels in healthy individuals and diabetic patients are both in the millimolar range, the complicated blood matrix causes interferences in glucose detection, requiring a sample dilution procedure before the analysis. Therefore, for point-of-care glucose level detection, a highly sensitive method is needed (Ma et al., 2016).

CNTs are used in amperometric biosensors due to their induced properties such as high aspect ratio, control adsorption, reactivity, thermal stability, flexibility, and electronic conductivity. Amperometric enzymatic electrodes based on glucose oxidase represent an excellent alternative for detecting glucose levels. Glucose oxidase catalyzes the oxidation of glucose to gluconolactone in the presence of the natural mediator, oxygen, and generates hydrogen peroxide, which is subsequently oxidized at the electrode, producing a current proportional to the concentration of the analyte (Comba et al., 2018). Despite the benefits that its application provides, care should be taken with the use of CNTs due to some of their physicochemical properties, such as nanoscale size, high aspect ratio and substantial biopersistence, as CNTs are breathable fibers, making them act as foreign bodies after inhaling material containing CNTs (Dong, 2020).

Sun, Liu & Wang (2022) developed a thermal self-regulatory biosensor through the integration of a phase change material (PCM) and CNT as an electroactive layer on a SiO_2 shell surface. It is known that enzyme-based biosensors are thermal sensitive due to their strong temperature dependency on catalytic activity (Arcus et al., 2020). The device was constructed with the resultant electroactive microcapsules with GOD as a redox enzyme and showed a high sensitivity of 5.95 $\mu A \cdot mM^{-1} \cdot cm^{-2}$ and a LD of 13.11 μ mol L^{-1} at 60C°, achieving a thermal self-regulation capability to enhance the biosensing detection of glucose under in-situ thermal management even at higher temperatures. Comba et al. (2018) developed an amperometric biosensor formed by mucin and CNT for the detection of glucose in human plasma. Human blood plasma samples with glycemic values between 80 mg dL^{-1} and 304 mg dL^{-1} were analyzed, obtaining transformation factor R^2 = 0.991. The developed biosensor presented an average systematic error of 4.0% (% BIAS) when compared with the values determined by the standard, providing a residual standard deviation (% RSD) of 2.2% for the set of evaluated evaluations. After 21 days, the comparison was repeated, with R2=0.989, showing %BIAS of 3.7 and %RSD of 4.7%. CNTmuc composite provided a sensitivity of 0.44±0.01 $mA.M^{-1}$, a response time of 28±2s and LD of 3 μ mol L^{-1}. According to the authors, the inclusion of CNT in the enzymatic system of the matrix would contribute to increasing the transmission of reactive species and, thus, improve the response time of sandwich-type biosensors.

Zou, Wang & Qiu (2020) developed a biosensor formed by a glassy carbon electrode with a 1-methyl imidazole based on a composite of graphene and CNTs functionalized in liquid, with the aim of immobilizing horseradish peroxidase (HRP) and GOD for glucose detection. The sensor response to different glucose concentrations ranging from 10 to 100 μ mol L^{-1}, showed that increasing the glucose concentration, the peak oxidation current increases correspondingly. According to the relationship between the glucose concentration and the response current, it is verified that the linear response range of the sensor is between 0.004-5mmol L^{-1}, $R^2 = 0.997$, the sensitivity is 53.89 μA mmol L^{-1} cm^{-2}, and the LD is 3.99×10^{-7}mol/L (S/N=3). The sensor shows excellent performance, stability and reproducibility results after two weeks, showing a response of 90% compared to previous results. The functionalization of graphene and CNT significantly increases the surface area and forms a three-dimensional network structure, providing more enzymatic binding sites. CNTs can act as molecular threads to connect graphene sheets and improve electron transfer efficiency.

