

Exploiting plug-and-play electrochemistry for drug discovery

Electrochemistry has emerged as a powerful analytical technique for chemical analysis of living cells, biologically active molecules and metabolites. Electrochemical biosensor, microfluidics and mass spectrometry are the most frequently used methods for electrochemical detection and monitoring, which comprise a collection of extremely useful measurement tools for various fields of biology and medicine. Most recently, electrochemistry has been shown to be coupled with nanotechnology and genetic engineering to generate new enabling technologies, providing rapid, selective, and sensitive detection and diagnosis platforms. The primary focus of this review is to highlight the utility of electrochemical strategies and their conjunction with other approaches for drug metabolism and discovery. Current challenges and possible future developments and applications of electrochemistry in drug studies are also discussed.

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Background

Electrochemistry is the branch of physical chemistry that studies the relationship between electricity and chemical reactions, containing advantages of instrumental simplicity, moderate cost and portability. As early as the year 1800, the first chemical battery was invented by Volta, which has now gone through 200 years of development. Recent trends in the field of electrochemistry involve electrochemical analysis, chemical power supply, electrochemical synthesis, photoelectric chemical and others [1,2]. Electrochemistry has a number of different uses and its applications are ranging from biomedical chemistry to clinical diagnostics.

Electron transfer occurs in a redox reaction [3,4]. Commonly, an electrochemical cell measurement involves a system of three electrodes, including the working, reference and counter electrodes, which are immersed in a solution containing the compound of inter-

est and a supporting electrolyte (Figure 1A). These electrodes are connected to a potentiostat, which controls the potential of the working electrode and measures the resulting current. The working electrode, also called research electrode, makes contact with the analyte and applies the desired potential in a controlled way to facilitate the transfer of charge to and from the analyte. The reference electrode provides a fixed potential against the potential applied to the working electrode. The counter (auxiliary) electrode is used for voltammetric analysis or other reactions in which an electric current is expected to flow [5]. Basic analysis using three-electrode system gives the information of oxidation/reduction, resistance values and reversibility details of the material. As an alternative to the traditional electrodes, screen-printed electrodes (SPEs) (Figure 1B), the devices produced by printing different inks on various types of plastic or ceramic

Lixia Gao¹ & Yong Teng^{*2,3}

¹School of Life Sciences, Chongqing University, Chongqing 400044, PR China

²GRU Cancer Center, Medical College of Georgia, Augusta University, Augusta, GA30912, USA

³Department of Oral Biology, Dental College of Georgia, Augusta University, Augusta, GA30912, USA

*Author for correspondence:

Tel.: +1 706 721 5257

Fax: +1 706 721 1671

yteng@augusta.edu

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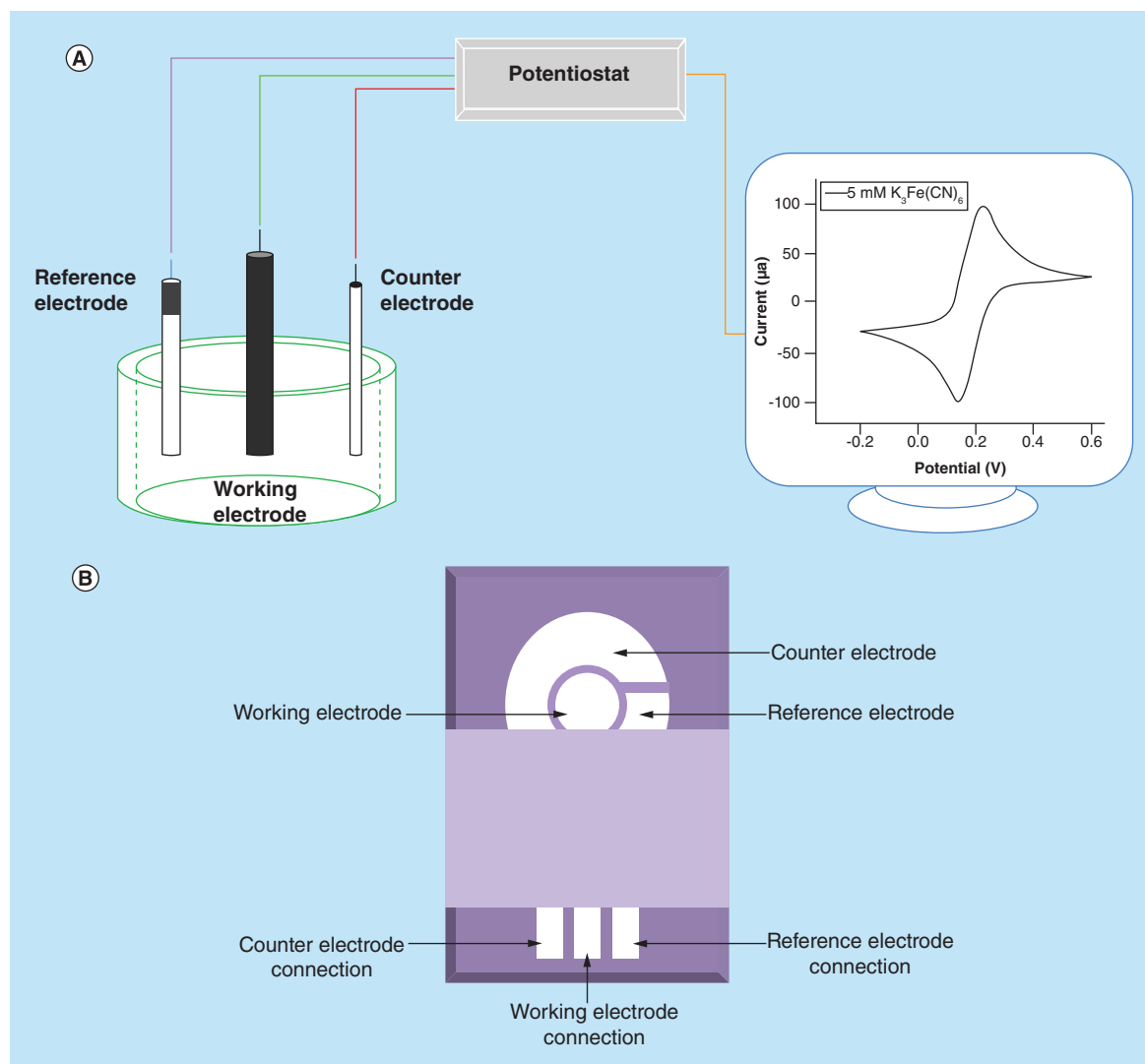


Figure 1. The structure diagram of traditional electrochemical cell with a three-electrode system (A) and screen-printed electrodes (B).

substrates, enable researchers to generate great interest due to their low cost and simple preparation process [6]. According to the types of materials used to modify the working electrode, SPEs can be categorized into unmodified, film-modified, enzyme-modified and antigen/antibody-modified SPE [7]. The great versatility presented by the SPEs lies in the broad range of ways in which the electrodes may be modified. Unlike three-electrode system, a two-electrode potentiostat only permits the use of a working and counter electrode, and the more versatile four-electrode potentiostat uses two working electrodes [8].

Electrochemical parameters & techniques in medical research

Many processes of life phenomenon are all accompanied by electron transfer reactions, electrochemical methods are thus the better way to reveal the essence of

life for research electron transfer and related processes in biological systems. In medical research, electrochemistry provides a cost-effective, rapid and clean system for detection of the reactivity of drug metabolites toward biomolecules. For example, methylglyoxal is a biomarker in human plasma and there are many methods reported for its determination. For quantitative analysis of methylglyoxal, a single-walled carbon nanotube modified glassy carbon electrode has been developed [9]. This modified electrochemical sensor exhibits its potent and sustained electron-mediating behavior, by which a well-defined reduction peak in response to human plasma methylglyoxal can be observed in a wide linear range from 0.1 to 100 μM and a higher sensitivity of 76.3 $\text{nA } \mu\text{M}^{-1}$. Therefore, this effective system facilitates the laboratory detection of methylglyoxal and elucidation of its role in diabetes-related complications. Johnson *et al.* have shown that elec-

trochemical detection of various organic compounds on Pt electrodes can be dramatically improved using square-wave potential pulses [10]. Nouri-Nigjeh and his coworkers present the benefits of electrochemical oxidation drug metabolism by square-wave potential pulses for the oxidation of lidocaine [11]. By means of square-wave potential pulses, electrochemical production of drug metabolites can be collected with higher selectivity and more yield based on the optimization of cycle times and potentials. Therefore, electrochemical methods are frequently used to obtain critical information about drug molecules and their mechanism of action in the discovery of valuable leading compounds of new drugs [12,13].

In particular, voltammetry and electrolytic methods are more relevant to research electroactive drugs, drug metabolites and metabolites interactions with biomolecules [12]. The techniques of cyclic voltammetry and linear sweep voltammetry have grown enormously in popularity over the past few years since they can robustly assess the electrode processes and redox mechanisms [14]. Stripping voltammetry has been widely used for ultrasensitive detection by preconcentrate analytes [15–17]. Amperometry has been applied in electrophysiology to study vesicle release events using a carbon fiber electrode [18,19]. Other techniques such as differential pulse voltammetry and square wave voltammetry are particularly important in the determination of trace amounts of electroactive compounds in pharmaceuticals and biological samples [20–22].

