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Review Article

Nanomaterials in Label-free Impedimetric Biosensor: Current Process and Future Perspectives

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Abstract Today, the application of nanomaterials has been gaining prominence in the field of biosensing technologies as it offers more and more impact studies to improve the sensitivity, specificity, speed of response and cost effectiveness. Especially in electrochemical impedance spectroscopy technique, an outstanding electrical biotransducer, nanomaterials have been extensively used in almost all studies over the past decade. The nanostructures used in the construction of sensing devices vary in size, shape and physicochemical properties, such as nanoparticles, nanotubes, nanosheets, nanoelectrodes or nanochannels. The use of nanomaterials in impedimetric biosensor involves two key principles: (1) to develop a new sensing substrate via surface modification techniques with the aim of increasing the impedimetric response and (2) to develop a compatible sensing platform to facilitate the detection based on the resemblance in size between targets and signal transducers. Herein, the review shows the recent trends of using engineered nanomaterials in impedimetric biosensors as a factor of signal amplification. The detection events are diverse from biomolecular recognitions including enzymes, proteins, nucleic acids, etc. to whole cells monitoring. Additionally, the shortcomings of current techniques and future perspectives of impedimetric point-of-care devices are also included.

Keywords: Electrochemical Impedance Spectroscopy (EIS), Nanomaterials, Impedimetric biosensors, Signal amplification, Label-free

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Introduction

Label-free Impedimetric Biosensor

As soon as the impedimetric spectroscopy technique has been applied to the field of biosensor development in the 1980s[1,2] the new generation of advanced detecting and monitoring devices has been raised. The number of researches on impedimetric biosensor has increased significantly, focusing on the advantages of versatility, facile manipulation, rapid response, miniaturization capability and readiness for Lab-on-a-chip (LOC) integration. By using a small amplitude sinusoidal excitation signal, impedimetric biosensors offer the high sensitivity, low cost, undameged and continuous measurement to detect very low concentrations (as low as femtomoles) of clinically relevant analytes[3]. The targets for detection are diverse in form and size, including enzyme[4], antibodies[5], nucleic acids[6], protein biomarker[7], mammalian cells[8] and microorganisms such as bacteria[9] and viruses[10]. Each detection target requires different sensing strategies and device configurations. Three basic parts which construct biosensor system are biorecognition element, signal transducer and a readout display. The operation of entire system can be described in three specific steps. First, the analyte of interest and biorecognition elements will interact and bind together via various interaction mechanisms. The alteration of surface components then leads to the increase or decrease of electron transfer between analyte and electrode, which will be recorded by the electrical transducer. Eventually, the difference in sig-

nal will be presented by the readout display module after several steps of interpretation, filtering and amplification. The indication can be either in numeric or colorimetric display.

The most common way to present impedance data obtained by EIS measurements is using a Nyquist plot as showed in Figure 3C. Fundamentally, the real part (Z') and imaginary part (Z") of impedance data will be plotted in a coordinate axes, forming a semi-circular trace, which reflects the conductive behaviour of the sensor at different frequencies. For a particular approach, the features of the Nyquist plot can be related to the equivalent circuit of which components represent the specific impedance changes upon the analyte binding. The common equivalent circuit includes the solution resistance (R_s)-which affected by the conductance of ions in the bulk solution, the constant phase element (CPE) in parallel with the charge transfer resistance (R_{et})-both can be altered by analyte binding due to the alteration of insulating layer on the sensor surface, and the Warburg impedance (W)-only observed in the case of Faradaic sensors, reflecting the electron mediator diffusion to the sensor surface at a finite rate. Thus, R_s and CPE are the parameters which are often plotted against analyte concentration for constructing calibration curves of the sensor. The value of individual component can be calculated by extrapolating the trace back to the axies or using supporting software (e.g. ZView, Scribner Associates Inc.).