In the study by Hossain & Slaughter (2020), a biosensor based on graphene nanocomposites and CNT for selective glucose detection was also developed. In this study, GOD was immobilized on the nanostructured electrode and subsequently coated with nafion for selective glucose detection. The manufactured hybrid biosensor exhibited good electrocatalytic activity towards glucose with a linear dynamic range from 0.5 m mol L^{-1} to 13.5 m mol L^{-1} with a fast response time of <5 s. A high sensitivity of 26.5 μA m mol L^{-1} cm^{-2} and a low LD of 1.3 μ mol L^{-1} was observed. The obtained results thus indicate that the nanostructured composite material offers a large surface area and high electrocatalytic activity towards glucose and is a potential candidate material for glucose biosensors. Riwichai & Phanichphant (2022) developed biosensors for glucose detection. In this research, the authors developed the biosensor from a composite film of poly(3-aminobenzylamine) and functionalized MWCNT. The developed biosensors presented electrochemical characteristics of PABA/f-CNTs that confirmed that the presence of f-CNTs in the composite contributed to the improvement of the electrochemical properties of PABA after the detection of glucose in several concentrations (0.56-2.8 mmol L^{-1}) and under common interference. Amperometry was employed to investigate the sensitivity, selectivity and stability of the electrode prepared from electrospun PABA/f-CNTs. It can be concluded that the PABA/f-CNTs electrode showed high selectivity, a sensitivity of 0.40 ^A·mm^{-2}·mM^{-1} with LD of 0.067 mM in the linear range of 0.562.8 mM and good stability up to 1 week. This electrode obtained can be developed as a detection platform for future use in analysis of real samples.

Choi et al. (2019) developed a biosensor from a chitosan-CNT hybrid to electrochemically detect glucose. The biosensor reacted with various glucose concentrations (0.0, 0.1, 1.0, 5.0, 10.0 and 20.0 mmol L^{-1}) without corresponding oxygen saturation. The current density in our electrode was shown to change from 270 μA cm^{-2} to 157 μA cm^{-2}. These results showed that it has 3 to 10 times more response compared to other chitosan-based biofuel cells. The authors suggest that this optimized biosensor can be considered a great promise for use in biosensor applications in healthcare.

3.4 Virus

Viruses are obligatory intracellular parasites that require a suitable host for replication and that are constantly changing their genetic composition as a mechanism to evade the host immune system, which potentially leads to small illness symptoms or major diseases (Mahmood et al., 2020). Currently, the problem of viral diseases, in particular, emerging diseases, such as dengue (Palomar et al., 2020), yellow fever (Kalinke et al., 2023) and SARS-CoV-2 in recent years (Zamzami et al., 2022; Thanihaichelvan et al., 2021), leads to a growing number of studies with the objective of developing biosensors for the electrochemical determination of these viral diseases. Throughout history, there have been several pandemics caused by viruses, the most recent and devastating being the one caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (Choi, 2020). On January 2020, the World Health Organization declared the outbreak of COVID-19 a global public health emergency of international concern and, as of June 2023, over 767 million confirmed cases and over 6.9 million deaths have been reported globally (WHO, 2023).

The SARS-CoV-2 has high transmission capability and its clinical manifestation ranges from mild illnesses such as fever, cough and dyspnea to life-threatening syndromes, including pneumonia, acute respiratory distress syndrome and death.

Therefore, early and precise diagnosis is important to reduce the risk of more serious complications (Choi, 2020). Although nowadays the vaccine production and application has progressed considerably, considering the virus variations and the possibility of even more in the future, the COVID-19 is still a cause for concern (Patel et al., 2023). In viral diagnosis, virus isolation is the gold standard, as it is the most sensitive method, however, it is also laborious and time consuming (3–7 days), while serological investigation for antibodies against viral antigens are less sensitive and specific (Abid et al., 2021). The PCR assay, which is the standard method for SARSCoV-2 identification, is an expensive test that also requires time and specialized laboratories with expert personnel (Choi, 2020).

