Integrated Biosensors for Personalized Medicine

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ABSTRACT

Biosensors are heterogenous devices, incorporating biological structures combined with electronics, optical or other readout systems. They have been developed for detecting different biomolecules and/or pathogens and represent a key technology for advanced and point-of-care diagnostics as well as patient monitoring. In this paper we present a systematic classification of biosensors described in literature, particularly focusing on nanotechnology-based sensing. Then, we present our approach to develop electrochemical biosensors for measuring metabolites and anticancer drugs, based on a platform for multiple target detection. This platform is modular and achieves a clear separation between the chemical and the electrical components, thus easing design and manufacturing. It shows superior performance thanks to the excellent properties of electron transfer and selectivity showed by enzymes immobilized on carbon nanotubes.

Categories and Subject Descriptors

J.3 [**Life and Medical Sciences**]: Health

General Terms

Measurement

Keywords

biosensors, personalized medicine, point-of-care, integration

1. INTRODUCTION

The integration of biosensors with electrical data acquisition chains and information systems opens new opportunities for health management. In particular integrated biosensors are key elements for *advanced diagnostics*, including portable and disposable devices, and for *monitoring* metabolites and/or drug concentrations, thus enabling (possibly remote) treatment of chronic patients. Drug monitoring in human fluids is important to increase the effectiveness of therapies, and specifically in the case of personalized treatment. Indeed, standard drug therapies are based on randomized clinical trials, and treatments are chosen according to the best mean efficacy, with improvements in the 20 to 50% patients, while the rest may not completely benefit from the assigned treatments [10]. For all these reasons, the development of an integrated platform to monitor the drug metabolism and the concentration of endogenous compounds in physiological fluids is highly requested. Optimized treatments and follow-up therapies can be easily tuned by using point-of-care devices, which represent a potent and innovative tool for personalized medicine.

Biosensors have been developed for diverse biomolecule and pathogen detection. Disposable electrodes are by far one of the most popular strategy coupled with electronics to develop point-of-care devices. However, system miniaturization becomes highly important and conventional approaches, like disposable electrodes, are a bottleneck for decreasing the size of the system. A potent approach to address this limitation is the integration of the biological layer with the electronic portion of the system. A benefit of integration is better performance with respect to signal-to-noise ratio, especially favorable when dealing with biological signals that are typically weak and noisy. High-density arrays of biosensors and multiple detection can be achieved by reducing the sensor area with microfabrication techniques. Finally, system miniaturization increases also sensor response and requires small samples.

System integration is a key issue for self contained biosensors. Power source, transducer circuitry, control unit, wireless communication are some of the blocks that can be potentially used in biosensing systems. However, the integration of all units may not be a satisfactory solution. Scaling trends for the analog circuit, the digital unit, and the biosensor itself are different, and so heterogeneous technologies may be required [17]. A platform-based design style using heterogeneous components and compositional rules eases the design process and reduces the *non-recurring engineering* (NRE) costs of biosensing systems, thus enabling the introduction of new approaches in the medical arena.

In this paper we present a strategy to develop the sensing block of a biosensor for the detection of endogenous compounds and anticancer drugs, and we compare its performance with the state-of-the-art devices. Before presenting the comparative results, we give an overview of the biosensors used in clinical practice, with particular emphasis on those suitable for integration.

2. CLASSIFICATION

Biosensors are a subgroup belonging to the wide family of chemical sensors. Biosensors may be classified in different ways. IUPAC recommends their classification according to the biological recognition mechanism or the transduction principles [48]. Hereunder, we want to propose an essential classification of biosensors that have been proposed in literature during the last decade.

