CHAPTER THREE

Electrochemical Biosensor Based on TiO₂ Nanomaterials for Cancer Diagnostics

Tina Mavrič*, Metka Benčina†, Roghayeh Imani‡, Ita Junkar†, Matjaz Valant§,¶, Veronika Kralj-Iglič‖, Aleš Iglič*

*Laboratory of Biophysics, Faculty of Electrical Engineering, University of Ljubljana, Ljubljana, Slovenia
†Jožef Stefan Institute, Ljubljana, Slovenia
‡Structural Chemistry, Ångström Laboratory, Uppsala University, Uppsala, Sweden
§Materials Research Laboratory, University of Nova Gorica, Nova Gorica, Slovenia
¶Institute of Fundamental and Frontier Sciences, University of Electronic Science and Technology of China, Chengdu, China
‖Laboratory of Clinical Biophysics, Faculty of Health Sciences, University of Ljubljana, Ljubljana, Slovenia
1Corresponding author: e-mail address: metka.bencina@ijs.si

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Abstract

Regardless of the high awareness of the importance of early cancer diagnosis, a high mortality rate is still present due to cancer discovery at a high advancement of the disease, where it has already metastasized. One of the fields where improvement is needed are the current diagnostic methods that cannot discern the cancerous lesions when these are very small. Thus one of the important fields, where the advancement is crucial, is novel diagnostic methods such as biosensors. Here biomaterials are combined with...
inorganic transistors to detect and produce a signal once a binding of an analyte occurs. With the development on the field of nanomaterials and their applications as transducers, cancer detection via biosensors could soon become a reality. Material with several advantages, such as high bioinertness and resistance to corrosion from bodily fluids is TiO₂, which already showed potential in biosensing applications. On the other hand, there is much research focused onto the understanding of the role of extracellular vesicles in cancer progression, therefore the combined effort of having a biomarker for cancer detection and a sophisticated small detection system, such as a (nano)biosensor based on TiO₂ materials, could facilitate the progress in the diagnostics. In this chapter, we offer an overview of the TiO₂-based biosensors, which were applied for human cancer biomarker detection.

1. INTRODUCTION

Cancer has a major impact in today’s society, because the effects are not only physiological, but also psychological, economical, and social [1]. The statistical figures alone are cause for concern—according to estimations there has been 14.1 million cancer cases in 2012 worldwide and this number should only increase to 24 million until 2030. Even though the cancer can be cured, in 2012 there have been over 8 million deaths ascribed to cancer [2]. This makes cancer is one of the leading causes of death, coming second only to cardiovascular diseases [3]. Most common cancers are lung and colon, however the cancers that take the highest toll are gender specific—breast and prostate cancer [2,4]. The increased incidence over the past years is attributed to increased environmental risks, such as exposure to UV irradiation and environmental pollution, and also to certain lifestyle risks, such as obesity, lack of regular exercise, smoking, and alcohol consumption. However, cancer can occur also due to certain medications, occupational exposure, genetic predisposition, or chronic inflammation [4]. An important factor is also age, because the risk of being diagnosed with cancer is different for different periods of a person’s life [5]. Even though some cancers occur specifically with children, most cancers are developed after the age of 50, which can be due to the accumulation of mutations and the decline of native antitumorigenic mechanisms [4]. It is an important aspect, since the population is aging, especially in the developed world [6]. Regardless of age, cancer incidence is expected to increase and prevention is the first choice and necessary step to battle this disease [7]. There is a two-step approach regarding prevention—primary and secondary prevention [6]. In the first stage of prevention, there is the need to increase the awareness of the risk factors and
the importance of changing such conditions (e.g., healthier life, exercise, cease smoking, decrease environmental pollution). The secondary prevention measures are set on early detection of cancer [6]. When prevention fails, early detection is of crucial importance for patient survival, since at its early stage cancer is localized and can be contained. Once the disease is spread, the chances of survival become drastically lower [1]. Several cancer screening processes are established for cancers with high incidence and mortality [8]. These tests have shown a promising effectiveness in early detection as they can tag the already formed precancerous lesion. The screening disadvantage is that it does not take into consideration the heterogeneity of the disease, the tests are cancer specific therefore they do not reveal other possible pathologies in the body [1]. False-positive and false-negative results given by the screening tests are also an important issue that leads to unnecessary investigations, overtreatment, and additional costs [9,10]. Also, in the current typical diagnostic methods are composed of imaging techniques and morphological analysis of suspected cancerous tissue [11]. The histopathological examinations are made only after the symptoms of cancer appear; hence the disease has already advanced. In addition, some of the imaging techniques are not reliable when the cancer is in the early stages and cannot be discerned from healthy surrounding tissue [12,13]. Thus there is still an enormous demand for a highly reliable, general, noninvasive low cost diagnostic tool that would allow pinpointing the prognosis. Also, hand in hand with the relevant progress in the field of understanding the cancer development mechanisms, the need for point-of-care diagnostic methods arise, which would cater the individual, their needs, lifestyle, and specific exposure to risk factors. In this regard, nanotechnology has been accepted as the tool of the future not only in cancer diagnostics but also in targeted drug delivery [14]. The advances in nanotechnology have enabled the development of biosensors, devices that are able to detect and directly assess the abnormalities in physiological fluids, such as blood, saliva, urine, and serum. These composite devices can detect the so-called biomarkers (or cells or even mutated DNA) that are (over)expressed when a tumorous or pretumorous tissue is developing [15]. Nanoparticles can have one of the essential roles in biosensors, since they can significantly improve the sensitivity by enhancing the conductivity of the system, amplify the signal, and offer more active site for biological material to attach due to its favorable surface to volume ratio [15,16]. From the vast array of available nanoparticles, TiO$_2$ is a very desirable choice due to its nontoxicity, biocompatibility, relatively good conductivity, and low cost. It can also be grown in several different morphologies
(nanoparticles, nanospheres, nanorods, nanotubes, nanobelts, etc.), which can be exploited for specific needs for specific biosensor. In this chapter, we present some basic aspects of biosensors, the application of nano-TiO$_2$ in biomedicine and an overview of TiO$_2$-based biosensors that can potentially be used for early cancer detection.

2. CANCER

2.1 Pathology

Understanding the outset, mechanisms, and progress of cancer is the foundation of prevention and more efficient treatment of the disease [17]. Cells, as tissue building blocks, are programmed to carry out certain tasks while maintaining an internal and intracellular homeostasis, which is vital for the normal cell functioning [18]. Disruptions that the cell is facing during its lifetime are detected through a series of molecular events inside the cell that trigger an appropriate response through adaptation of the cell’s metabolism [19]. Cells can adapt through modifications that promote life (e.g., atrophy, hypertrophy, hyperplasia, and metaplasia) or they can instigate a programmed cell death when repair is not possible [20,21]. Although cell survival and proliferation are a finely tuned mechanisms with several check points for mistake and aberration detection, which allow the elimination of highly damaged cells via apoptosis, it can still fail. This can result in the disruption of tissue homeostasis, namely the tissue hosts damaged cells that differ in their appearance and function in comparison to the original cell type [17,22]. The abnormal cells in the tissue can lead to several diseases, including cancer [23]. Evolution of tumorous tissue therefore begins with a cell, which defied all the built-in security mechanisms for mistake elimination [23]. Furthermore, the successful development of tumors is based on genetic changes on three types of genes: protooncogenes, DNA repair genes, and tumor suppressor genes. Mutations on these genes lead to loss of primary function of the cells and increased, unrestrained proliferation, which is a trait characteristic for tumors [24,25].

Every abnormal group of cells that lack shape, size, and nucleus uniformity of the inherent surrounding tissue is not yet characterized as cancer. Such group of cells can be determined as dysplasia. The tissue can be well discerned from the normal tissue under the microscope, but does not necessarily evolves into cancerous tissue. It can actually regress, if the stressor causing it is eliminated [20]. One such example is the cervical tissue aberrations due to certain HPV serotypes—low grade dysplasia can regress and
disappear [26], while high grade dysplasia can evolve into malignant tumor [27]. The latter is determined as precursor to tumor formation, the cells however do not protrude the membrane of the surrounding tissue [28]. The cell mass resulting in tumor formation is known as neoplasia [17]. Neoplastic tissue can be identified by irregular cell pattern and the loss of cellular differentiation [17]. All tumor tissues are composed of two parts—the proliferating tumor cells (parenchyma) and the surrounding supporting tissue consisting of connective tissue and blood vessels [4,17]. These lesions continue to lead an independent life and can be classified as benign tumors, therefore a localized formation of abnormal cells, or malignant tumors, characterized by aggressive infiltration behavior toward the surrounding healthy tissue and the metastasis ability (transfer of tumor cells to other sites) [4]. Benign tumors actually have well-differentiated cells, meaning the cells are very similar to the ones of the surrounding normal tissue. Since they are not prone to spreading, they are easier to remove with surgery and rarely reoccur. In contrast, malignant lesions can metastasize to distant sites where they begin to newly invade healthy tissue and thus present a great challenge to control [28]. Malignant tumors are generally divided into two groups, carcinomas, derived from epithelial cells, and sarcomas that originate from mesodermal layer [4].