Impedimetric biosensor is the latest subset of the electrochemical biosensing technology, which can employ a wide range of bioreceptors, including enzymes, nucleic acids, antibodies or even whole cells, due to the negation of using a redox-active or charged analyte as being used in conventional biosensor system. Instead of being subject to the enzymatic reaction to alter levels of redox-active product, impedimetric biosensing technique directly exploits the interfacial properties change, such as electric current flow, charge transfer resistance or double layer capacitance upon analyte binding. Hence, the impedimetric sensing appear to be a predominant technique used for the investigation of both bulk and interfacial physicochemical properties of the electrode systems. The change in impedance value is proportional to both biochemical reaction rate and the related analyte concentration. Impedimetric sensor is a general term used to describe a group of sensing devices of which operation is based on the impedance changes of desired target. For more detail, there are several ways to categorize and cluster these systems. One such taxonomy centers on the target of detection e.g. enzyme, antibody, DNA, foodborne pathogen and cellular impedimetric biosensors. This taxonomy presents a paranomic view of the diversity in application usage, however, there is an overlap in the aspects of detection mechanism. Another, more common way, is based on the biorecognition elements, which is assessed as the most influential part of every label-free biosensor system. By this point of view, biosensor using impedance measurement is categorized into various subsets, including antibody-antigen based (immunosensors), aptamer-based (aptasensors), enzyme-based and cell-based impedimetric biosensor[11]. This kind of arrangement is more preferable by the researchers who are focusing on improving the key performance parameters of sensing devices, such as sensitivity, specificity, stability and multiplexing capabilities for parallel recognition.

Since the trends of biosensor research and development have been arisen from 1990s, there are number of efforts to optimize and improve the operating conditions of impedance biosensors, making such devices more functional and reliable. Various strategies have been proposed and studied, including the engineering of novel electrode design, the using assisted nanomaterials to functionalize transducer surface and the tethering of bioreceptor. In the contemporary time, the emergence of nanotechnology has opened up new horizons in every aspect of scientific research. Nanostructured materials have been extensively used in almost all biosensor models as the biomolecule immobilizing matrices/supports to improve electrochemical detection[12-14]. Particularly, the invention of silica-based and carbon-based nanomaterials has narrowed the gap between materials sciences and the biosensor related studies. Silica based nanomaterials have been proven to be an ideal protein host since they are highly chemically and thermally stable[15,16] with large surface area for functionalization and fine suspendability in aqueous solution. In biomedical-oriented research, they are also environmentally inert and nontoxic at low dosages[17,18]. In another trend, carbon based nanomaterials (e.g. graphene-GR, carbon nanotubes-CNTs and carbon nanofibers-CNFs) are popular used for electrode matrixes fabrication as well as in nanoprobe, nanocarrier engineering due to their advantages of high electrical conductivity[19], large surface area[20], easy functionalization[21,22], biocompatibility[23,24] and a low cost.

The use of nanomaterials in impedimetric biosensors is widespread and diverse, however, the general view has not yet stated. Herein, the current state-of-the-art sight of using nanomaterials as signal enhancement factor for impedimetric biosensors will be provided and exemplified with some of the most relevant researches in the last five years. In conclusion, the situation of commercialized products and the ultimate goal

towards personal impedimetric point-of-care devices are also brought to discussion.

Nanomaterials Used in Impedimetric Biosensors: Identification and Categorization

Impedimetric biosensor systems are relied on the Electrical Impedance Spectroscopy (EIS) technique[25], which combines the analysis of both resistive and capacitive properties which related to the biorecognition events occurring at the interface between electrode and analyte. In the context of this review, we classify the purpose of nanomaterials applications within the impedimetric biosensors into two aspects. The more popular one is that nanomaterials can be exploited to construct new sensing platforms via electrode modifications with the aim of increasing the surface area for receptor-analyte interactions and/or facilitating the involved electroanalytical activities. A range of nanomaterials have been employed in this way with remarkable successes in signal enhancement, including nanoparticles, nanotubes, nanowires and nanostructured silicons/polymers. This aspect is preferably used in EIS system to amplify the impedimetric response upon target binding. The other aspect focuses on the development of nanoengineered sensing structure (i.e. nanoelectrodes, nanopores and nanochannels) which facilitate the detection based on the resemblance in size between targets and signal transducers (Figure 1). In fact, there is no obvious boundary between these different applications when coordination model of multifunctional nanomaterials has become a recent promising trend of almost all biosensor systems, making it more efficient and user-oriented[26].