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2.1 Targets

The development of a biosensor is strictly connected to the target we want to detect. In clinical applications there are lots of analytes which are interesting to follow. **DNA** is one of the most important targets that have been studied during the last decades. Applications range from medical diagnosis and genome sequencing to food, pollution, and environmental analysis [6]. There are several methods to detect DNA: the most widely used is typically based on microarray technique, which consists on nucleic acid hybridization and optical readout [35]. Another technique quite popular involves electrical DNA biosensors, based on capacitance measurements [45].
Many researches have been focusing for long time on the detection of **molecules**, too. The most studied metabolite over the last fifty years is by far glucose, which lends to point-of-care device development and self-management for chronic diseases. Glucose biosensors are generally based on electrochemical principles, with a disposable sensing element and a permanent readout device [30]. However, there are other interesting molecules to detect for clinical interest. Over the last two decades there have been proposed biosensors for lactate [31], cholesterol [43], glutamate [38], creatinine[21], etc.
Biomarkers are another large family of biomolecules arising interest, since they are able to point out if a biological process, a disease, or a response to a therapeutic intervention is in progress. Currently, the most popular are the cancer biomarkers, including proteins, peptides, and tumor-related metabolites. One outstanding example is the *prostate specific antigen* (PSA) detection, related to prostate cancer [58], and the carcinoma antigen 125 (CA-125), related to ovarian cancer [47]. Autoimmune diseases present also distinctive biomarkers, typically antibodies or auto-antibodies. *Surface plasmon resonance* (SPR) has showed promising results for the detection of such biomarkers [11]. Biosensors are also promising tools for widespread and cheap screening of infectious diseases by detecting the RNA sequence of virus (for example dengue fever virus) or hepatitis B antigen. Recent works have presented encouraging results for protein detection to achieve the diagnosis of acute myocardial infarction and presence of coronary plaque [11].
Drugs are a further big category of molecules that can be sensed by using biosensors. Their monitoring in patient blood can reveal drug absorption, so that drug supply can be optimized according to the individual, enhancing the therapeutic efficacy. Some examples are detectors for paracetamol (analgesic and antipyretic), theophylline (used as therapy for respiratory diseases), chlorpromazine (antipsychotic), salicylate (antimicrobial agent) [53]. Multi-panel drug biosensors were also proposed for the detection of drugs by using cytochrome P450 in different isoforms. Benzphetamine (used in anti-obesity treatments), cyclophosphamide (used in anti-cancer therapy), Dextromethorphan (cough suppressant), Naproxen and Flurbiprofen (anti-inflammatory compounds) were detected with an electrochemical-based biosensor [9].

2.2 Sensing element

The sensing element is strictly related to the target. Biological systems represent the most selective element and they typically confer specificity to the biosensor. Several biosensors are based on **enzymes**. They are complex macromolecules, largely formed by protein structure, which are able to catalyze a chemical reaction. Generally, the chemical reaction is then transduced in a signal that can be measured and correlated to analyte quantity. The enzymes bind the analyte next to the active site. Enzymes needs a cofactor to work, which is bound to the protein itself. The cofactor is the part of the enzyme typically involved in the oxidation or reduction reaction [44].
Antibodies are another common sensing element. They are able to specifically bind the corresponding antigen, but they do not promote or catalyze any chemical reaction. The antigen, i.e. the target, can be a molecule or a cell (for example a bacteria) [11]. ELISA (Enzyme-Linked ImmunoSorbant Assay) is maybe the most popular analytical assay based on the complex antibody-antigen. An enzyme can be coupled to the antibody as transducer to promote a colorimetric reaction, for example, even if the sensing element remains the antibody [25].
DNA biosensors are primarily based on **nucleic acids** as sensing element. The specificity is conferred by the base-pairing and the strand of nucleic acids can detect genetic diseases, viral infections, and cancer [12]. The strands are often labeled with radioactive or fluorescent compounds, as well as enzymes or electroactive species. The transduction mechanisms, then, is strictly correlated to the used label.
The last category of sensing elements is represented by **receptors**, which are basically cell-membrane proteins. The detected signal is typically electrical, since a charge-flow is measured through an ion-channel [46]. Drugs are mostly the target, even if there are still evident problems to incorporate such receptors onto biosensors [34].