A very important aspect of malignant tumors is their ability to metastasize. Metastases are ascribed about 90% of deaths related to cancer [29]. The route through which cancer spreads throughout the body is via the lymphatic system to the neighboring lymph nodes, and through the bloodstream [29], the cells can also seed through the surrounding tissue of the cancer [4]. One way of the short distance cell communication with surrounding tissue is by way of forming nanotubes toward the surrounding cells, an example of such mechanism is presented in Fig. 1 [30]. The diameter of these cell-originating nanotubes is less than 100 nm, the length can however range up to 20 μm [31]. This mechanism enables material and information to be efficiently supplied to the surrounding cells, even if this means the transfer of infection.

Long distance information transport is possible through the bloodstream and can deliver cells and genetic material to distant organs. In this regard, not only metastases can travel this distance, but also corrupt cell constituents that are incorporated in microvesicles. These entities are one of the three main classes of extracellular vesicles (EVs) and are present in all bodily fluids regardless of the presence of the disease [30]. In Fig. 2, EVs isolated from blood and pleural fluid can be observed. They form through the budding
of the membrane of the parent cell and are pinched off through chemical stimulus or mechanical influence [30,33]. The budding is well represented in Fig. 3A and B, where the difference between healthy red blood cells and the same cells exhibiting extensive budding found present in a blood sample [32]. Fig. 3C shows multiple EVs budding from an urothelial cancerous cell.

Fig. 1 Communication of neighboring cells through nanotubes, formed by elongation of membrane buds. Adapted with permission from V. Kralj-Iglič, Membrane microvesiculation and its suppression, in: A. Iglič, C.V. Kulkarni, M. Rappolt (Eds.), Advances in Planar Lipid Bilayers and Liposomes, vol. 22, Academic Press, 2015, pp. 177–204. Copyright (2012) Elsevier.

Fig. 2 Extracellular vesicles isolated from different body fluids. Specifically, in (A) there are extracellular vesicles isolated from peripheral blood of a healthy human donor (microvesicles are indicated with arrowheads, residual erythrocytes are indicated with an arrow) [32], and in (B) extracellular vesicles isolated from pleural fluid of a lung cancer patient can be observed [35]. Panel A: Reprinted with permission of Dove Press. Panel B: Reprinted with permission from American Chemical Society.
Owing to the process of their creation, EVs are carriers of the mother cell information and can contain, e.g., proteins, lipids, metabolites, and nucleic acids, mRNA, microRNA or DNA [30,35]. The function of EVs can range from communication between cells to excretion of unwanted materials from within the cell [36]. One of the downsides of this otherwise healthy and welcome mechanism for sustaining overall system communication is the long distance transfer of malignant cells or other aberrant information in order to seed the disease in different organs in the body [35]. It has been observed that there is an increased release of EVs with certain cancer types. In their review, Ogorevc et al. [34] have concluded that cancer cells are especially inclined to microvesiculation, not only via the aberrated proteins, lipids, and nucleic acids, but also through mutant growth factor receptors and should be considered as an important mechanism regarding understanding the cancer advancement and spreading. On the other hand, it needs to be emphasized that EVs can be applied also to other diseases and conditions besides cancer [34].

The transfer process of cancerous cells and the following deposition into the secondary organ is very fast, within 24 h, the formation of metastases occurs within weeks. However, the cells do not necessarily proliferate and form metastases, they can stay dormant in the secondary organ and only regain growth when appropriate stimuli are present—weeks to years later [29].

2.2 Cancer Diagnostic: Current Issues

It cannot be stressed enough how either discovery of precancerous lesions or the early diagnosis, especially of aggressive cancers, does affect the survival...
rate of cancer patients. Regrettably, it happens too often that an individual approaches a physician only when experiencing a health problem or when first symptoms of the disease start expressing. According to the symptoms, the patient is then subjected to different types of analysis that usually include a combination of several diagnostic procedures, from laboratory testing, imaging techniques endoscopic, and biopsy examinations. The diagnostics heavily relies on imaging techniques, such as CT and PET scans, X-ray, MRI, and ultrasound, to confirm the formation of an abnormal tissue. These techniques are very proficient, but are not able to discern cancer at its very early stages [11,13]. As for biopsy examination, these procedures can be painful and invasive, and can even lead to tumor metastasis [16].

One of the important early detection programs is based on cancer screening tests and is commonly employed for people who feel or appear healthy (average risk individuals) [37]. There are limited screening programs that are associated with decreasing the cancer death toll [38]. For example, cervical precancer abnormalities can be efficiently detected through Pap test or smear. Colon precancer lesions can be identified through fecal occult blood detection. Mammography is used for early detection of breast cancer [13]. Lung and prostate cancer have received attention for regular screening test, where for the lung cancer there are indications that regular screening tests could prevent cancer-related deaths, there is no such confirmation for prostate screening test (with PSA) [39]. Overall, although they have an important impact on prevention, they are accompanied with several downsides. The tests target specific cancers, though the disease is very heterogeneous and thus the screening tests can mistakenly raise alarm over the dormant or slow growing cancer and disregard the aggressive ones [40]. They lead to overtreatment that is the consequence of overdiagnosis or false-positive results [41]. Screening tests are basically only preliminary findings, which subject the patient to further diagnostic procedures [42], some of them can potentially be of harm due to radiation (e.g., CT, PET) [43,44]. The test can also give false reassurance and decrease in self-care due to a false-negative result [45]. There are also still several cancer pathologies for which there are no reliable routine screening tests, e.g., for leukemia, kidney, liver, pancreatic, thyroid, and urinary bladder [38]. Since some of these cancers are asymptomatic or project nonspecific symptoms, cancer can metastasize freely [46].

Since the screening tests are often associated with abundant costs, not only for medical examinations, but also of the whole surrounding support apparatus (database, invitations, tests, diagnosis follow-up, etc.), they are reserved for target groups with higher risk for cancer. There is another
cost-related aspect correlated to cancer problematics. Some of the established diagnostic methods are considered expensive already in the wealthy or high-income countries, which makes them practically unattainable in the low-income countries [7]. In such regions, the cancer incidence presents over half of the world’s cases [47], therefore a large amount of cancer burden could be averted if low cost and easy accessible early diagnostic method would be available. We already mentioned EVs and their role in cancer diffusion. Since the knowledge of their presence in bodily fluids and their specificity regarding their origin, EVs are the rising new potential for the much needed novel, efficient, fast, and minimally invasive diagnostic tool [35].

Of great assistance to cancer diagnostics are several already established assay techniques that use antibodies for determination of substances, such as enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), electrochemiluminescence (ECL), surface plasmon resonance (SPR) immunoassays, and fluorescence in situ hybridization. Although these techniques are accomplished and have high accuracy, they also have some drawbacks. They are expensive, sophisticated instruments that require time and trained personnel [15,48].

3. TOWARD NOVEL METHODS OF CANCER DIAGNOSIS

Radical resection of the tumor at its early stage is the only way to cure cancer. However, currently many cancers are diagnosed only after they have metastasized throughout the body. For those patients, chemotherapy and radiotherapy are needed [49].