Virus biosensing can be a good diagnosis alternative, as it can use highly specific biomolecules as viral analytes, immobilized onto transducers to act as functional sensors. That target analyte involves viral antigenic part that can be a whole virus, viral proteins, viral nucleic acids or viral-specific antibodies, while the most common sensing constituents are whole cells, peptides, nucleic acids, aptamers and antibodies (Caygill, Blair, & Milner, 2010). Zamzami et al. (2021) developed an electrochemical biosensor based on CNT field-effect transistor (CNT-FET) for SARS-CoV-2 surface spike protein S1 detection in saliva samples. The biosensor was developed on a Si/SiO2 surface by CNT printing with the immobilization of an anti-SARS-CoV-2 S1, while a commercial SARS-CoV-2 S1 antigen was used to characterize the electrical output of the CNT-FET sensor. The SARS-CoV-2 S1 antigen was rapidly detected (2-3 min) at concentrations of 0.1 fg mL^{-1} to 5.0 pg/mL, with a 4.12 fg mL^{-1} LD. The selectivity test was performed by using target SARS-CoV-2 S1 and non-target SARS-CoV-1 S1 and MERS-CoV S1 antigens and the biosensor showed high selectivity for the SARS-CoV-2 S1 antigen (no response to SARS-CoV-1 S1 or MERSCoV S1 antigen), indicating good selectiveness. Another interesting method was observed by Thanihaishelvan et al. (2021), with the utilization of the reverse sequence of the RNA-dependent RNA polymerase gene of SARS-CoV-2 onto the CNT channel of a FET. Tests with synthetic positive and control target sequences showed a selective sensing response to the positive target sequence with a LD of 10 *fm*, indicating that the proposed CNT-FET based biosensor could be further developed into a reliable, fast, and cheap alternative for existing COVID-19 diagnostic techniques.

Yellow fever is considered endemic in tropical regions, it is caused by the prototype of the genus Flavivirus (family Flaviviridae), which contains approximately 70 single-stranded and positive-stranded RNA viruses, most of which are transmitted by arthropods (mosquitoes and ticks). It can cause hemorrhagic viral fever, in addition to other symptoms such as headaches, malaise, skin rashes, nausea, vomiting and diarrhea (Fenollar, & Mediannikov, 2018). Kalinke et al. (2023) developed an electrochemical biosensor for the detection of yellow fever virus cDNA. Custom additive manufacturing filaments were used using recycled lactic acid (rPLA, 65% by weight), polyethylene succinate (PES, 10% by weight), carbon black (CB, 15% by weight) and COOH-MWCNT (10% by weight, by size - short (0.5–2.0 μm) and long (10–30 μm) Detection of the Yellow Fever virus target sequence Using ASWV, showed a linear dynamic range (LDR) of 0.5–15 μM (R2=0.9995), sensitivity 11.9 ± 0.1 μA $μM^{-1}$, LD 0.138 μM (SN=3). Repeatability was tested by measuring six separate detection platforms with the incubation of 1 μM of yellow fever virus cDNA, the results showed an RSD of 2.10%, evidencing the reliability of the system. Stability was analyzed, showing a minimal decrease in the first 4 weeks and then a decrease to 69,2% of the original sensor response 8 weeks after production. Three concentrations (1.00 μm; 5.00 μm; 10.00 μm) of yellow fever virus cDNA in human serum samples were also analyzed. The proposed additive manufacturing sensor platform obtained good recovery values from 95.6 to 105% (RSD 2.17 - 4.17%), indicating good applicability of the biosensor.