Electrochemical methods for *in vitro* assessment of drug metabolism

The research of drug metabolism is essential for successful drug discovery [23]. Pharmaceutical compounds can be converted to therapeutically active or toxic metabolites *in vivo*. Although experimental models are required for the research of drug metabolism and disposition, the proper selection and applications of correct detection techniques are critically important in decision-making and successful advancement of drug candidates. In the past two decades, electrochemical

techniques have been developed for different purpose and widely applied for fast evaluation and mimicry oxidative reaction of drug metabolism and studying reaction mechanisms of electroactive drugs [24]. These techniques include electrochemical biosensors, electrochemistry in conjunction with microfluidics and MS. Electrochemical biosensor can be used to monitor drug metabolites and bioactivation pathways, and electrochemical MS (EC–MS) is always chosen for drug elemental analysis and configuration, while electrochemical microfluidics have advantages in the evaluation of drug efficacy and pharmacokinetic properties. As proposed by Nouri-Nigjeh and colleagues, sub-second square-wave potential pulses promoted the transformation of phenacetin to acetaminophen by the electrochemical oxidation [25]. This method is a potential analytical technique for the imitation of oxidative drug metabolism during the early stages of drug discovery and development. Recently, electrochemical impedance spectroscopy has captured more and more attention because of its high sensitivity and label-free characteristics during the measurement of the dielectric properties [26,27].

Electrochemical biosensors for drug metabolism

Quantification of biological or biochemical processes is of utmost challenge for biological, biomedical and biotechnological applications. Electrochemical biosensors function by converting a biological response into an electrical signal, providing an attractive means to analyze the content of biological samples or drugs through detecting, transmitting and recording information regarding a physiological or biochemical change [28]. Compared with other electrochemical sensors, electrochemical biosensors have good specificity, high sensitivity and ease of production [13,29]. A typical biosensor comprises sample, bioreceptor, transducer and processing (Figure 2). The biological recognition element usually contains enzymes, antibodies, cells, tissues or micro-organisms. The transducer includes electrochemical, optical and piezoelectrical elements.

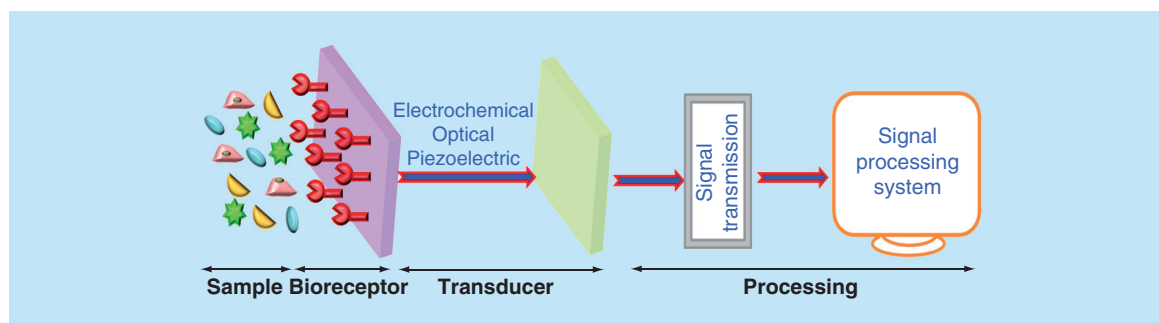


Figure 2. Basic components of the biosensor.

Signal transmission and signal processing system are two important elements in data collection and processing. The recognition element of the biosensor can determine the selectivity to target drugs. However, the sensitivity of biosensor is greatly influenced by the transducer, which is used as an evaluation standard for the sensor performance [30,31].