Since chemotherapy drugs target all rapid dividing cells, including healthy cells, they can cause great damage to the patient’s body. Therefore, developing new methods to detect cancers at their early stage and directly target cancerous cells without affecting normal ones are of vital importance [50,51]. However, most cancers are asymptomatic during their early stage. Also, conventional clinical cancer imaging techniques, such as X-ray, CT, and MRI, do not possess sufficient spatial resolution for early detection of the disease. Since distinct morphologic changes are absent in most early neoplastic disorders, they can easily avoid being detected by these imaging techniques [11,13]. The analysis of biomarkers in blood, urine, and other body fluids is one of the methods applied in the detection of the cancer in early stage, and cancer biomarkers are extensively used in oncology for cancer diagnosis and prognosis [52].
Biomarkers are biological indicators of a state within the body, which can be objectively measured [53]. These entities have specific functions, expressions, or activities that can deviate from their expected modus operandi due to pathological changes in the body [54]. Biomarkers of cancer include proteins overexpressed in blood and serum or at the surface of cancer cells that facilitate diagnosis. Cancer biomarkers are present at a very low concentration in first stages of cancer and are difficult to detect [55]. More recent techniques use immunoassays to detect cancer biomarkers, and these are carried out in hospital laboratories. However, in most cases, only one biomarker is used as an indicator of the disease. Immunoassay tests are not sufficiently sensitive for the detection of low-level marker concentrations, which exist in the early stages of the disease. In addition, immunoassays can be time-consuming and expensive [56]. Therefore, it is necessary to develop devices which can detect such low concentrations of biomarkers. Furthermore, it would be beneficial to be able to detect and target cancer cells in blood and human tissues. Biosensor could be a suitable choice for this problem.

EVs are ideal tumor biomarkers as they offer a low-invasive diagnostic method [30], providing a leap in the supporting technology—biosensors. Moreover, the charm of using EVs lies in its application; they offer various diagnostic methods as well as use in drug delivery systems. Thus EVs can be used for detecting abnormalities in a body and could offer the targeted drug delivery [35]. It is important to thoroughly understand the mechanism of vesiculation because this process can be suppressed, therefore, e.g., cancer spreading could be contained or even prevented [30].

### 3.1 Biosensor

Once we get to know the biomarkers for specific condition or disease, the next step is to construct a device that would be able to detect these species from biofluids—a biosensor. This device is therefore a constructed microsystem composed of the bioreceptor connected to a transducer [57,58]. The bioreceptor allows the attachment of analytes onto the biosensor. Perfézou et al. [59] proposed that this analyte would not only present a cancer biomarker for disease detection, but would also offer a prediction at what stage the disease is. The analyte can be a degenerated protein or a surface-based overexpressed receptor of the cancer cell [59]. This analyte is bound to the bioreceptor through specific catalytic or affinity interactions [57]. For the bioreceptor role, several biological components were adopted—antibodies, nucleic acids, membranes, and enzymes [60].
the analyte becomes attached onto the biosensor, a signal is produced and intercepted and converted by the transducer part of the biosensor [56,59]. Depending on their nature, transistors can be electrochemical, optical, thermometric, piezoelectric, magnetic, or micromechanical [59]. One of the important biosensor advantages is the efficiency in discerning the designated analyte or a group of analytes at very low concentrations without the need of pretreatment (e.g., labeling) [61]. These advantages combined with the knowledge that electrochemical devices usually allow simple data collection and interpretation steadfast, make biosensors very attractive [61].

In Fig. 4, a schematic diagram of a biosensor is presented—it is visible that a biosensor is a composed system. Besides the recognition element (bioreceptor) and the signal transducer, biosensor also needs a signal processor “that relays and displays the results” [62].

### 3.1.1 Molecular Recognition Element in Biosensor

Bioreceptor is an indispensable part of the biosensor, since it is the component that enables the binding of the target analytes. For the binding to occur, there has to be an attraction between both species, thus the recognition elements on the biosensor need to be considered carefully. Mostly, proteins,
antigens, antibodies, enzymes, and nucleic acids are applied as recognition elements [62]. First, they were isolated from natural biological systems, nowadays however synthetized recognition elements offer better stability and reproducibility [62].

3.1.2 Transducers in Biosensor
A transducer is the device that converts recognition signal events into electrical (often digital) signals—and can be electrochemical (amperometry, potentiometry, conductimetry/impedimetry), optical (colorimetric, fluorescence, luminescence, interferometry), calorimetric (thermistor), mass change (piezoelectric/acoustic wave), or magnetic in nature [63]. Electrochemical transducers are the most widely used in sensor technology.

3.2 Electrochemical Biosensor and Electrochemical Detection Techniques
Electrochemical biosensors are characterized by the electrochemical transducer (e.g., electrode), which transforms the signal to allow for quantitative or semiquantitative analytical information [64,65]. These biosensors have been defined as portable, simple, easy to use, cost effective and disposable, all this are features that make them ideal for point-of-care devices [66]. Development in the field of electrochemical devices has allowed the reduction of size of the accompanying detection infrastructure that needs to be used together with biosensors in order to make a reliable analysis, hence the biosensor-based analysis can be performed either at the doctor’s practice or even at home [67]. The most known example of an electrochemical biosensor is the glucose biosensor “based on a screen-printed amperometric disposable electrode” [69].

Labib et al. [65] have stated that voltammetric/amperometric, impedimetric, conductometric, potentiometric, and field-effect transistor-based biosensors are main techniques used for the cancer marker detection. Below we offer a short description of the principles on which methods are based. Some parallels that apply to all this methods can be drawn. Electrochemical sensing is made possible through a three-electrode system. Besides the working electrode, which is the workhorse of the biosensor applied as transduction element for the biochemical reaction, counter electrode, and sometimes even reference electrodes (usually Ag/AgCl), are also required [68]. The obtained output signal (e.g., peak, plateau) usually corresponds to the concentration or the amount of detected interactions with the bioreceptor [69].
3.2.1 Voltammetric/Amperometric Biosensors
Voltammetric and amperometric biosensors are based on current measurement by either ramping the potential at a given rate on the working electrode (with respect to the reference electrode) or keeping the potential constant, respectively, for each method, while monitoring the response of the system \([65]\). The main instigator of the detected current are the oxidation or reduction reactions unfolding between the sample and the bio-receptor on the working electrode; the success of the reactions is conditioned by the ability of the transport of analyte molecules toward the surface of the electrode \([65,69]\). Since the development of biosensors was based primarily on the amperometric transducers, amperometric biosensors are most employed devices on the field \([70]\). They can be based on direct measurement of analytes and products of reactions near the working electrode, they can also use alternative oxidizing agents (e.g., ferricyanide, ferrocene) or they function due to direct transfer of electrons between the analytes and the electrode \([70]\). With this method there are different parameters, which can be chosen to employ and be manipulated: potential \((E)\), current \((I)\), charge \((Q)\), and time \((t)\); depending on the combination of the utilized parameters and alteration of different conditions we have a number of different techniques from cyclic voltammetry, linear sweep voltammetry to different stripping voltammetries, an overview of which is offered from Labib et al. \([65]\).

3.2.2 Impedimetric Biosensors
Electrochemical impedance spectroscopy (EIS) can determine both the resistive and capacitive (dielectric) properties of materials \([65,71]\). All such a system requires for obtaining a signal is the excitation or perturbation of the chosen measured environment by a small amplitude sinusoidal alternative current (AC) excitation signal \(\sim 2–10 \text{ mV}\) \([65]\). The impedance spectrum is obtained by changing the frequency over a wide range AC \([59,71]\). However, this also is a drawback of the method, since obtaining a full impedance spectrum is time consuming \([71]\).

3.2.3 Conductometric and Capacitive Biosensors
Conductometric biosensors detect the change in conductive properties of the sample when there is a reaction in progress \([68,72]\). Several authors emphasize the association of conductometric biosensors with enzymes due to the ionic change in the medium, which instigates the conductivity \([68,72,73]\). During a measurement, several interferences are avoided because
this technique does not need a reference electrode and can thus apply a differential mode of measurement [73].

**3.2.4 Potentiometric Biosensors**

Potentiometric biosensors are based on the potential difference between indicator and a reference electrode, or two reference electrodes, as a response of certain reactions taking place in the medium at (nearly) zero current [64,68,74]. Transducer in the potentiometric biosensor is sensitive to the solution-based ions [75]. For the purpose of generating ions, enzymes are predominantly used as bioreceptors [64]. The measured signal is, through logarithmic function, correlated to the analyte concentration [75].

**3.2.5 Field Effect Transistor (FET)-Based Biosensors**

FET-based biosensors are used for detection of potential change in the analyte solution by using a three-electrode system [76]. A semiconductor and an insulator material are employed, the former for creating a channel between source and drain electrode, while the latter provides an effective barrier for separation of the gate electrode from the channel one [76]. The semiconductor presents a channel for the charge carriers transition, thus when there is an outside event that causes the electric field of the environment to change, this is reflected in the charge carrier conductivity [77]. FET-based biosensors offer high sensitivity and fast response, but are difficult to manufacture in small sizes, are difficult to operate for nonprofessionals, and are not cost effective [78].