2.3 Transduction mechanism

The transduction mechanism is another big section for a detailed classification. The purpose of the present work is not to give a detailed description of all the mechanisms used for biosensing, but we want to present an exhaustive overview on the main techniques.
Optical biosensors are typically enzyme-based, since the transduction mechanism is a chemical reaction producing changes in spectroscopic or spectrophotometric properties. Another strategy to perform the detection is by labeling secondary antibodies and DNA strands with fluorescent agents to confer the optical readout [20]. **Surface plasmon resonance**-based biosensors belong to the family of optical sensing and they have increasingly arisen interest in biosensing applications during the last years. This technique consists of the excitation of the interface between a metal and a dielectric by using light waves. If the excitation frequency matches the oscillation frequency of surface charge density, electromagnetic waves propagate along the interface, called surface plasmons. The dielectric medium can be functionalized with biological elements: as soon as the dielectric changes (because the target molecules bind the receptor), there is also a change in the refractive index [56]. Metal layer is mainly functionalized with antibody for the detection of antigens and hormones [11].
Piezoelectric biosensors typically detect mass variation and they are commonly known as *quartz crystal microbalance* (QCM). The quartz disk, or the microcantilever for nanoscale sensors, oscillate because of the application of an alternating electric field. The resonance frequency depends also on system mass. If the surface of the quartz is coated with sensing elements, once the sensing element binds the target, the mass of the system varies and shifts the resonance frequency. Such biosensors have been reported for DNA and pathogens detection and for immunoassays [13].
Surface modification with sensing elements can result in mass variation, as in the case of piezoelectric biosensors, but it can also result in the variation of the **electrical** properties of the surface. If the surface is an electrode, it is possible to quantify such electrical change by measuring the variation of impedance. Two sub-groups belong to the family of impedimetric biosensors. Capacitive biosensors are sensitive to capacitance variation: DNA detectors and immunosensor for tumor biomarkers are often developed according to these principles [50]. The Faradic impedimetric biosensors foresee to

couple the antibody with a redox probe: the measured property is the charge transfer resistance [37].
The last category is represented by the **electrochemical** biosensors, which are by far the most reported devices in literature. It is possible to distinguish three sub-groups based on electrochemical sensing. The catalyzed reaction promoted by the enzyme can result in a variation of the electrode potential, while no current flows. Such technique is call *potentiometric*. Ion-selective sensors belong to that family. Potentiometric biosensors have been developed for urea detection in blood, creatinine in biological fluids and immunosensor assays [23]. *Ion charge* or *Field-Effect-Transistors (FET)* are another category of transduction mechanisms where the ion charge variation is monitored. Conventional FET can be modified for biosensing purposes by functionalizing the gate terminal with probes, for example. The binding between probes and targets results in a variation of electric charges at the gate terminal [24]. The functionalization can be applied also to the channel, especially when it is replaced by nanostructures, as nanowires or nanotubes (as discussed in Section 2.4). In this case the binding mechanism is transduced in a conductivity variation of the channel [22]. Finally, there are the *amperometric* biosensors, where current variation is monitored as result of the redox reaction promoted by the enzyme with the target. Amperometric biosensors have had great success in the market, because they can be produced quite easily and inexpensively, and they lend to be integrated in portable devices. Since their development is quite inexpensive, the sensing element (enzyme and electrodes) can be disposable, guaranteing uncontaminated and safe self-measurements. Amperometric biosensors have been developed for many applications: metabolite (especially glucose) and drug monitoring are by far the most common [53].