Dengue fever is a neglected disease that affects the population of many tropical regions around the globe. The methods used for diagnosing dengue have high technical complexity, in addition to intrinsic miniaturization challenges. Such characteristics pose difficulties for mass population testing, which is an important step in controlling the spread of infectious diseases. Consequently, new bioanalytical methods emerge as potential diagnostic tools. Among such methods, biosensors are receiving more and more attention, as they allow the development of a sensitive, portable and easy-to-diagnose methodology (Khristunova et al., 2020). Palomar et al. (2020) developed a biosensor for the voltametric detection of the dengue virus based on a composite of MWCNT decorated with Au nanoparticles. After optimization, the system exhibits a high sensitivity of − 0.44 ± 0.01 μA per decade with wide linear range between 1×10^{-12} and 1×10^{-6} g mL^{-1} at a working potential of 0.22 V vs Ag/AgCl. The extremely low LD (3×10^{-13} g mL^{-1}) ranks this immunosensor as one of the most efficient reported in the literature for the detection of recombinant viral dengue virus 2 NS1. This biosensor also offers good selectivity, characterized by a low response to various non-specific targets and assays in human serum. The outstanding performances and the reproducibility of the system place the biosensor developed among the best candidates for future medical applications and for early diagnosis of dengue fever.

3.5 Hydrogen peroxide

CNT-modified electrodes are widely used in the catalytic and electrochemical industry for biomolecule detection. Hydrogen peroxide (H_2O_2) is an analyte of biological interest due to biological, environmental and industrial issues. In the human body, H_2O_2 can be converted into hydroxyl radicals (•OH), the overproduction of reactive species in cell interstices promotes cell damage. H_2O_2 detection forms the diagnostic response of medical devices such as glucose sensors, as in the presence of oxygen, H_2O_2 is produced by the action of glucose oxidase. When the bioenzymatic glucose biosensor is inserted into the glucose solution, the glucose molecules will be oxidized into acidic glucose by the preserved oxygen, while the glucose oxidase is changed to a reduced state. Glucose reduced to oxidase is not as stable and is also oxidized by oxygen, producing hydrogen peroxide. At the end of the reaction, hydrogen peroxide is reduced to water under horseradish peroxidase (HRP) catalysis (Guerrero et al., 2020; Zou, Wang, & Qiu, 2020). H_2O_2 acts in cell growth signaling and contributes to numerous diseases such as cancer, diabetes, neurodegeneration and aging. Analytical methods for the accurate detection of H_2O_2, including spectrophotometry, chemiluminescence, capillary zone electrophoresis, and chromatography, have limitations of being time consuming, needing a sophisticated expensive instrument, and tedious measurement. Electroanalytical H_2O_2 detection has advantages in terms of safety, simplicity and feasibility for real-time analysis, making it an emerging trend for H_2O_2 detection (Murphy et al., 2019).

Alvarez-Paguay et al. (2022) developed an electrochemical biosensor based on a glassy carbon electrode (GC) modified with MWCNT modified with hydroxyapatite (HAp) and HRP, for the detection of H_2O_2. Transmission electron microscopy (TEM) showed an average diameter of 12±2 nm of fCNTs and HAp nanoparticles are located on the walls of fCNTs with size in the range of 3 to 5 nm. According to the results, a LD of 1.91 and 4.45 μmol L^{-1} H_2O_2 was obtained, and sensitivity of 0.063 and 0.028 μA (μmol/ L^{-1}) for the electrodes HRP/HAp5-fCNT/ GC and HRP/HAp20-fCNT/GC, respectively. The average percentage of RSD was 2.39% for the HRP/HAp5-fCNT/GC and 5.18% for the HRP/HAp20-fCNT/GC, which shows that the HRP/HAp5-fCNT/GC electrode has greater precision. In the raw milk sample, H_2O_2 was not detected, and in commercial milk, 5.56 μmol/L^{-1} of H_2O_2 was detected, presenting an analytical precision of the recovery percentage (R%) of 98.5% and 102.4%, demonstrating good accuracy of the method. In conclusion, the use of HAp5-fCNT improves the immobilization of the HRP enzyme in the HRP/HAp5-fCNT/GC biosensor, which was associated with its smaller particle size and better distribution of particles in the nanotube, presenting better detection limit, quantification and sensitivity.