Overall, voltammetry-based techniques are on the forefront of use, since they are most adaptable and produce clear results [65]. Development of electrochemical devices was evolved from DNA hybridization research [79]. Nowadays, the detection of cancer biomarkers is often conducted using electrochemical biosensors that screen for gene mutation and protein biomarkers [80]. However, much of the technology is still at the research stage. Amperometric and potentiometric transducers have also been employed very often, additionally, with the desire for new approaches, label-free impedance-based transducers are also on the rise [81].

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**4. NANOSIZED MATERIALS**

A nanometer is one billionth of a meter—$10^{-9}$ m. For insight, a strand of hair is about 80,000–100,000 nm wide, hepatitis B virus cores are 32–36 nm in diameter [82], and spherical hemoglobin molecule is 5.2 nm [83].
According to the definition adopted by the International Organization for Standardization (ISO), nanomaterial is: “Material with any external dimension in the nanoscale or having internal structure in the nanoscale,” where nanoscale is defined as “Size range from approximately 1 nm to 100 nm” [84]. Nanosized materials exhibit different chemical and physical properties than bulk materials of similar masses due to (i) their increased surface to volume ratio and (ii) quantum confinement effect (QCE). Nanomaterials have more exposed atoms at the surface than bulk materials, thus have large surface energy, which significantly changes nanomaterials’ physiochemical properties, such as boiling point, vapor pressure, solubility, and reactivity. Contrary, the QCE defines electronic structure and absorption spectra of the semiconductor materials and is exhibited at small crystallite size, when the size of the material is comparable to the electron wavelength. Unique properties of nanomaterials have been well used by nature, for example water striders nanostructured legs poses water repellency mechanism [85], extraordinary colors of butterfly wings are due to interaction of light with nanostructured surface [86], and intermolecular van der Waals forces between nanofibers on Gecko feet and other materials are responsible for superior adhesion and for the self-cleaning ability [87]. Natural nanomaterials produced by biomineralization process have also various functions for the organisms, for instance magnetotactic bacteria use magnetite nanoparticles for navigation [88], while others for respiration facilitation [89] and tissue hardening.

Physical, chemical, optical and electronical, thermal and mechanical properties that are size, shape, and composition dependent are great advantage of synthetic inorganic nanomaterials. Besides, the size of nanomaterials is similar to that of most molecules and structures in human biological system (e.g., the diameter of DNA is around 2 nm) and a majority of biological processes occur at the nanoscale. Therefore, biomedicine has been taking advantage of the nanotechnology and miniaturization of the materials to nanolevel, which made even more possible for biomimetics to imitate natural materials/structures and processes. Very important aspect of nanotechnology is miniaturization of biomedical devices, e.g., biosensors for early stage detection of diseases, target drugs for specific attack of disease cells on site and nanorobots. More than 247 nanomedicine products have already been approved by the Food and Drug Administration (FDA) and are currently in various stages of clinical study [90].

In the following sections, we will discuss biomedical applications of TiO$_2$ nanomaterials and characteristics they have to fulfill as biosensors.
4.1 TiO₂ Nanomaterials in Biomedicine

Fabrication of novel nanomaterials and their biomedical applications present an extremely fast growing field in science. Among all novel biomedical materials, Titanium (Ti), and its alloys exhibit a unique combination of strength and biocompatibility, which makes them the most used metal compounds in medical applications [82,83,91]. Ti biocompatibility arises mainly due to the formation of a stable and inert oxide layer on its surface which has, in comparison with pure Ti, much larger specific surface area [85]. The latter is important since interactions with biomaterials are actually the interactions between the surface of biomaterial and biological environment.

Among various TiO₂ nanomorphologies, TiO₂ nanotubes (NTs) attracted significant scientific interest for biomedical applications. TiO₂ nanotubes can be synthesized by atomic layer deposition [92], hydrothermal method [93], and electrochemical anodization of Ti foil in an organic electrolyte [94]. The formation mechanism of self-ordered arrays of TiO₂ nanopores/nanotubes by electrochemical anodization method is shown in Fig. 5. The length, diameter, nanopore/nanotube formation, and open top morphology of TiO₂ nanotubes can be controlled by varying synthesis

![Fig. 5](image)

Fig. 5 Extension of specific surface area of TiO₂ by TiO₂ nanotubes formation with electrochemical anodization method from Ti foil. (A) Initial TiO₂ compact layer formation, (B) solvatization of TiO₂ layer by F-ions from the electrolyte and propagation of TiO₂ into the Ti foil (C) pore formation, and (D) H₂O dependence of nanopores/nanotubes formation [95]. Reprinted with permission of The Electrochemical Society (ESC).
parameters, e.g., anodization time, applied voltage, and electrolyte composition [94], as shown in Fig. 6.

TiO$_2$ nanomaterials are widely studied for utilization in biomedical applications. Graphene oxide (GO)/TiO$_2$ thin films were utilized as nanocomposite photocatalysts for degradation of $E.~coli$ bacteria in an aqueous solution under solar light irradiation [96]. TiO$_2$ nanoparticles loaded with daunorubicin, first-line treatment for hematological malignancies and solid tumors, through the formation of daunorubicin/TiO$_2$ nanocomposites were studied for smart pH-responsive drug delivery system [97]. Owing to their morphological benefits, TiO$_2$ nanotubes attracted considerable attention for such drug delivery systems [98–100]. The drug eluting mechanism of TiO$_2$ could be exploited also for coronary stents [101,102] and orthopedic implants [103]. Surfaces with various TiO$_2$ morphologies such as nanopores [102–104] and nanocrystals [105], nanosheets [106], sol–gel matrices [107], but mostly nanotubes (NTs) [108–110] were studied for biosensors applications. These nanostructures present promising interface to assemble different kinds of proteins, e.g., antibody, enzyme. Such biosensors can qualitatively and quantitatively analyze biological

![Fig. 6 SEM images of TiO$_2$ NTs synthesized by electrochemical anodization method—effect of anodization parameters on pore diameter and length of nanotubes: (A) HF-based electrolyte as a source of F-ions, anodization voltage = 58 V, anodization time = 2.5 h and (B) NH$_4$F-based electrolyte, anodization voltage = 58 V, anodization time = 17 h.](image-url)
molecules, such as tumor makers [111,112] and glucose [113]. The advantages of nanotubes before other morphologies for biosensor construction are a high surface area, favorable transport pathways, and very good adhesion to the substrate [114].

4.2 Important Characteristics of TiO$_2$ Nanomaterials for Biosensor Applications

To improve sensitivity of TiO$_2$ biosensors the following aspects have to be considered: (i) adsorption behavior of biological materials and (ii) electron transfer rate. Important material properties that affect binding of the biologic molecules on the TiO$_2$ surfaces are specific surface area/morphology and wettability. To induce proper surface physiochemical conditions for binding biological material, surface functionalization is often required. Recently, we demonstrated that that oxygen plasma treatment of TiO$_2$ nanotube surfaces significantly influences the adhesion and morphology of osteoblast-like cells in comparison to untreated nanostructured surfaces [102]. Interaction of TiO$_2$ with biological material is although influenced also by other factors. For instance, the adsorption of horseradish peroxidase (HRP) and thionine (Th) on NTs was found to be calcination temperature dependent [114]. It is believed that the reason for better adhesion of molecules on calcined samples is the presence of Ti$^{3+}$ states, which are formatted under such high-temperature condition. The methods and mechanisms of Ti$^{3+}$ formation will be discussed in the next sections.

4.2.1 Specific Surface Area

Very important parameter of nanomaterials that can be especially utilized in biomedicine is the surface area to volume ratio, which is far larger at nanoscale in comparison with bulk materials; since surface area of the material increases, a greater amount of the material can come into contact with surrounding environment, thus affecting reactivity. Generally, surface roughness and surface area increase with decreasing the size of the material [91,115].

For instance, it was reported by Smith et al. [116] that TiO$_2$ NTs with 70–90 nm in diameter increase adsorption of blood plasma proteins and adhesion of platelets and their activation in comparison with Ti. Similarly, Kulkarni et al. [117] synthesized TiO$_2$ nanostructures with nanopore (NP) and nanotube (NT) surfaces with electrochemical anodization and showed that NTs with higher length have a higher total surface area, and therefore protein adhesion is higher. Small diameter NT can bind more small-sized
positively charged proteins per surface area, e.g., histone (Fig. 7). Theoretical modeling [91,115,118–121] showed that this can be due to the higher density of sharp edges of the small diameter TiO$_2$ NTs that lead to an increased magnitude of the negative surface charge density at the wall edge and thus to more histone adhesion. This was confirmed [118] by comparing different nanostructures with similar diameter, i.e., NPs and NTs, and although they possess similar surface area, results showed higher histone protein binding to NTs with 100 nm diameter (Fig. 7).