2.4 Nanotechnology-based biosensors

According to many authors [8], [15], the new frontier of biosensing is nanomaterial employment. Nanomaterials exhibit many interesting properties for biosensing, including dimensions comparable with sensing elements, high electron transfer rate [51], considerable electronic emission [28], and high surface area [2], due to their 3-D structure.
Nanoparticles (NP) are typically metallic, showing interesting electrical and magnetic properties for biosensing applications. NP applied in the biosensing field are often made of gold, because of the numerous ways to modify Au surfaces to obtain high affinity with biomolecules. Silver and platinum are other two reported metals used for NP synthesis with similar behavior. They have presented proper optical properties to be used in biosensing, but also interesting electrical features, as high sensitivity in voltammetry and improved limit of detection in potentiometric techniques [36]. Quantum dots are semiconductor crystals whose size is within 10 nm. Quantum confinement confers different properties to quantum dots with respect to larger particles. In fact, they have remarkable optical properties, suitable to be used as labels for sensing elements [27]. *Core shell* are a subfamily of NP, with a metallic core and an organic or inorganic shell, to improve biocompatibility and reduce particle aggregation [2].
With the improvement of micro and nanofabrication techniques, **nanowires (NW)** have been arising more interest in biomedical applications, since they can interact with biomolecules at the nanoscale. They can be metallic or semiconductor, according to the transduction mechanism. NW are often employed in conductive measurements, when functionalized with proteins, enzymes or antibodies, or field-effect-transistors, as discussed previously in Section 2.3 [39].
Finally, **carbon nanotubes (CNT)** have shown to possess interesting electrochemical properties. The electron current through the nanotube is based on ballistic conductivity, so the measured mean free-path results to be two orders of magnitude higher than the best macroscale conductor [26]. Electron transfer depends on surface conditions: many works have been published regarding emission properties of tips and walls of CNT [7], [29] to explain their high rate. Moreover, they have been largely reported for the absorption of proteins onto their walls, resulting in an excellent immobilization method [4]. Surface modification by using carbon nanotubes can be accomplished in different manner and for several purposes. Directly growing of aligned carbon nanotubes have been proposed on different substrates [15]. Another way to obtain aligned CNT is by self-assembly: surfaces are generally modified with thiols or other functional groups to link nanotubes [40]. Carbon nanotubes can be also randomly dispersed on the electrode surface and many efforts have been addressed to find the right solvent to disperse CNT. Wang *et al.* showed that well-dispersed CNT solution can be achieved by adding Nafion [54]. Carbon nanotubes can be used as a forest of nanomaterials, patterned arrays or as single-sensors.
Nanowires and carbon nanotubes can be used to replace the channel in field-effect-transistor for biosensing purposes . As in the case of electrochemical biosensors, biological sensing elements can be adsorbed on NW or CNT surface and modify the conductivity of the channel [52].

2.5 Electrode technology

Disposable biosensors are by far the most common tool sold in the market. They avoid common drawbacks like cleaning process, sterilization procedures, and contamination. On the other hand, fully-implanted monitoring is not possible with disposable electrodes, hampering the development of definitive solutions for the treatment of diabetes. Biosensor integration is definitely needed for such applications. Electrochemical-based sensing is the most suitable approach for the development of integrated biosensors. Amperometric, potentiometric, and impedimetric detection can be easily achieved with CMOS circuits next to the transducer. CMOS technology brings some interesting advantages, especially for electrochemical biosensors, where the signals are weak while the noise is quite high. Signals involved in such measurements are often analog, so the integration of analog-to-digital converters is required as well. CMOS circuits are typically covered with one or more passivation layers, to isolate the chip from the outside and from contaminants. CMOS applied in biology are much more subject to contamination and the wet environment does not guarantee proper working conditions. So, integrated biosensors need hybrid solutions. A really interesting and innovative solution for integrated biosensors was proposed by Guiducci *et al.* [17]: they propose a 3-D integrated system with vertically stacked layers and thru-silicon vias among the different layers. This solution treats each layer with different technologies, particulary suitable for the layer in contact with the biological environment. The authors propose a disposable biolayer, which is not suitable for fully-implanted devices, but can represent a step towards the development of permanent systems. Instead, the other layers designed for the readout, the transmission, the power supply, and the post-processing are permanent.

3. CNT-BASED BIOSENSOR

In the present section we describe one possible strategy to develop a platform of biosensors with the perspective to integrate the electrodes and the electronics in an unique device. Following the classification presented in Section 2, our biosensor can be described as following:

- *Target*: molecules, drugs
- *Sensing element*: enzymes
- *Transduction mechanism*: electrochemical (amperometric)
- *Nanotechnology-based*: carbon nanotubes
- *Electrode type*: disposable, integrated

Afterwards, we compare the performance of our developed biosensors with others found in literature. We will focus on enzyme-based electrochemical biosensors with similar modification of the electrode surface by using CNT and functionalization with the same type of protein.