Biosensor-related research has experienced explosive growth over 20 years, and now electrochemical biosensors have been broadly applied to mimic drug metabolism and the reactivities of metabolites toward biomolecules. Several methods have been successfully used to immobilize biological recognition molecules onto sensing surfaces with full functionality in biosensor-binding assays, such as enzyme-based electrochemical biosensor, which is an effective tool for the study of metabolic reactions by recording the electron transfer signal [32]. CYP450 enzymes are a superfamily of mono-oxygenases and have important position after glucose oxidase and cytochrome C [33,34]. They are the major drug metabolizing enzymes present in the liver, as they can metabolize from 75 to 90% of all drugs currently on the market. Sheila *et al.* reported that suppression of CYP-mediated drug metabolism by a concomitantly administered second drug led to drug–drug interactions in humans using bioelectrochemistry [35]. This observation indicates that as a CYP isozyme, CYP3A4 is the most important in terms of drug metabolism. Victoria and his coworkers analyzed current–voltage characteristics, stoichiometry of the electrocatalytic cycle, redox thermodynamics and the peroxide shunt reaction of CYP through bioelectric catalysis-based screening of potential substrates or inhibitors of CYP [36]. Electrochemistry of CYP in combination with nanotechnologies, a novel high-throughput screening method, enables to miniaturize assay time and analysis steps and reduce research costs. Alexey *et al.* evaluated the activities of oxazolinyl derivatives using highly sensitive electrochemical method [37]. As the potential CYP17A1 inhibitors, these oxazolinyl derivatives suppress growth and proliferation of prostate cancer cells through binding to CYP17A1, which have important implications for the development of new drugs [37]. To avoid the unnecessary costs associated with ineffective therapies, many efforts have been focused on the development of new technologies for the therapeutic drug monitoring (TDM). However, the TDM is restricted to few facilities mainly because the traditional analytical techniques (e.g., chromatography and immunoassay). Taking advantages of multi-walled carbon nanotubes as very promising nanomaterials for enhancing electron transfer in biosensing, Baj-Rossi *et al.* introduced a graphite SPE using multi-walled carbon nanotubes

and microsomal CYP1A2 as the electrode material [38], which provides a novel approach for the development of a fully operational therapeutic biosensor. Therefore, the TDM practice can greatly benefit from the electrochemical biosensors owing to portability, high sensitivity and extreme simplicity. Since a CYP-catalyzed reaction is a common instance, CYP enzyme films have been used to modify electrodes, which can be used as a screening tool to study drug metabolism of electrocatalytic activity [39–41]. To understand the direct electrochemical properties at the interfaces between various carbon electrode materials and human liver microsomes (HLM), Walgama *et al.* developed a novel microsomal bioreactor by adsorption of HLM directly onto polished basal plane pyrolytic graphite, edge plane pyrolytic graphite, glassy carbon or high-purity graphite electrodes. Considering the importance of HLM in pharmaceutical drug development and toxicology fields, such a bioanalytical platform will be very useful for inexpensive drug metabolism and inhibition assays [42]. To date, more efforts have been focused on direct electronic delivery by enzyme catalytic cycle, which facilitate biosensor development without the need of redox transfer proteins and cofactors.

Electrochemical microfluidics for drug metabolism

Microfluidics has the potential to revolutionize the conventional method of cell biology [43], and it can better detect cell growth in microenvironment. Microfluidics has become a promising technology with a wide range of applications in engineering, biology and medicine [44]. Early microfluidic concept can be traced back to the 1970s. Terry *et al.* presented a micromachined gas chromatograph in a silicon wafer, and later it was developed into microfluidic capillary electrophoresis and microreactors [44–46]. One critical feature of microfluidics is a microscale fluid environment with unique properties, such as laminar flow and drops. With these unique fluid phenomena, microfluidics can be achieved by conventional methods which are difficult to complete a series of micromachining and micro-operation. Currently, microfluidic technology and electrochemistry have been combined to improve the automatic determination of the experimental data quality and reduce analysis time and cost by complex electrochemical interactions with biologically important molecules [45,46].

Electromicrofluidics is a relatively young field, but now is considered to have great development potential and broad prospects in biomedical research [47–50]. It has achieved substantial successes in the drug metabolism. For instance, continuous-flow electrosynthesis has been used to detect metabolites of several commer-

cial drugs for various chemical reactivity such as aromatic hydroxylation, alkyl oxidation and glutathione conjugation [51]. The use of electrochemistry within microfluidic structures made measurements from a 10 to 100 mg scale of pure isolated metabolites per hour. Gu *et al.* established a simple but robust droplet-based microfluidic system with a concentration gradient for dose-response enzyme inhibition assay of acetylcholinesterase by electrochemical method [52]. Compared with traditional enzyme inhibition assay methods, the sample consumption in this system reduced 1000-fold, which is applicable to many biochemical reactions (e.g., drug screening and kinetic studies) as long as one of the reactants or products is electrochemically active. Dopamine is an important neurotransmitter in the autonomic nervous system and involved in the reward, mood and addiction, as well as neurological disorders such as Parkinson's and Huntington's diseases [53]. The most popular methods for monitoring dopamine release *in vivo* are impossible to measure dopamine and its metabolites simultaneously, making it a challenge to investigate the effect of drugs or other treatments on the dopamine metabolic pathway. Given separations employing microchip electrophoresis are fast (subminute), highly efficient and require low sample volumes (from pL to nL) [54]; it has been developed for the separation and detection of analytes in the dopamine metabolic pathway [55]. This electrophoresis-based microchip consists of a 5 μm polydimethylsiloxane separation channel in a simple configuration, may open a new window for detecting compounds in specific metabolic pathway and online monitoring of brain microdialysis samples.