In another study, we have also shown that the adherence of oral streptococci to TiO$_2$ NTs surface can be modified by variation of the surface topology. We have tested the adhesion of two oral bacterial species *Streptococcus sanguinis* and *Streptococcus mutans* on nanoporous and nanotubular surfaces and shown that the lowest adhesion of these two bacteria species was observed for small diameter TiO$_2$ nanoporous/nanotubular surfaces [99]. This coincides with the highest osteoblast adhesion on small diameter nanotubular/nanoporous surfaces shown in previous works [87].

### 4.2.2 Wettability

It was showed that the surface morphology and roughness at the nanoscale level affect the wettability properties of the materials [122]. Lai et al. [123] demonstrated the difference in water contact angle, which is the measure of
surface wettability properties, between smooth TiO$_2$ film and nanorough TiO$_2$ NTs. In latter, water rapidly spread and wetted the surface due to side penetration of the liquid by capillary forces, which proved superhydrophilic surface properties. On the contrary, the water droplet on smooth TiO$_2$ superhydrophobic surface hardly comes to rest. Moreover, authors demonstrated reversible surface hydrophilicity of NTs by UV irradiation. Kulkarni et al. [122] studied the wetting behavior and kinetics of the contact area of water droplet on macroscopically flat, nanoporous, and nanotubular TiO$_2$ surface topologies under similar evaporation conditions. Their experiments demonstrated that the surface morphology and roughness at the nanoscale level substantially affect the TiO$_2$ surface–water droplet interaction, opposing to similar previous observations on microscale structured surfaces. Ishizaki et al. [124] investigated the effect of the extreme wettability surface on cell adhesive behaviors. Their results showed that the even cells adhered and proliferated on both superhydrophobic and superhydrophilic surfaces, the cells easily adhered and proliferated on the superhydrophilic surface immediately after seeding. Similarly, the protein adsorption on the hydrophilic surface was much greater than those on the hydrophobic surface.

4.3 Interactions TiO$_2$ Nanomaterials: Biological Material

4.3.1 Effect of Gaseous Plasma Surface Treatment

Plasma is considered as one of the four fundamental states of matter, besides solid, liquid, and gas. It is generated by supplying energy to a natural gas; when electrons or photons of sufficient energy collide with feed gas atoms and molecules, the latter dissociate into a high energetic state of matter, consisting of positively and negatively charged ions, free electrons, and other neutral species [125,126]. Plasma can be generated by a number of methods, such as combustion, flames, electrically heated furnaces, electric discharges (corona, spark, glow, arc, microwave discharge, plasma jets, and radio frequency plasma), and shocks (electrically, magnetically, and chemically driven) [127]. Depending on the conditions in which they are created plasma can be classified as a high temperature (thermal) and a cold (nonthermal). Thermal plasma is obtained at high pressure (≥105 Pa) and need substantial power (up to 50 MW) to be observed [128]. It is characterized by high and approximately equal gas and electron temperature, which present almost local thermodynamic equilibrium [128]. Example of natural occurring thermal plasma is the sun [129], otherwise this plasma type can be found in plasma torches and in electric arcs [128]. Cold plasma can be generated by electric discharges at lower pressures and by using less power [128].
The applied energy mostly produces energetic electrons whereas the majority of gas atoms, ions, and molecules remain in a low-energetic state causing a low plasma temperature. In such plasma, the gas temperature is low, but electron temperature is high, thus there is no local thermodynamic equilibrium. Cold plasma can be generated by radiofrequency (RF), dielectric barrier, corona, and gliding arc discharges [128]. For example, RF plasma in a flow of gas is produced by an oscillating electromagnetic field (EM) generated by an inductive or capacitive discharge. In inductive discharge EM field is generated by an induction coil, which surrounds the reactor, whereas capacitive discharge uses separate electrodes to generate EM.

Nonthermal plasmas have been considered very promising for a variety of biomedical applications like wound healing [130], blood coagulation [130,131], cancer treatment [130,132–134], sterilization of skin [135], and sterilization and decontamination of surfaces [136]. Plasma applications include modifications of material surfaces—change of physical and/or chemical surface properties, e.g., to increase surface wettability, optimize adhesion of living cells, and sterilization of materials and biomedical devices.

Although the TiO$_2$ surfaces disclose biocompatibility, the quality of the oxide layer plays an important role in the biological response. Plasma treatment is increasingly used in biomedical applications, since plasma-treated surfaces improved the biological response. By plasma treatment of TiO$_2$ surfaces it is possible to induce alterations in chemical surface composition, which can influence on cell–surface interactions; like improved proliferation of desired cell type [137] or even reduced adhesion of bacteria [138]. Our previous results showed that treatment with oxygen–plasma altered surface properties of TiO$_2$ NTs, which resulted in a removal of surface contaminants and an increase in oxygen content on the surfaces [102]. The results showed potential of such approach in medical applications, where it is necessary to promote adhesion of one cell type over another. By fine tuning, the surface topography and plasma treatment conditions it would be possible to design appropriate surface conditions for improved/reduced growth of different cell types. We also showed that modification of TiO$_2$ nanotube surfaces with oxygen plasma has a beneficial effect on MG-63 cell adhesion and proliferation (Fig. 8).

4.3.2 Effect of Electron Transfer Rate
Enzymes usually lack of electrical conductivity, therefore they are often coupled with conducting metal nanoparticles, e.g., silver [139] and gold [106,139], to enhance electron transfer to the electrode. However, several
TiO$_2$-based metal free biosensors demonstrated direct electron transfer between protein molecules and electrodes [104,140–142]. Sensitivity of TiO$_2$ biosensor is highly influenced by the creation of Ti$^{3+}$ defects, which affect the electronic properties of electrode surface. Ti$^{3+}$ defects are not responsible only for improvement of the electron transfer rate of TiO$_2$ [114], but also for adsorption of enzyme or proteins since they represent active adsorption sites and controlling the hydrophilic property [143]. Zhang et al. [144] found that the enzyme adsorption amount of CO-annealed TiO$_2$ NTs was almost three times higher than that adsorbed on O$_2$-annealed TiO$_2$ NTs due to the creation of Ti$^{3+}$ defects. Ti$^{3+}$ can be generated by reduction of Ti$^{4+}$ either by photoinduction, e.g., UV irradiation [145] or loss of O$_2$ from the TiO$_2$ surface induced by thermal annealing [146] and/or reducing conditions [147], vacuum heating [148], plasma treatment [149], and chemical treatment [150]. Some selected mechanisms of Ti$^{3+}$ formation are presented in the next paragraphs.

UV irradiation cause the formation of electrons and holes in the valence (VB) and conduction (CB) band of TiO$_2$ (Fig. 9). Excited electrons can be trapped and reduce Ti$^{4+}$ cations to Ti$^{3+}$ states, while holes oxidize O$^{2-}$ anions and from O$^-$ trapped states or O$_2$ [152] as follows:

$$\text{TiO}_2 + h\nu \rightarrow e^{-}_{\text{CB}} + h^{+}_{\text{VB}}$$
$$e^{-}_{\text{CB}} + \text{Ti}^{4+} \rightarrow \text{Ti}^{3+} \text{ trapped electron}$$
$$h^{+}_{\text{VB}} + O^{2-} \rightarrow O^{-} \text{ trapped hole}$$
$$4h^{+}_{\text{VB}} + 2O^{2-} \rightarrow O_2$$

Ti$^{4+}$ to Ti$^{3+}$ reduction can be achieved also by thermal treating of TiO$_2$ with reducing gasses that generate electrons or cause removal of lattice
oxygen atoms. Liu et al. [153] proposed formation of Ti$^{3+}$ by H$_2$ treatment in such way: at the temperature below 300°C hydrogen physically interacts with the adsorbed oxygen on the surface of TiO$_2$. Above this temperature, the electrons are transferred from the H atoms to the O atoms in the lattice of TiO$_2$. The formation of H$_2$O through leaving O and H atom causes the generation of oxygen vacancies. Above 450°C the electrons are transferred from oxygen vacancies to Ti$^{4+}$ ions, and then Ti$^{3+}$ ions are formed.