3.1 Sensor description

Some biosensors are developed by using carbon paste *screen-printed electrodes* (SPE) (Dropsens, Spain) as disposable electrodes. The SPE consist of graphite working and counter electrodes, and Ag reference electrode. Working electrode has an area equal to 13 mm^2. Other molecules are detected by using a microfabricated chip, consisting of five Au microelectrodes, Au counter electrode, and Pt reference electrode. Each working electrode presents an area equal to 0.25 mm^2. Microfabrication details are described in [3]. All the electrode surfaces are modified with *multi-walled carbon nanotubes* (MWCNT - diameter 10 nm, length 1-2 μm - Dropsens, Spain) and functionalized with the enzyme-probe. Two families of enzymes are used for the experiments: oxidases are used for the detection of glucose, lactate, and glutamate, while *cytochrome P450* (CYP) is used for arachidonic acid, ifosfamide, *cyclophosphamide* (CP), and Ftorafur®. Oxidase-based detection is investigated by chronoamperometry with microfabricated Au electrodes and a drop cast solution of MWCNT dispersed in Nafion 0.5%. The working electrode potential is set at +650 mV and the current variation is recorded, since it is proportional to the target concentration. CYP-based sensing, instead, is carried out on screen-printed electrodes modified with MWCNT dispersed in chloroform. A linear-sweep potential is applied forward and backward within a certain potential window, while continuously monitoring the current. The hysteresis plot gives qualitative and quantitative information about the detected target. In particular, the peak hight is proportional to drug concentration and calibration curves can be plotted. Table 1 summarizes the main characteristics of our developed biosensors.

Table 1: Features of different metabolite biosensors.

Target	Probe	Technique
GLUCOSE	Glucose oxidase	Chronoamperometry
LACTATE	Lactate oxidase	
GLUTAMATE	Glutamate oxidase	
ARACHIDONIC ACID	custom-CYP	Cyclic voltammetry
FTORAFUR®	CYP1A2	
CYCLOPHOSPHAMIDE	CYP2B6	
IFOSFAMIDE	CYP3A4	

3.2 Results of detection

Here following we will focus on the comparison between our sensors and other similar biosensors found in literature. Table 2 summarizes the main features of the discussed biosensors, like the sensitivity, the linear range, and the limit of detection.

3.2.1 Glucose biosensor

Glucose biosensors have been extensively investigated. Examples regarding diverse surface modification and functionalization are largely reported in literature. Focusing on CNT-based biosensors using *glucose oxidase* (GOD) as sensing element, our biosensor shows the best performance for both sensitivity and limit of detection compared to similar sensors reported in literature. We achieve a sensitivity of 55.5 μA mM^{-1} cm^{-2} in a range from 0 to 1 mM, with a detection limit of 2 μM. Wang *et al.* [55] evaporated a thin Au film onto grown MWCNT and they drop cast GOD on top of the nanotubes, showing a sensitivity of 14.2 μA mM^{-1} cm^{-2}. Another approach can be to mix CNT and GOD in the same solution, with the addition of Nafion to increase the solubility of nanotubes. Tsai *et al.* proposed this approach in [49], where they drop cast the mixed solution on glassy carbon electrodes. In a linear range from 0.025 to 2 mM, they got a sensitivity of 4.7 μA mM^{-1} cm^{-2}. Regarding sensitivity, quite similar results were shown by Ryu *et al.*: CNT forme a network on the electrode (defined mat) and GOD is covalently bound to the nanotubes [42]. The highest result in terms of sensitivity was presented in [18], showing 23.5 μA mM^{-1} cm^{-2} for MWCNT-based sensor with *butyric acid* (BA) functionalization and GOD immobilization.