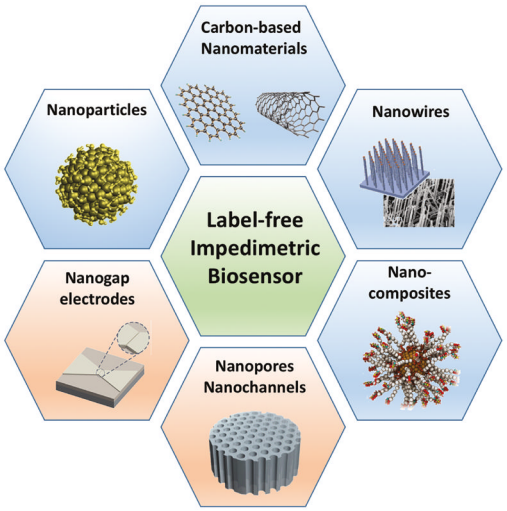


Figure 1. Applications of nanomaterials in label-free impedimetric biosensors.

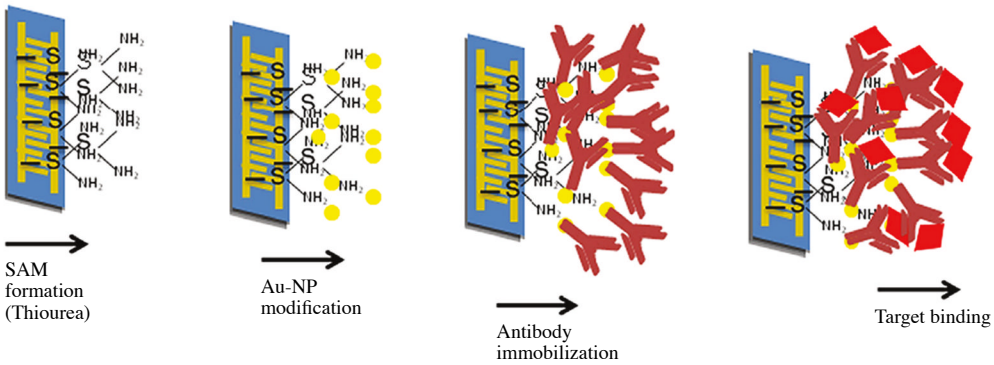
Using Nanomaterials as the Electrode Modification Factors

Metal and Metal-oxide Nanoparticles

Up to now, nanoparticles absolutely rank as the predominant materials used for electrode modification and still highly attract the research interest in biosensor technology. There are several good reasons to justify the notice, including high surface-area-to-volume ratio, easy rationally designed capability (geometry, size and distribution), flexible surface for molecular functionalization, electrocatalytical properties and facilitation of direct electron transfer in mediatorless biosensor devices. Precious metal, such as Au[27], Ag[28], Pt[29] and Pd[30], have been preferably selected for electrode modification due to their inertness against oxidation reactions and good biocompatible properties. Additionally, metal oxide nanoparticles (i.e. ZnO[31], CuO[32], NiO[33] and TiO_2[34]) with similar advantages but require lower cost and simple fabrication protocols, have been also extensively used in faradaic biosensors to promote faster electron transfer kinetics between the electrode and the active sites of biomolecules. These nanoparticles can be employed to modify the sensing substrate by either arranged on the electrode surface or mixing with the other components in the composite electrode matrix (e.g. polymeric or sol-gel materials)[35].

One of the main goals of using nanoparticles is that the high and flexible surface area allows the larger biomolecule target loading upon immobilization, creating a higher impedance response in non-faradaic sensing models. Altintas *et al*. have amplified the sensitivity of capacitive sensor platform via interdigitated electrode (IDE) transducer modified using gold nanoparticles (GNPs)[36] (Figure 2). Carcinoembryonic antigen (CEA) and epidermal growth factor receptor (hEGFR) could be successfully detected in the concentration range of 20-1000 pg mL^{-1} while cancer antigen 15-3 (CA15-3) was detected in the range of 10-200 U mL^{-1}. The sensitivity increased by six-fold with respect to those not modified.