Recently, Bharti et al. [149] reported Ti$^{3+}$ formation by air plasma surface modification of TiO$_2$. The results derived by X-ray photoelectron spectroscopy (XPS) revealed that the peak area of Ti$^{3+}$ increases by 30%, whereas the peak area of Ti$^{4+}$ decreases by 12%. Since the result is quite new, the exact mechanism of Ti$^{3+}$ formation is not known, although authors suggest that Ti$^{4+}$ reacts with the electrons generated either from plasma or from the formation of oxygen vacancies in the surface layer by the air plasma treatment [154].

Contrary to above methods, Zhou et al. [155] prepared blue-colored Ti$^{3+}$ self-doped TiO$_2$ via a simple one-pot solvothermal method, by which they controlled Ti$^{3+}$ doping level. The incorporation of Ti$^{3+}$ into TiO$_2$ matrix was directly derived from the TiCl$_3$ precursor, which is increased the doping concentration of Ti$^{3+}$ ions and oxygen vacancies in TiO$_2$. Similarly, Liu et al. [150] synthesized rice-shaped Ti$^{3+}$ self-doped TiO$_{2-x}$ nanoparticles by mild hydrothermal treatment of TiH$_2$ in H$_2$O$_2$ aqueous solution. The high concentration doping of Ti$^{3+}$ was achieved throughout the bulk and surface of TiO$_2$. They proposed “surface oxide-interface diffusion–redox” reaction mechanism of Ti$^{3+}$ formation. Briefly, the surfaces of TiH$_2$ powders were firstly oxidized by H$_2$O$_2$. A surface layer, that was a mixture of

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**Fig. 9** Light-induced charge separation in anatase TiO$_2$ particles [151]. *Reprinted with permission of PLoS.*
various titanium oxides and oxohydrides, was formed on TiH$_2$ particles. The intermediate products, composed of unreacted TiH$_2$, represent the cores and titanium oxides and oxohydrides represent the shells. Under hydrothermal condition, solid interface diffusion—redox reaction occurred on the TiH$_2$ and titanium oxides/oxohydrides that was considered to be responsible for the formation of Ti$^{3+}$ ions. With the extension of reaction time, the oxidation products, Ti$^{3+}$ self-doped TiO$_{2-x}$, were formed in increasing quantities and the shells grew thicker and peeled off from the TiH$_2$ surface. The TiH$_2$ precursor gradually disappeared and was completely transformed to TiO$_{2-x}$ with a further prolongation of reaction time. As a result, phase-pure rice-shaped Ti$^{3+}$ self-doped TiO$_{2-x}$ nanoparticles were formed.

Ti$^{3+}$ states in TiO$_2$ can be obtained with vacuum-activated process in a relatively low temperature [156,157]. After annealing at 300°C for 3 h, Liu et al. [158] were able to form Ti$^{3+}$ states. They suspect that TiO$_2$ loses the bonding oxygen during vacuum treatment to form O$_2$ and leave an oxygen vacancy in the lattice. Remaining oxygen then reduces the neighboring Ti$^{4+}$ of oxygen vacancy to Ti$^{3+}$ in order to eliminate the charge imbalance. Similarly, Guillemot et al. [159] report increasing Ti$^{3+}$ concentration in TiO$_2$ with increasing annealing temperature.

5. TiO$_2$ BIOSENSORS

5.1 TiO$_2$ Biosensors for Human Cancer Detection

On the basis of the advancement on the field of biomarkers, there has been an extensive research dedicated to the development of biosensors for said biomarkers. TiO$_2$ nanomaterials have been extensively researched on the field, not only for detection of tumor markers, but also for mutated DNA and cell capture. Even though there is a need of a general cancer detection sensor, the research often focuses on specific cancer pathologies for detection of cancer-specific indicators (markers, cells, mutated DNA, etc.). Nevertheless, the efforts achieved are an important step toward efficient early cancer diagnosis without the accompanying downsides of existing diagnostic methods. For the purposes of giving a concise presentation of the latest relevant reports on cancer-related TiO$_2$ biosensors, we have limited our research on the articles reported from year 2010 onward and included the ones which present the results directly applicable to human cancer pathologies. In this spot, we should however mention that the mechanism of
cancer information throughout the body via EVs was first mentioned in Kralj-Iglič et al. [160].

Usually TiO$_2$ nanostructures, as electrochemical platforms, are complemented with other semiconductors, metals, molecules that highlight the underlying potential of titania. As discussed previously, nanomaterials and noble metal nanoparticles have long been recognized as very good agents for immobilization of biomolecules, such as enzymes, antibodies, antigens, DNA, etc. In Fig. 10, we present an integrated example of how the majority of reported biosensors are constructed. We present some of the possible scenarios, where nanoparticles, nanotubes, or thin films are deposited. It has to be taken into account that all the presented situations can be intertwined, to obtain an improved result, here these are separated only for the purpose of schematic representation. The deposition often takes place over a platform/electrode that can be indium tin oxide (ITO), glass carbon electrode (GCE), graphene, carbon cloth, or Ti foil. The electrode is afterward often equipped with an agent that promotes electron transfer, better connection, or immobilization (gold nanoparticles (NP) or quantum dots (QDs), cysteine,

**Fig. 10** Schematic representation of different approaches of biosensor construction.
ionic liquid) of the biological molecular recognizer, which is the last component deposited on the electrode for the detection and immobilization of the cancer-specific indicator from the environment. An agent that blocks the nonspecific sites (e.g., bovine serum albumin—BSA) can also be deposited.

In Table 1, we gathered the current research regarding designated cancer pathology, investigated indicator, biosensor type, limit of detection (LOD), and the synthesis procedure used for TiO\textsubscript{2} part of the sensor.

Fan et al. [161] have reported a complex TiO\textsubscript{2}–NTs/CdS:Mn/CdTe sensor. Titania nanotubes were used as the base. The sensor was applicable for detection of MMP-2, the overexpression of which is associated with different cancers and is also the basis for the established biomarker-based expression techniques (ELISA, RIA, and IHC). Owing to synergistic effects of the components of this TiO\textsubscript{2}-based system, it is described to have pronounced electron transfer and effective inhibition of charge recombination, attributed to the cascade effect of the charge carriers through the system. In addition, they have applied SiO\textsubscript{2} coated with MMP-2 antibodies for signal amplification. Thus the system exhibited a very low detection limit of 3.6 fg/mL for MMP-2 detection. Also for use of detection of several malignancies, Li et al. [48] structured a photoelectrochemical immunosensor, where they deposited commercial TiO\textsubscript{2} (P25) followed by anti-AFP antibody deposition. The state of the art of their sensor however was the signal amplification bioconjugate, which they constructed from CdTe QDs, modified with AFP and glucose oxidase (GOD) (GO\textsubscript{x}). The reported LOD was 0.13 pg/mL. A SPR biosensor was used for detection of the immunoglobulin G (IgG), associated with secretion from tumor cells to promote their existence. A layered sensor was constructed over a prism through which light was able to penetrate. Over a sol–gel titania membrane the authors deposited Au nanoparticles, which they were able to obtain about eight times lower detection than that obtained without AuNP. As the receptor rabbit antihuman IgG was imbedded as molecular recognizer. The sol gel represented a waterish environment for biomolecules, therefore it could retain its biological activity for a long term and enhance the sensitivity of biosensor [162]. Another important biomarker that attracts attention for early diagnosis of several cancers (breast, lung, and colorectal) is vascular endothelial growth factor (VEGF). Zhao et al. [163] took advantage of a p–n heterojunction comprised of p-type BiOI nanoflakes (NFs) array and n-type TiO\textsubscript{2} nanotubes (NTs) array for constructing a photoelectrochemical sandwich immunoassay for detection of VEGF. BiOI NFs have been reported to function as the large surface area light harvesting agent and
<table>
<thead>
<tr>
<th>Sensor Name</th>
<th>Type of Sensor</th>
<th>Analyte/Pathology</th>
<th>LOD</th>
<th>TiO₂ Component Synthesis</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TiO₂ nanotubes</td>
<td>Photoelectrochemical</td>
<td>MMP-2 antibodies</td>
<td>3.6 fg/mL</td>
<td>Electrode—obtained via anodic oxidation</td>
<td>[161]</td>
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<tr>
<td>TiO₂-NTs/CdS:Mn/CdTe</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITO/TiO₂/antibody</td>
<td>Photoelectrochemical immunosensor</td>
<td>Chitosan, anti-AFP antibody</td>
<td>0.13 pg/mL</td>
<td>Commercial TiO₂ (P25)</td>
<td>[48]</td>
</tr>
<tr>
<td>Layered sensor consisting of titania membrane, Au nanoparticles</td>
<td>Surface plasmon resonance (SPR) biosensor</td>
<td>Rabbit anti-human IgG</td>
<td>/</td>
<td>Sol–gel, vapor deposition</td>
<td>[162]</td>
</tr>
<tr>
<td>p–n heterojunction BiOI NFs array/TiO₂ NTs array</td>
<td>Sandwich-type photoelectrochemical immunoassay</td>
<td>Chitosan, VEGF antibodies</td>
<td>/</td>
<td>Electrochemical anodization</td>
<td>[163]</td>
</tr>
<tr>
<td>Au–TiO₂ nanoparticles on the NiNPs/nano–Au–modified GC electrode</td>
<td>Electrochemical immunosensor</td>
<td>Anti-CEA</td>
<td>0.06 ng/mL</td>
<td>Commercial TiO₂</td>
<td>[164]</td>
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</table>