EC-MS for drug metabolism

Conventional metabolism studies in early stages of drug development include *in vitro* tests on the basis of hepatic cells and cell extracts, as well as animal model tests. The possibility of simulating the oxidative metabolism of drugs causes the strongly increasing attraction in combined use of EC-MS [56,57]. Electrochemistry thus benefits from the identification of structural information and the quantitation of reaction products in nanomolar levels by means of MS [58]. MS in conjunction with electrochemistry began in the early 1970s. Early studies in this field were aimed at the detection of reaction products of one particular drug under conditions. Recent publications have reported a comparison of the effect of different solution conditions (i.e., pH and electrolyte) and the electrochemically assisted Fenton reaction [59,60].

Oberacher *et al.* determined the reduction of $[(\text{C}_5\text{Me}_5)_2\text{Mo}_2\text{O}_5]$ and related complexes using online EC-MS based detection and successfully identified

mono- to tetranuclear organometallic molybdenum oxides [61]. This work may open up avenues for the development of new catalytic redox chemistry and the analysis of metal complexes [61]. Jahn *et al.* investigated drug candidates by use of ESI-MS and inductively coupled plasma-mass spectrometry for the qualitative and quantitative analysis of drug metabolites [62]. In this study, the complementary measurements have been inevitable for metabolite identification and revealed N-dealkylation as the main metabolism reaction for the drugs amiodarone and toremifene. EC-MS can also be used to elucidate molecular structure. For instance, two curcumin analogs (designated as NC2067 and NC2081) with a structural backbone of 1,5-diaryl-3-oxo-1,4-pentadiene have been identified and their diagnostic product ions have been qualified and quantified using EC-MS [63].

Applications of electrochemical strategies in drug discovery

The journey from drug discovery to its commercialization is an expensive, lengthy and incremental process, which promotes the development of increasingly selective, reliable and rapid high-throughput screening assays for the early stage of drug discovery. Cell-based assays for high-throughput screening provide an early indication of the toxicity characteristics and efficacy of the drug candidates. If we consider a living cell as an electrochemical system, electron generation and charge transfer caused by redox reactions and the changes of ionic composition and concentration in a living cell can be used to characterize cell viability in a homogeneous solution [64,65]. Cell-based biosensors integrate biological recognition elements and electrochemical transduction units based on cellular activity and function, cellular barrier behavior, or recording/stimulation of electric potential of electrogenic cells [64]. Compared with other methods, these biosensors have the ability to provide physiologically relevant data in response to an analyte and to detect the bioavailability of the analyte, with the advantages of higher biocatalytic activity, lower production costs and enhanced stability [66,67].

In drug discovery, the specialized electrochemical strategies have been used to determine drug safety and efficacy. For example, Ding *et al.* developed a bio-inspired gel for immobilization and electrochemical study of cells [68]. Using this electrochemical method, the oxidation peak in K562 leukemia cells can be detected when the cells are attached to the gold nanoparticle modified carbon paste electrode. This work provides a new avenue for electrochemical investigation of antitumor drug sensitivity through the determination of cell adhesion, proliferation and apoptosis. Since reactive oxygen species (ROS) and

reactive nitrogen species (RNS) play a critical role in the mechanism of many drugs and have been shown to be involved in electron transfer [69–71], electrochemical sensors and biosensors have been developed for assessing ROS and RNS of interest in clinical and physiological analysis [72]. Numerous studies have shown that the utility of electrochemistry to monitor cell free radical levels may lend insights into a potential effective approach for cell biology and drug screening. For example, BRAFV600E inhibitor PLX4032 (vemurafenib) is a US FDA-approved new drug for the treatment of metastatic melanomas, which inhibits melanoma cell growth. The involvement of superoxide and nitric oxide in PLX4032-induced growth repression has been identified by electrochemical biosensors [73]. In this study, superoxide radicals $O_2^{\bullet-}$ and nitric oxide (NO) released from PLX4032-challenged

BRAFV600E-mutant A375 cells were monitored using electrochemical sensors and conventional fluorescein staining techniques (Figure 3). Interestingly, the scientists also found that PLX4032 treatment decreased the mitochondrial membrane potential in BRAFV600E-mutant melanoma cells, which indicates electrochemical methods can be used to detect early cell apoptosis induced by biomolecules including lipids, proteins, DNA and small chemical compounds.