3.2.2 Lactate biosensor

Lactate biosensors have been less studied compared to glucose biosensors, but there have been proposed diverse modification and functionalization, as well. Our sensor shows a sensitivity of 25.0 μA mM^{-1} cm^{-2} within a range from 0 to 1 mmM, and a detection limit of 11 μM. Goran *et al.* [16] drop cast successively N-doped CNT, LOD, and modified-Nafion onto glassy carbon electrode. They obtained higher sensitivity than us, because carbon electrode has better performance than metallic electrodes for the detection of H_2O_2. In fact, we have already showed similar sensitivity as [16] in our previous work by using carbon paste SPE [5]. However, the linear range is very narrow (from 0.014 to 0.325 mM), which cannot fit with physiological lactate concentration. CNT can be also incorporated with mineral oil to form a paste and used as electrodes [41]. However, the resulting sensitivity is quite low, 0.204 μA mM^{-1} cm^{-2}, in an extended range from 0 to 7 mM. MWCNT have been also incorporated into sol-gel film to form a further matrix for the immobilization of the enzyme. Huang *et al.* deposited the obtained matrix onto glassy carbon electrode, but they still showed a sensitivity ten times lower that our results [19]. In the last example, the authors used titanate instead of carbon nanotubes [57]. The obtained sensitivity is much lower than the previous case, suggesting that carbon gives better performance not only for the nanoscale structure, but also for the material itself.

3.2.3 Glutamate biosensor

Glutamate is a neurotransmitter and its monitoring can be crucial for neurochemical experiments. Lots of microsensors have been proposed to be implanted in the brain and most of them are not based on carbon nanotubes. Pan *et al.*, for example, covered a Pt electrode with Nafion matrix to entrap glutamate oxidase. The detection was carried out in a really narrow range within 1 and 13 μM, obtaining a sensitivity of 16.1 μA mM^{-1} cm^{-2}. Alternatively to Nafion immobilization, Zhang [59] described the entrapment of GlOD in chitosan, with a sensitivity of 85 μA mM^{-1} cm^{-2} within 0 and 200 μM. Similar linear range was also explored by Ammam *et al.* [1]. Differently, they used MWCNT and polyurethane (PU) onto Pt electrodes to increase the sensitivity (384 μA mM^{-1} cm^{-2}) and GlOD was dispersed in polypirrole (PP). All the previously de-

Table 2: Comparison of electrochemical enzyme-based biosensors.

	Modification	Sensitivity	Linear range	Limit of detection
GLUCOSE	CNT mat + GOD [42]	4.05 μA mM^{-1} cm^{-2}	0.2 - 2.18 mM	–
	MWCNT/Nafion + GOD [49]	4.7 μA mM^{-1} cm^{-2}	0.025 - 2 mM	4 μM
	MWCNT + GOD [55]	14.2 μA mM^{-1} cm^{-2}	0.05 - 13 mM	10 μM
	MWCNT-BA + GOD [18]	23.5 μA mM^{-1} cm^{-2}	0.01 - 2.5 mM	10 μM
	MWCNT/Nafion + GOD	55.5 μA mM^{-1} cm^{-2}	0 - 1 mM	2 μM
LACTATE	MWCNT/mineral oil + LOD [41]	0.204 μA mM^{-1} cm^{-2}	0 - 7 mM	300 μM
	Titanate NT + LOD [57]	0.24 μA mM^{-1} cm^{-2}	0.5 - 14 mM	200 μM
	MWCNT + sol-gel/LOD [19]	2.1 μA mM^{-1} cm^{-2}	0.3 - 1.5 mM	0.3 μM
	N-doped CNT/Nafion + LOD [16]	40.0 μA mM^{-1} cm^{-2}	0.014 - 0.325 mM	4 μM
	MWCNT/Nafion + LOD	25.0 μA mM^{-1} cm^{-2}	0 - 1 mM	11 μM
GLUTAMATE	Nafion + GlOD [33]	16.1 μA mM^{-1} cm^{-2}	0.001 - 0.013 mM	0.3 μM
	Chit + GlOD [59]	85.0 μA mM^{-1} cm^{-2}	0 - 0.2 mM	0.1 μM
	PU/MWCNT + GlOD/PP [1]	384 μA mM^{-1} cm^{-2}	0 - 0.14 mM	0.3 μM
	MWCNT/Nafion + GlOD	0.9 μA mM^{-1} cm^{-2}	0 - 2 mM	78 μM
ARACHIDONIC ACID	MWCNT + CYP	1140.0 μA mM^{-1} cm^{-2}	0 - 0.04 mM	0.4 μM
CYCLOPHOSPHAMIDE	MWCNT + CYP	102.0 μA mM^{-1} cm^{-2}	0 - 0.07 mM	2 μM
IFOSFAMIDE	MWCNT + CYP	160.0 μA mM^{-1} cm^{-2}	0 - 0.14 mM	2 μM
FTORAFUR®	MWCNT + CYP	883.0 μA mM^{-1} cm^{-2}	0 - 0.008 mM	0.7 μM