Continued
### Table 1  Human Cancer-Related Biosensors Involving TiO$_2$ as the Inorganic Component—cont’d

<table>
<thead>
<tr>
<th>Sensor Name</th>
<th>Type of Sensor</th>
<th>Biological Element (Receptor/ Molecular Recognizer)</th>
<th>Analyte/ Pathology</th>
<th>LOD</th>
<th>TiO$_2$ Component Synthesis</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Au–graphene/CS–Fc + TiO$_2$ nanocomposite multimembrane</td>
<td>Electrochemical immunosensor</td>
<td>Chitosan–ferrocene (CS–Fc), anti-CEA</td>
<td>CEA/ colorectal carcinoma</td>
<td>0.71 ng/mL</td>
<td>Deposition on glass carbon electrode (GCE)</td>
<td>[165]</td>
</tr>
<tr>
<td>Au–TiO$_2$ nanoparticles/GCE</td>
<td>Electrochemical immunosensors</td>
<td>Anti-CEA</td>
<td>CEA</td>
<td>12 pg/mL</td>
<td>Functionalization of nano-TiO$_2$</td>
<td>[166]</td>
</tr>
<tr>
<td>CdSe/TiO$_2$–RGO</td>
<td>Photoelectrochemical biosensor</td>
<td>CEA antibody</td>
<td>CEA</td>
<td>1.38 pg/mL</td>
<td>Ultrasonic and acid treatments, deposition on indium tin oxide (ITO) electrode</td>
<td>[167]</td>
</tr>
<tr>
<td>Thi–TiO$_2$–Gr–Nf composite</td>
<td>Electrochemical immunosensor</td>
<td>Anti-CEA</td>
<td>CEA</td>
<td>0.01 ng/mL</td>
<td>Hydrothermal</td>
<td>[112]</td>
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<tr>
<td>Carboxylated g–C$_3$N$_4$/TiO$_2$ nanosheets</td>
<td>Photoelectrochemical immunosensor</td>
<td>Anti-CEA</td>
<td>CEA</td>
<td>2.1 pg/mL</td>
<td>Surfactant self-assembly method</td>
<td>[168]</td>
</tr>
<tr>
<td>Au–TiO$_2$/CdSe/melamine network</td>
<td>Sandwich-type photoelectrochemical (PEC) immunoassay</td>
<td>Anti-CEA</td>
<td>CEA</td>
<td>5 pg/mL</td>
<td></td>
<td>[169]</td>
</tr>
<tr>
<td>TiO$_2$ nanofibers on silicon wafer</td>
<td>Cell capture assay</td>
<td>Anti-EpCAM antibody</td>
<td>Colorectal, gastric cancer</td>
<td>3–19 CTCs/0.5 mL blood</td>
<td>Electrospun nanofibers-deposited substrate coated with cell capture agent</td>
<td>[170]</td>
</tr>
<tr>
<td>Material/Device</td>
<td>Functionalization</td>
<td>Antibody/Target</td>
<td>Concentration</td>
<td>Detection Method</td>
<td>Reference</td>
<td></td>
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</tr>
<tr>
<td>CdS QDs/TiO₂ NTs electrode</td>
<td>Photoelectrochemical immunosensor</td>
<td>PSA antibody</td>
<td>PSA/prostate cancer</td>
<td>0.5 ng/mL</td>
<td>Electrostatic adsorption method, Ti foil [171]</td>
<td></td>
</tr>
<tr>
<td>TiO₂ nanoparticles coated multiwalled carbon nanotubes (TiO₂/MWCNTs)</td>
<td>Chemiluminescence (CL) immunoassay</td>
<td>PSA antibodies</td>
<td>Prostate-specific antigen (PSA)</td>
<td>0.8 pg/mL</td>
<td>TiO₂/MWCNT sol-gel method [172]</td>
<td></td>
</tr>
<tr>
<td>Nano-3D Au–TiO₂ membranes (MN)</td>
<td>Electrochemical cytosensor</td>
<td>Cysteine, lectine</td>
<td>T47D, MCF7/breast cancer</td>
<td>10 cells/mL</td>
<td>Electrode—via electron beam physical vapor deposition method [173]</td>
<td></td>
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<tr>
<td>DNA biosensor based on (Nb,V) codoped TiO₂ (NVTO) nanoparticle</td>
<td>Electrochemical DNA biosensor</td>
<td>Chitosan, ssDNA</td>
<td>BRCA1/breast cancer</td>
<td>1.09 \times 10^{-16} M</td>
<td>Acid-mediated hydrothermal method [174]</td>
<td></td>
</tr>
<tr>
<td>CdS-capped TiO₂/IL/APTES/Au NP/antibody</td>
<td>Electrochemiluminescence (ECL) immunosensor</td>
<td>HepG2 antibodies</td>
<td>HepG2/Liver cancer</td>
<td>396 cells/mL</td>
<td>Hydrothermal [176]</td>
<td></td>
</tr>
<tr>
<td>Au-capped nano-TiO₂ nanospheres/graphene-Cys-Nf</td>
<td>Sandwich-type electrochemical immunosensor</td>
<td>Cysteine, anti-ProGRP</td>
<td>ProGRP/lung cancer</td>
<td>3.0 pg/mL</td>
<td>APTES-treated nano-TiO₂ added into colloidal gold solution [177]</td>
<td></td>
</tr>
</tbody>
</table>
electron generator; TiO$_2$ NTs array would later ensure their fast transportation. They have labeled this p–n junction as a spontaneous “exciton pump.” Titanium NTs were obtained through electrochemical anodization of Ti foil. Onto them BiOI NFs were deposited. For easier immobilization of VEGF antibodies chitosan–glutaraldehyde (CS–G) was used as a bridge.