Recently, single-cell-based analysis of drug metabolites has been established by electromicrofluidic detection platform, which can be used to accurately monitor the cell-to-cell heterogeneity during the response to external cues such as drug treatment [74,75]. The study from Yeon's group presents the use of electrochemical impedance sensor array integrated into the bottom of a microtiter plate to assess cell growth as

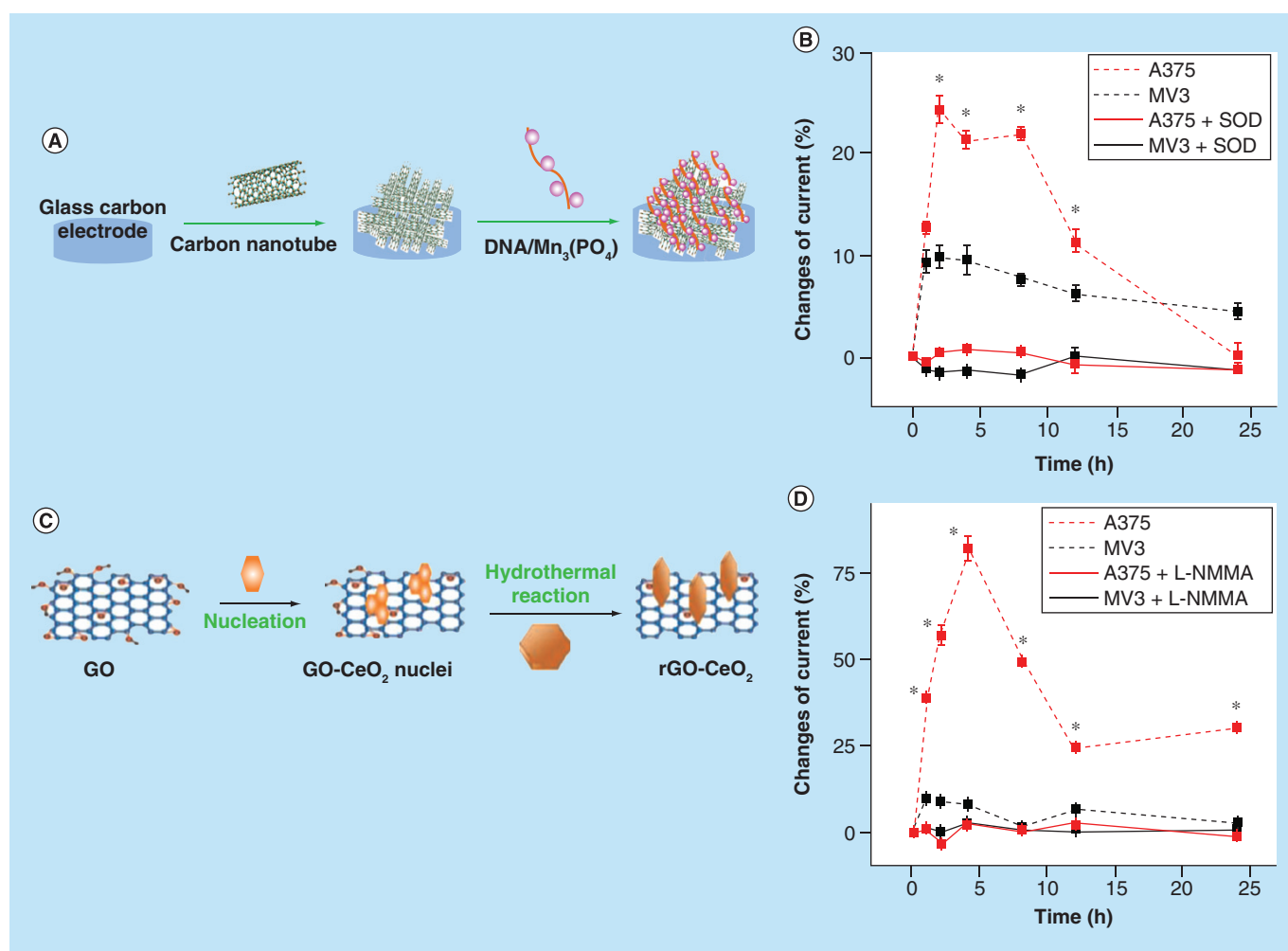


Figure 3. Modified electrode process and electrochemical signal levels. (A) DNA/Mn₃(PO₄)₂@CNT nanomaterial modified glassy carbon electrode for O₂^{•−} detection. (B) Effects of PLX4032 treatment on O₂^{•−} generation from A375^{BRAFV600E} (A375) and MV3^{BRAFV600E} WT (MV3) cells; (C) rGO-CeO₂ nanomaterial modified glassy carbon electrode for NO detection. (D) Effects of PLX4032 treatment on NO production of A375 and MV3 cells.