Similarly, Guerrero et al. (2020) developed a biosensor with a modified glassy carbon electrode of multiple walls of CNTs with titanium dioxide (TiO_2) nanoparticles, using Prussian blue (PB) as an electrocatalyst. The sensor was also the basis for the construction of the H_2O_2 biosensor using horseradish peroxidase (HRP) immobilized on the TiO_2-fCNT film. The average diameter of the fCNTs was 5±2 nm and TiO_2 was 6.1±1.3

nm. The average value of zeta potential fCNTs was -51.84±9.66 mV and TiO_2-fCNT was 9.93±0.73 mV. The chronoamperometric detection of H_2O_2 on the PB–TiO2/fCNT/GC electrode was performed for concentrations from 0.5 to 4.3 mmol·L^{-1} (R^2 = 0.997) and 0.04 to 0.9 mmol·L^{-1} (R^2 = 0.991). The LD was 0.088 mmol·L^{-1} in the oxidation zone, while in the reduction zone the LD was 0.092 mmol·L^{-1}. The chronoamperometric detection of H_2O_2 in PB-fCNT/GC, for concentrations in the range of 0.05 to 0.8 mmol·L^{-1} (R2 = 0.992) and 0.05 to 0.9 mmol·L^{-1} (R^2 = 0.997), with LD of 0.024 mmol·L^{-1} were obtained in the oxidation zone, while the LD was 0.015 mmol·L^{-1} in the reduction zone. According to these results, the PB–fCNT/GC electrode showed better LD, but in the PB-fCNT/GC electrode, the potential affected the stability. The reproducibility of the modified electrodes demonstrated acceptable values with a mean relative standard deviation of 3.6% for the current determined at 2 mol·L^{-1} of H_2O_2. After 30 days, PB–TiO_2/fCNT/GC still retained 80% of its response to H_2O_2, indicating good stability, however PB-fCNT/GC retained only 15% of its response to the analyte. Chronoamperometric responses obtained from the HRP–TiO_2/fCNT/GC electrode showed that HRP exhibited a very fast response to H_2O_2, reaching about 90% of the steady-state signal in 10s. The biosensor exhibited a linear response range (R^2 = 0.9997) between 0.5 mmol·L^{-1} and 7.5 mmol·L^{-1}. The detection limit was 0.81 mmol·L^{-1}. According to the authors, the modified PB–TiO2/fCNT/GC electrode was considered the best for H_2O_2 detection in terms of operability.

Murphy et al. (2019) developed an electrochemical biosensor with a glassy carbon electrode (GCE) for the detection of H_2O_2 using cytochrome c (Cytc) covalently immobilized with functionalized carboxyl hybrid (TPP-HA[TFSI]) from MWCNT. Cytc/TPP-HA[TFSI]/MWCNT/GCE showed excellent electrocatalytic capacity, reaching steady state in <3s, obtaining detection response with wide linearity in the concentration range from 20 μM to 892 μM, with detection limit (S/N=3) of 6.2 μm. The Cytc/TPP-HA[TFSI]/MWCNT/GCE biosensor showed better sensitivity, with a value of 0.14 μA mM^{-1}cm^{-2}, compared to Cytc/TPP -HA[TFSI]/GCE which presented a value of 0.016 μA mM^{-1}cm^{-2}, according to the authors, this increase is due to the highly conductive

TPP-HA[TFSI]/MWCNT platform. Regarding stability, the biosensor maintained 95% and 92% of its initial performance even after 30 days. An electrocatalytic reduction current was tested in the absence and presence of 30 μM H_2O_2, the results showed RSD of 1.9% in the absence and 2.4% in the presence, demonstrating excellent reproducibility for Cytc/TPP-HA[TFSI]/MWCNT/GCE. The determination of H_2O_2 by Cytc/TPPHA[TFSI]/MWCNT/GCE was carried out in real samples of milk and apple juice, obtaining recovery values in the range of 99% to 101.5%. Concluding that the biosensor demonstrated excellent selectivity and sensitivity for H_2O_2 determination along with remarkable stability and reproducibility.