Several immunosensors were built for carcinoembryonic antigen (CEA) detection, which is most commonly associated with colorectal cancer, but can also represent an early warning for lung, ovarian, and breast cancer [178,179]. One of the sensors consisted of GCE onto which gold was deposited followed by nickel hexacyanoferrate nanoparticles (NiNPs). This particles exhibited high affinity to nano–Au and had useful redox properties regarding charge compensation, besides having a high surface-to-volume ratio, relevant for species adsorption. Since NiNPs showed poor stability and short lifetime, the authors have assembled onto the existing setup the Au–TiO$_2$ nanoparticles for NiNPs protection. Bioreceptor anti–CEA was immobilized before last. Onto this the BSA was added [164]. Han et al. [165] also reported an immunosensor-based TiO$_2$ film used for CEA detection. It was constructed by subsequent deposition of chitosan–ferrocene and TiO$_2$ onto GCE followed by the deposition of gold nanoparticles–graphene and immobilization of anti–CEA. The sensitivity of the immunosensor has been improved in comparison to ELISA, which was attributed to the synergistic effect of favorable electron conduction promoted by graphene–gold nanoparticle. LOD for this sensor was reported to be 0.71 ng/mL. Another immunosensor for CEA detection was reported by Yang et al. [166], where they reported a GCE-based uniformed assembly of 3D Au–TiO$_2$ nanoparticles. Primary antibodies (anti–CEA) were deposited on nanoparticles, which were reported to have a double task of capturing the target CEA besides a facile pathway for the electron transfer. BSA was also deposited afterward to assure the bloc of attachment of nontarget molecules. The Au–TiO$_2$ had been reported to be successful in retaining the bioactivity of the protein. Amplification of the signal was assured by a system constructed of hollow Pt nanospheres which were functionalized by HRP-labeled antibodies. This contributed to high sensitivity and a low detection limit (12 pg/mL) compared with conventional label methods. Zeng et al. [167] presented a complex sensor for CEA antigen detection. TiO$_2$ and GO were deposited on ITO electrode. Additionally, CdSe QDs were deposited. Horseradish peroxide was used as means of signal amplification. According to the authors, the sensor holds potential for tumor marker detection and for construction of versatile photoelectrochemical
biosensing platforms. Reported LOD was 1.38 pg/mL. In addition, also for colorectal cancer, for CEA detection a sensor based on TiO\textsubscript{2} and graphene was presented by Huang et al. [112]. Hydrothermally prepared and Nafion (Nf)–treated TiO\textsubscript{2}–GO nanoparticles were deposited onto GCE. Cationic dye thionine (Thi) and Au NPs were deposited afterward. Thionine was able to adsorb onto the electrode through electronic exchange with Nafion. Finally, anti–CEA was dispersed on top, followed by BSE. Immunosensor exhibited a wide linear response and a relatively low detection limit of 0.01 ng/mL. Another CEA immunosensor was based on 2D TiO\textsubscript{2} nanosheets and carboxylated graphitic carbon nitride (g-C\textsubscript{3}N\textsubscript{4}) deposited on ITO. The authors have shown that 2D TiO\textsubscript{2} nanosheets produced higher photoelectrochemical signals than TiO\textsubscript{2} nanoparticles. First, carboxylated g-C\textsubscript{3}N\textsubscript{4} was attached to 2D TiO\textsubscript{2} nanosheets, which drastically enhanced the photocurrent measured in these separate materials. The deposition of anti–CEA on TiO\textsubscript{2} leads to the decrease of photocurrents. The detection limit was 2.1 pg/mL [168]. Li et al. [169] used Au–TiO\textsubscript{2} as substrate on ITO. CEA antibody was immobilized directly onto the Au–TiO\textsubscript{2}. After this step, the photoelectric conversion efficiency increased significantly. TGA-stabilized CdSe QD-melamine network were applied as the signal amplificator, detected LOD was 5 pg/mL. Zhang et al. [170] constructed a cell capture assay by electrospinning titania nanofibers, with addition of polyvinylpyrrolidone, onto silicon platform and proceeded to calcine them. Agents for circulating cell capture (anti-EpCAM) were grafted on the fibers. To the enhancement of the cell capture efficiency from the deposited antibodies greatly contributed the rough nanoscale topography from the deposited TiO\textsubscript{2} nanofibers, which increased the adhesion of circulating cancer cells (CTCs). The assay was able to catch from 3 to 19 CTCs per 0.5 mL blood sample of gastric cancer patients.

Zhao et al. [171] applied their manufactured photoelectrochemical immunosensor for PSA detection, an important tumor marker for prostate cancer. The sensor was fabricated from TiO\textsubscript{2} nanotubes, decorated with thioglycolic acid–capped CdS QDs. It was observed that the strong coupling effect between CdS QDs and TiO\textsubscript{2} NTs, and immunogold used as an amplifier of the signal, lead to the detection limit of 0.5 ng/mL. Overall, photoelectrochemical sensors often include CdS or CdSe semiconductors with the intention to achieve the adsorption of light into visible range. Other authors have also employed TiO\textsubscript{2} nanoparticles coated onto multiwalled carbon nanotubes (TiO\textsubscript{2}/MWCNTs) in PSA detection. In this case the TiO\textsubscript{2} was not used as a part of the working electrode establishment, but
applied as a signal amplification tag to label signal antibodies. The electrode (m-PCLI) itself was constructed from chitosan membrane modified paper working zone onto which capture antibodies were covalently immobilized. They reported a sensor with sensitive response to PSA that showed high consistency, with 0.8 pg/mL LOD [172].

Zanghelini et al. [173] have constructed a gold-coated TiO$_2$ butterfly like tridimensional nanomembrane sensor onto which they immobilized lectin and cysteine proteins with high binding efficiency. With the help of the well-known characterized tumor-derived mammary epithelial cell lines they valuated the efficiency and specificity of the sensor. They were able to distinguish between highly invasive and less invasive breast cancer cell lines. They have also achieved significantly low LOD—10 cells/mL for every cell line. Other authors have also employed their sensors for breast cancer detection through BRCA1. An electrochemical DNA biosensor was manufactured where DNA probe was immobilized on a nonporous chitosan-(Nb,V) codoped TiO$_2$ (CHIT-NVTO) deposited on an ITO-coated glass plate. The DNA biosensor displayed very high selectivity for hybridization detection and had a much wider detection range and lower detection limit for the target DNA assay in comparison to other reported DNA biosensors [174]. Also, Ali et al. [175] assembled an electrode for potential use in electrochemical biosensors that consisted of porous hierarchical graphene foam (GF), which they modified with electrospun and physically adsorbed carbon-doped titanium dioxide nanofibers (nTiO$_2$). Onto nTiO$_2$ antibody of ErbB2 was deposited through EDC–NHS chemistry followed by oxygen plasma treatment. Anti-ErbB2 for detection of ErbB2 gene, the excessive signaling of which is an indication of breast cancer. Through this setup they obtained high charge transfer resistance, large surface area, and porous access to the sensing surface by the analyte, resulting in new possibilities for the development of electrochemical immunosensors.

Wang et al. [176] have manufactured a CdS-capped TiO$_2$/IL/APTES/Au–NP/antibody ECL immunosensor based on CdS-capped TiO$_2$ nanoparticles deposited on ITO electrode. Nanoparticles were then covered with ionic liquid, onto which Au NP and HepG2 antibodies were deposited for detection of human liver hepatocellular carcinoma cells. Ionic liquid had a double function of efficient immobilization of CdS–TiO$_2$ on the electrode firmly, and facilitate the electron transfer between the electrode and the solution.

For detection of lung cancer subtypes, Zhou et al. [177] formed a sandwich-type electrochemical immunosensor. The detection was made
through the increased concentration of tumor marker ProGRP (progastrin-releasing peptide). Onto the GCE, they deposited Nafion–cysteine functionalized graphene sheets. Graphene increased the surface area and improved the electronic transmission rate to stabilize a large amount of antibodies on the electrode. TiO$_2$ was also in this case used as the module for signal amplification. Au–TiO$_2$ nanospheres were obtained via APTES-treated nano–TiO$_2$ and codeposition of gold. Onto the Au–TiO$_2$ GOD and ferrocene were attached as secondary antibodies.

### 6. CONCLUSIONS

Cancer is a critical issue for the society, since the incidence of the disease is increasing worldwide. The most successful weapon against it is prevention. However there is still a possibility for its occurrence, because the risk factors are abundant. Second best thing to occur to a cancer afflicted individual is the early discovery of the disease, when therapy is accompanied by high rate of survival. Nowadays the methods for cancer diagnostics are based on well-established techniques that range from laboratory testing, biopsy examinations to imaging techniques. Even though these methods keep increasing in efficiency, they are still associated with certain drawbacks. Together with the incessant developments on the field of nanotechnology, the possibilities of improved cancer diagnostic methods have arisen. One of such techniques, from which early cancer diagnostic would benefit greatly, is biosensors. These complex devices, consisting of coupled physicochemical inorganic transducer with biological material. There are several kinds of biosensors, and all can be used to detect certain analytes in the medium, for example overexpressed cancer biomarkers, such as proteins, in the blood or serum. In this chapter, we offered a brief review of certain electrochemical biosensors of which the main inorganic component was TiO$_2$, a well-known semiconductor, renowned for its biocompatibility, good charge transfer abilities, and cost effectiveness. The biosensors revised in this chapter have been manufactured with the intention of detecting human cancer pathologies. There have been several sensors reported for detection of biomarkers, antigens, or cells for breast, prostate, colorectal, lung, and liver cancers. The limits of detection reported for these biosensors were very low in comparison to the established conventional immunoassays (e.g., ELISA). Most popular analyte used for detection in test trials is CEA, antigen associated with several cancers. The inorganic component of the biosensors was often modified by addition of other semiconductors, metal QDs, or other...
organics, which had the function of better electron transfer through the system and offered better conditions for chosen antibody immobilization. Overall, these biosensors have a very good potential to become the new early cancer diagnostic method in practical everyday applications.

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Electrochemical Biosensor Based on TiO$_2$ Nanomaterials


Electrochemical Biosensor Based on TiO₂ Nanomaterials


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FURTHER READING