scribed sensitivities are higher (up to three orders of magnitude) than the one obtained by our biosensors. In fact, we achieve a sensitivity of 0.9 μA mM^{-1} cm^{-2} and a detection limit of 78 μM. On the other hand, we exploit a wider linear range (from 0 to 2 mM), useful for some particular applications like cell culture monitoring.

3.2.4 CYP-based biosensor

Arachidonic acid is a fatty acid abundant in liver, brain, and muscles. Its detection can be carried out by the isoform CYP102A1, for example. We got a customized CYP isoform from EMPA (St. Gallen, Switzerland) for the detection of fatty acids. Carbon paste SPE are modified as described previously by using MWCNT and the detection is performed by applying cyclic voltammetry. The developed biosensor shows a sensitivity of 1140.0 μA mM^{-1} cm^{-2} within a linear range from 0 to 40 μM, and a detection limit of 0.4 μM. In literature, the detection of such compound is typically optical, as presented by Giovannozzi *et al.* [14], and noone has described yet electrochemical detection by using CYP450.

Drugs can be detected using different isoforms of cytochrome P450. Here we present the results obtained from the detection of three drugs. Cyclophosphamide and ifosfamide are two alkilating agents, commonly used in anticancer treatments and as immunosuppressant. The third drug is a chemotherapeutic prodrug. Among these three drugs, CP is the only one for which electrochemical biosensors were previously developed. They are typically DNA-based and the signal variation is recorded when the CP interacts with DNA strands under differential pulse voltammetry [32]. We develop an enzyme-based electrochemical biosensor by using three different isoforms of CYP450 for the detection of such compounds. For CP we obtained a sensitivity of 102.0 μA mM^{-1} cm^{-2} in a linear range within 0 and 70 μM and a detection limit of 2 μM. For ifosfamide we got a sensitivity of 160.0 μA mM^{-1} cm^{-2} within a range from 0 and 140 μM, with a detection limit of 2 μM. Finally, Ftorafur® is detected with a sensitivity of 883.0 μA mM^{-1} cm^{-2} in a linear range within 0 and 8 μM and a detection limit of 0.7 μM. In conclusion, it is the first time that electrochemical biosensors based on MWCNT and CYP are used for the detection of the aforementioned compounds. These results are really promising for the development of integrated biosensors for monitoring of drug mixture in the blood, paving the way to innovative tools for personalized therapy.

4. CONCLUSIONS AND PERSPECTIVES

The aim of the present work is to give an overview on biosensing strategies and developed devices. In particular, our attention is focused on nanotechnology-based biosensors and the possibility of their integration in more complex systems. Then, we proposed one possible strategy to develop biosensors for the detection of some biomolecules and drugs, showing that surface modification of the electrode with nanostructures can enhance the performance in biosensing. The detection of multiple endogenous and exogenous compounds is essential for personalized therapy. All the presented results are really promising in the perspective to develop point-of-care devices. The variety of individual responses to the same treatment requires potent tools for the monitoring of metabolic mechanisms, to optimize therapy management and efficacy. Enhanced sensitivities and lower detection limit can satisfy these demands, as showed by our developed biosensors.

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