Huang et al. (2020) analyzed a composite formed from modified screen-printed carbon electrodes (SPCE) with MWCNT and polymeric nanoparticles (PAKB NPs-CNTs) to detect H_2O_2 using enzymatic biosensor through horseradish peroxidase activity (HRP). By SEM (scanning electron microscopy), PAKBNPs were spherical with particle sizes ranging from 90 to 200 nm with potential of −27.10 ± 1.12 mV. The results showed that the linear range ranged from 0.02 to 6.48 mmol L^{-1}, high sensitivity of 72.08 μA $mm^{-1}cm^{-2}$, and a short response time, <2s ($R^2 = 0.997$). The LD was estimated at 2.7 μm (S/N=3). Stability showed a final current response value greater than 90% of the initial value after 15 days and approximately 80% after 30 days, which demonstrates that the developed biosensors can function with good stability. Reproducibility was also analyzed; the results showed an RSD value of 6.17% indicating good reproducibility. In milk and water samples, the biosensor obtained recovery values of 96.7%, 100.5% and 100.4% with RSD values of 5.98%, 5.59% and 3.38% in samples of milk containing 0.02, 0.1, and 1 mmol L^{-1} H_2O_2, respectively, and had recoveries of 102.8%, 98.0%, and 96.4% with RSD values of 5.73%, 1.51%, and 0 .57% in lake water samples containing 0.02, 0.1 and 1 mmol L^{-1} H_2O_2, respectively.

With a different purpose, H_2O_2 detection was evaluated by Jiang et al. (2020). The authors evaluated H_2O_2 released from cancer cells through an electrode modified with horseradish peroxidase. The biosensor showed dynamic linear ranges of 0.1-1 μmol L^{-1}

(R^2 = 0.9974) and 1-600 μmol L^{-1} (R^2 = 0.9921), with a sensitivity of approximately 6149 μA m mol $L^{-1}cm^{-2}$, the LD was estimated at 0.028 μmol L^{-1} (S/N=3). The reproducibility of NH2-MIL-53(Fe)/HRP/MWCNTs/GCE for blank, 100 μM H2O2 and the difference between 100 μmol L^{-1} H_2O_2 and blank were evaluated, with RSD for blank of 6.62%, RSD for 100 μmol L^{-1} H_2O_2 of 2.16%, and the RSD of the current difference between the blank and 100 μM H_2O_2 is 2.13%. The detection performance of NH2-MIL-53(Fe)/HRP/MWCNTs/GCE at 15 °C can reach more than 90% stability in one week, and gradually decrease to less than 80% after two weeks. The NH2-MIL-53(Fe)/HRP/MWCNTs/GCEs response values for HeLa cell culture solutions (Control: 36.14 ± 3.42 μmol L^{-1}; Artemisinin (considered a promising treatment for cancer, slowing tumor cell proliferation through reactive oxygen species-mediated cell cycle arrest): 43.50 ± 5.28 μ mol L^{-1}) and HepG2 cells (Control: 55.17 ± 4.59 μmol L^{-1}; Artemisinin: 62.36 ± 6.03), indicate that the biosensor can be a new platform for detection of H_2O_2 released by cancer cells and proved to be significant for monitoring the change of H_2O_2 generated by the cell along with the change of the environment cellular.

4 CONCLUSION

Biosensors are devices capable of sensing a variety of compounds in different samples, which makes them useful for a wide range of applications, such as the prevention and treatment of diseases of all natures. In addition to a detection power comparable with the current standard clinical methods, these devices tend to be less expensive and simpler to use. Nanotechnology is an emergent and fundamental approach for biosensing development, as nanomaterials can enhance the efficiency of biosensors due to its known properties of higher surface area, size-dependent optoelectronic characteristics, electrical conductivity, high catalytic activity and biocompatibility. CNTs are unique materials that offer many advantages when serving as biosensor components, the main one being the superior electrical performance promoted by their unique structure. Even with all the evident promising potentials, biosensors still have some challenges before being widely applied to daily life, as the materials used as components, nano-scaled or not, need to be functional and efficient at physiological pH, room and body temperature and stable for longer periods, while maintaining the sensitivity.

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