The other expected goal is that metal and metal-oxide nanoparticles have been used to enhance the electron communication rate between redox active species and electrode surface in faradaic biosensors. Mashhadizadeh and Talemi have developed the magnetite and gold nanoparticle modified carbon paste electrode (CPE) for quantification of Hepatitis B virus (HBV) DNA[37]. The presence of gold nanoparticles and magnetite on the carbon paste electrode surface had synergistic effects, which enlarge the surface area and improve charge transport characteristics of the electrode,



	Standard assay (label-free methodology)	Au-NP modification
Concentration range (ng mL^{-1})	0.1-10	0.02-10
%|ΔC| for 10 ng mL^{-1} IL-6	~35	~195
%|ΔC| for lowest IL-6 concentrations	~32	~185
Detection limit (ng mL^{-1})	0.1	0.02
Signal increase (fold)	1	~6

Figure 2. The principle of the applied bioassay using the Au-NP modification and the comparison of IL-6 (Interleukin 6 protein) detection for standard (label-free methodology) and Au-NP modified capacitive sensor platforms. The capacitive relative change-|ΔC| upon target binding has significantly increased approximately 6 times when using electrode with Au-NP modification (Image reproduced from ref. 36 with permission. Copyright 2014, Elsevier).

increasing the sensitivity for DNA hybridization. The detection limit was at $3.1 (\pm 0.1) \times 10^{-13}$ M, which was greatly lower than the detection limit reported with the control electrode with magnetite alone.

Other studies attempted to couple different material types to maximize electron transfer facilitation. Anusha *et al*. combined the advantageous features of nanomaterials (ZnO + Pt) and chitosan biopolymer to enhance the glucose detection sensitivity (62.14 μA mM^{-1} cm^{-2}) in a wide linear range[38]. Final results indicated that Pt nanoparticles in the ZnO/Pt/CS/GOx electrodes demonstrated an excellent electronic conductivity as well as good biocompatibility and enhances the electron transfer between glucose and the electrode surface.

Carbon-based Sensing Platforms: Carbon Nanotubes and Graphene Nanosheets

Carbon nanomaterials (CNs) is the general term used to describe a group of nanosized, diverse allotrope materials originated from carbon, including graphene (GR), carbon nanotubes (CNTs), carbon dots (CDs), carbon nanofibers (CNFs) and buckminsterfullerence (C_{60}). With the great progress of nanotechnology in recent years, the roles of CNs in electrochemical biosensors have continuously expanded in various application aspects from base electrode materials to electrode modifications at the nano-scale. GR and CNTs are the most preferable materials used for impedimetric biosensors, thanks to the prominent advantages of high electrochemical activity[39], high electrical conductivity[40], large surface area[41], easy for functionalization and biocompatibility[42].

Up to the present time, many kinds of GR derivatives have been synthesized and utilized in electrochemical sensing protocols, including graphene oxide (GO) and reduced graphene oxide (rGO). GO can be synthesized by exfoliation of graphite oxide in water using sonication, ultimately producing single or few layer of graphene[43]. GO substantially increases the hydrophilicity of the graphene layers by the presence of oxygen-containing functional groups on its surface, which facilitate the functionalization of biorecognition elements by providing more surface area for molecular binding[41]. These oxygen-containing groups also can be eliminated via various ways (e.g. chemical treating[44], heating[45], linear sweep voltammetry applying[46]) to create rGO with excellent conductivity for electrode modifying purposes[47].

On another approach, single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotube (MWCNTs) are two basic forms of CNTs, which possess almost all of the aforementioned advantages of GR materials, making them become the second most