GO: Graphene oxide; rGO: Reduced graphene oxide.

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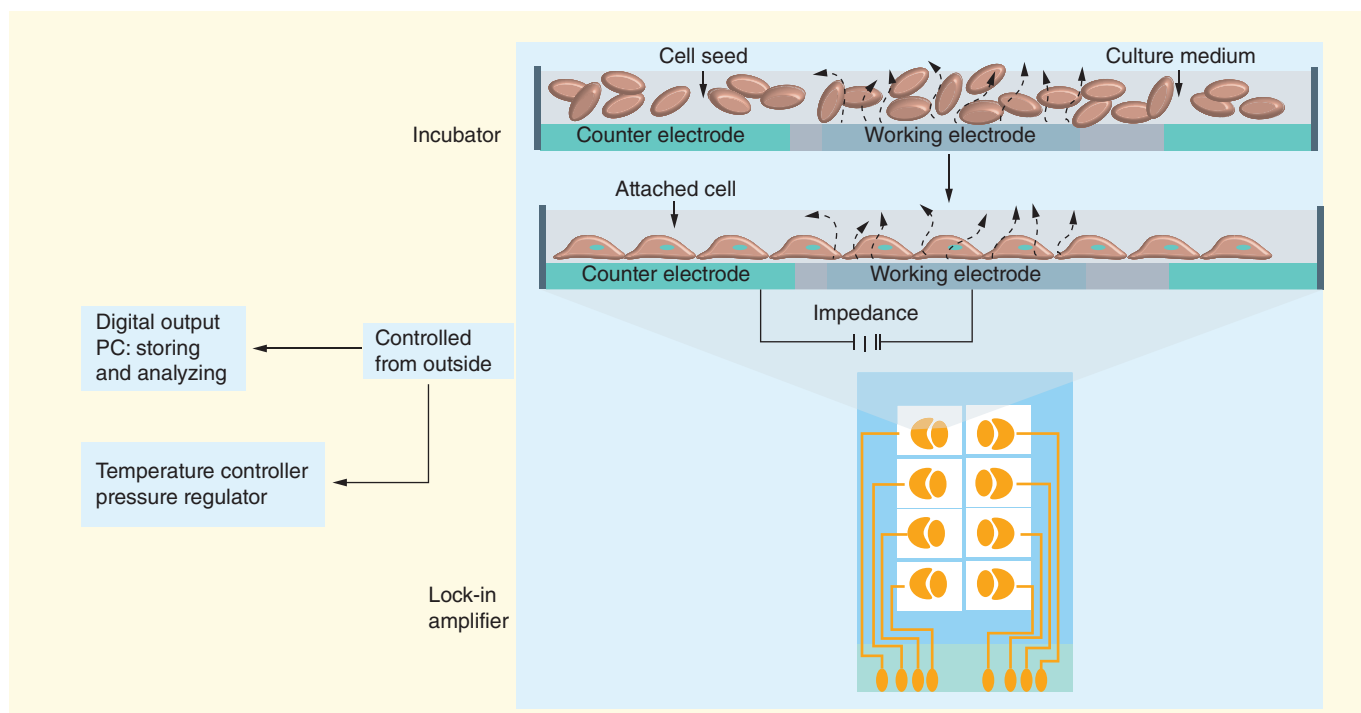


Figure 4. The electric cell-substrate impedance sensing method working principle.

a consequence of treatment with potentially cytotoxic agents [76]. The microfabricated cell chip system provides an easy and real-time monitoring tool for cytotoxicity test.