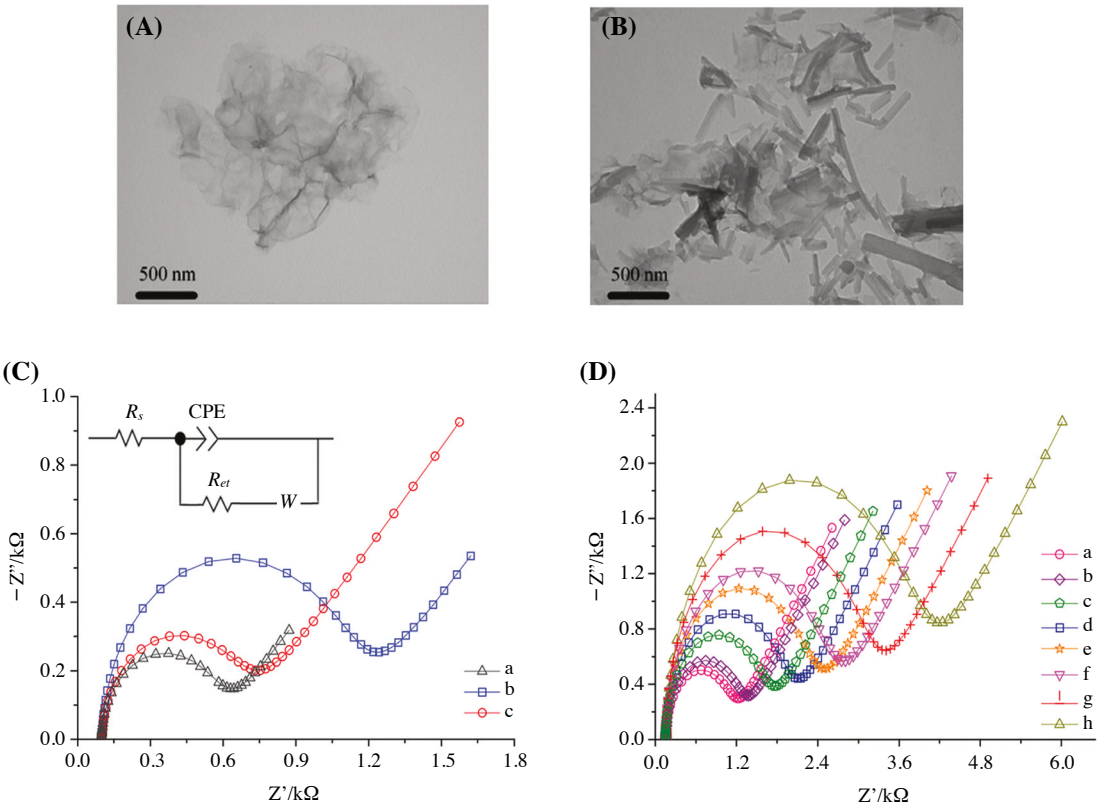


Figure 3. TEM images of (A) graphene and (B) PDI/graphene. (C) Nyquist diagrams obtained at bare GCE (a), graphene modified GCE (b) and PDI/graphene modified GCE (c). (D) Nyquist diagrams recorded at ssDNA immobilized PDI/graphene platform modified GCE (a) and after hybridization with its complementary HIV-1 pol gene sequences of different concentrations: 1.0×10^{-12}, 1.0×10^{-11}, 1.0×10^{-10}, 1.0×10^{-9}, 1.0×10^{-8}, 1.0×10^{-7} and 1.0×10^{-6} M (b-h). (Image reproduced from ref. 53 with permission. Copyright 2012, Elsevier).

popular nanomaterials for electrochemical biosensors. Moreover, thanks to the heterogeneity in 3D structure and composition[48], different electrochemical effects could be induced upon changing the orientation and arrangement of CNTs on the electrode surface[49,50].

Based upon the above advantages, GR and CNTs can be utilized as either a nanoprobe, relying on their intrinsic electrochemical properties, or a nanocarrier, relying on the other multiple properties (i.e. large surface area and easy modification). Constructing the graphene-based impedimetric biosensors are diverse in method and decipline, however, there are two main issues to be considered to optimize the sensitivity. The first one is that the composition of graphene layer immobilized on sensing substrate can affect its electrochemical properties. As reported by Ambrosi *et al.*[51], the impedimetric assay was strongly affected by the density of oxygen-containing groups due to the higher negative charge of the graphenic surface that made a higher initial charge transfer resistence R_{CT}. Bonanni & Pumera also developed a sensing platform with 3-4 layers of rGO for physisorption of hairpin DNA capture probe[52]. The final detection of complementary ssDNA are more robust and sensitive with LOD at 6.6 pM compared to 50 nM of a single layer platform. The second aspect to be considered is that the composition of graphene-related nanomaterials strongly affect the immobilization of biorecognition elements and the involved electrochemical activites. Hu's group constructed an efficient DNA impedance biosensing platform with chemically modified rGO using positively charged *N*,*N*-bis-(1-aminopropyl-3-propylimidazol salt)-3,4,9,10-perylene tetracarboxylic acid diimide (PDI), which introduce moieties for chemical coupling of DNA probes[53] (Figure 3). Impedance value of the modified graphene platform increased after probe DNA immobilization and hybridization to its complementary sequences. Another trending development is coupling model between graphene-related materials with metal nanomaterials (MNs) to improve the charge transfer event upon target binding. A model of impedimetric sensor based on an indium tin oxide (ITO)

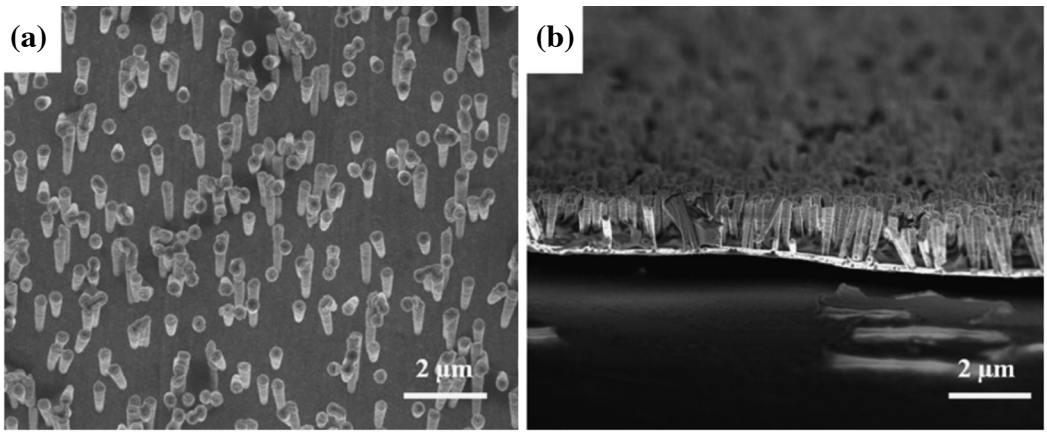


Figure 4. FE-SEM images of the AuNWsA (a) top view and (b) cross-sectional view. (Image reproduced from ref. 69 with permission. Copyright 2013, Elsevier).

electrode array functionalized with reduced graphene oxide-nanoparticle (rGO-NP) hybrid was developed by Yagati *et al*.[54]. The final results confimred significant changes in R_{CT} upon binding of C-reactive protein with the LOD at 0.08 ng mL^{-1} in human serum. Other studies using Ni[55], Cu[56], Sn[57], Zn[58], Ce-Pt[59] and Fe_3O_4[60] was also reported.

Nanowires: Enhanced Electrochemical Signals Provide Heightened Sensitivity

Nanowires (NWs) have emerged as one of the promising class of functional materials for their versatile roles, not only in high throughput optoelectronic devices but also in ultrasensitive, direct electrical detection of biological and chemical species[61,62]. Using nanoscale objectes as ensemble transducer, nanowire electrodes can generate nano- to microamps currents that are easily measured by simple and inexpensive instrumentation. Compared to other counterparts, NWs possess high surface area, high surface to volume ratio, unidirectional conduction channels with outstanding electrical transport behavior, and diameters which are commensurate with the sizes of molecules being sensed. These aspects have generated considerable interest to use NWs as an effective bioelectrochemical transducer. Both conducting and semi- conducting nanowires have been utilised in impedimetric biosensing; examples include gold nanowires[63] and gallium nitride nanowires for DNA sensing[64], titanium oxide nanowires for bacterial sensing[65] and silicon nanowires for the detection of hepatitis B and liver cancer biomarkers[66].

NWs based sensing platform can be constructed either in single-crystal, 1-D nanostructure[67] as in semiconducting field-effect devices or in 3-D collections of nanowires implemented as a sensing ensemble (nanowires array)[68]. Nanowires array structures are preferably used for impedance based measurement due to large target binding area and easy route to a nanostructured material without the need for expensive or difficult nanofabrication. Moreover, the conducted structures act as excellent electrodes, permitting the use of straightforward solution electrochemistry for sensitive biomolecular detection. In other approaches, the performance of nanowire arrays is highly dependent on the fabrication techniques, which control the structural parameters, such as length, diamater, crystallinity and ordered orientation. Ramulu *et al*. developed a DNA biosensor based on vertically aligned gold nanowires array (AuNWsA) by two steps electrodeposition (ED) method using a novel electrolyte and polycarbonate (PC) membrane interface[69]. The morphological studies of AuNWsA revealed that the nanowires were well-aligned and strongly attached to the Au thick film (Figure 4), providing a better electron transfer ability to detect specific hybridized DNA in a low concentration at 6.78×10^{-9} M, which is two times smaller than that of conventional technique. The development of novel materials for NWs electrode fabrication is also receiving significant attention as in the study of Mostafalu and Sonkusale[70]. Different types of metal nanowires with different lengths were grown on paper substrates for the first time, using a template-assisted electrodeposition and simple adhesive tape-based patterning at room temperature. The approach was used to make dry paper-based nanowire electrodes that exhibit excellent electrode-tissue impedance suitable for recording of electrocardiogram signals without any wet-gel adhesives.