Electric cell-substrate impedance sensing (ECIS) is another important electrochemical technique. The schematic diagram of ECIS method working principle is shown in Figure 4. As a bioactive platform for cell adhesion, the sensors can evaluate the dynamic information by monitoring current and voltage variations. When cells lose attachment, electric current in the form of ions can flow freely from the surface to the electrodes, while attachment of cells to the electrode surface can hinder the free electrons flowing to the electrode surface and increase the resistance system. Therefore, ECIS can be used for real-time monitoring of cell adhesion, spreading, mobile and other dynamic information [77,78]. ECIS has been widely applied to drug development such as early safety assessment and mechanism study of cellular metabolism [79–82]. Michaelis *et al.* have applied this technology to monitor the interference of soluble RGDS peptides with already established cell layers [83]. They also studied the attachment and spreading of epithelial Madin-Darby canine kidney (MDCK) cells on different protein coatings, and investigated the influence of divalent cations on spreading kinetics [54]. Bennet *et al.* reported a novel ECIS-based analytical tool for real-time measurement of the luminous effect on the time responses function of retinal ganglion

cells (RGC-5) [53]. Not only the protective effects of analyzed drugs (such as β -carotene, quercetin, agmatine and glutathione) on RGC-5 cells, but also the maximum drug activity of nontoxic safe dose was determined by ECIS, which was very consistent with standard biological measures. These studies implicate that electrochemical strategies would be of broad interest in the field of therapeutics.

Limitations of electrochemistry & challenges in drug development

Electrochemistry methods have strong potentials to be employed in a broad range of applications, but they cannot replace traditional metabolism studies in biological systems. As a particularly useful complementary technique for synthesis of metabolite standards, the absence of onsite measurement, long duration of measurement, specificity and lower accuracy remains challenging. For example, hydrogen peroxide and ascorbic acid are particularly serious interferences perplexing the electrochemical detection of $O_2^{\bullet-}$, as they are coexisting biological compounds widely existing in biological systems and possess electrochemical oxidative activity [84]. To avoid false signals and exclude interference, suitable electrode modified materials are required for the improvement of specificity in electrochemical assessment. Additionally, for stable and reactive metabolites, although metabolic pathways can be mimicked by electrochemistry oxidations, the related methods are not good candidates

as general tool to predict the metabolic fate of all drug molecules in biological systems [85,86]. To better explore and expand electrochemistry technology as a complement to traditional metabolism studies in drug discovery and development, continuous efforts should be made by coupling electrochemistry online with immobilized conjugative enzymes; modifying electrode surfaces with native enzymes or less complex enzyme mimics; evaluating the use of electrochemistry methodology with respect to reductive metabolism; combining electrochemistry oxidations with complementary nonbiological systems; advancing in the fields of miniaturized electrochemical cells for EC–MS and increasing commercial availability of preparative electrochemistry microfluidic systems capable of delivering electrochemical yields of >100 mg/h [87].

Conclusion

Electrochemistry provides a simple and robust platform for stability testing and identification of potential metabolites, making it ideal for characterization of complex samples, such as natural products, biological tissue and fluids, to achieve the lowest possible detection limits for oxidizable and reducible compounds. With the numerous advantages, electrochemical techniques hold great promise for: detecting and monitoring of biochemical processes in living systems *in vivo* and *in vitro*; developing of novel analytical approaches, suitable not only for laboratories, but also industry; the discovery of new drugs and many other goals in a cost- and time-effective means.

Future perspective

The ongoing goal of medicine to better treat patients requires a great deal of research from a wide variety of fields including electrochemistry. Electrochemistry has emerged as a robust alternative for drug evaluation and discovery. Currently, many suitable electronic bio-analytical tools and strategies have been developed and validated. The most frequently used techniques such as electrochemical biosensor, EC–MS and electrochemical microfluidics can be applied to analyze drug efficacy and properties. In addition, electrochemical methods can be applied for high-throughput drug screening and the high reliability of analytical information obtained has made it possible to speed up the process of drug discovery. Although electrochemical methods will not completely replace the conventional studies in biological systems, they help us further investigate the biological reaction pathways and interaction of biological macromolecules. The full potential of electrochemistry in drug studies is only just starting to be discovered, and significant new applications in this field can certainly be expected in the near future.

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Executive summary

- Electrochemistry is a complementary technique to conventional *in vivo* or *in vitro* metabolism studies, and delivers the oxidative metabolic fingerprint of a molecule/drug in a very short time.
- Compared with conventional methods, electrochemistry exhibits many striking advantages including generation and direct identification of both stable species and metabolites in a rapid, sensitive and clean manner.
- Various electrochemical methods have been widely applied to mimic drug metabolism and the reactivities of metabolites toward biomolecules before clinical trial.
- Electrochemistry in combination with other technologies such as microfluidics and mass spectrometry creates powerful platforms to simulate various oxidation and reduction processes, which promote the development of novel drugs.
- Electrochemical, biochemical and medical knowledge can be integrated to develop strategies for the development of redox-selective therapeutics and mechanistic studies.

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