Nanocomposite, a New Trend in Impedance-based Biosensors

Nanocomposites are solid materials composed of two or more phases where at least one of the phases is engineered in nanoscale dimensions[71]. The integral chemical and physical properties of composite materials, therefore, are the altogether unique and fascinating mechanical, electrical, thermal, optical, electrochemical, and catalytic properties of individual embedded phase. This outstanding feature makes nanocomposites currently become one of the most trending strategies in the development of impedimetric biosensors.

Basically, the nanocomposite consists of two composition: the solid matrix phase for platform shaping and the reinforcing phase for facilitation. In impedance based biosensors, conducting polymers such as chitosan[72], polyaniline (PANI)[73] and polypyrrole (PPy)[74], are mainly used to construct the matrix phase due to the attractive biocompatible, biodegradable, nontoxicity and excellent film-forming ability[75-77].

The reinforcing material, which aimed to enhances the impedimetric response, can be made up of nanoparticles (e.g. metal[78], metal oxides[79]), nanosheets (e.g. graphene oxide[77]) or nanotubes (e.g. CNTs[80]). Fu *et al*. developed a polyaniline/graphene oxide nanocomposite (PANI@GO) that was electrochemically codeposited on indium tin oxide (ITO) electrode for the detection of CSPG4-a tumor associated antigen expressed in malignant cells[73]. The results showed that the impedance signal increases, corresponds to expression of CSPG4 in both culture medium and lysate protein with alternative sensitivity to ELSIA and flow cytometry assay. In another study, glucose could be detected with LOD of 0.1 mM using simple composite material of GNPs/PANI on glassy carbon electrode (GCE)[81]. Using GNPs/PANI for oxidation of glucose instead of glucose oxidase enzyme (GOx), the authors successfully developed a non-enzymatic impedimetric glucose sensor with high sensitivity, good reproducibility, and 2-week stability at 20°C condition.

Engineered Sensing Structure at Nanoscale

Nanopores and Nanochannels Array

Detection of biomolecules using nanopores and nanochannels has attracted considerable attention due to the high surface area of the pores and similarity with nanopores in biological systems meaning there is the potential for new and advanced biosensing devices[82,83]. In nanochannels-based biosensing system, concentration of analytes can be evaluated by simply measuring changes in the electrical conductance between two compartments separated by a single microchannel or microchannel array when such analyte penetrate in and be anchored within the channel (Figure 5). The detection targets are diverse from DNA[84], protein[85], enzyme[86], pathogenic bacteria[87] to gases and vapours[88]. The impedance sensing can be performed using either faradaic or non-faradaic model. However, non-faradaic model is preferably used due to the eliminating the need of redox species or signal enhancer, making it particularly well-suited for the detection of binding events inside the pores.

The most popular nanochannels-based platform used with EIS technique are those consisting in anodic aluminum oxide (AAO) nanoporous membranes[89], since the electrical characteristics of AAO can be easily adjusted to an equivalent circuit[90]. AAO membranes prepared by electrochemical anodization process have been very attractive for development of nanopore biosensing devices, due to their uniform pore size, high aspect ratio, high surface area and straight-forward and inexpensive fabrication[91].

Nagaraj and his group was successful improved the detection of pharmaceutical contaminants in water by combining the strategies of EIS and nanochannels integration. The sensor was able to detect the presence of ibuprofen in a 100 mL sample within 15 minutes at 0.25 pg mL^{-1} concentration, without using redox probe or molecular tags[92]. Pathogenic bacteria such as *S. aureus* and *E. coli O157:H7* have also been evaluated using similar principles by Tan *et al*., reaching the LOD at 10^2 CFU/mL with high specificity[93].

In order to optimize the nanostructure for non-faradaic sensing model, Kant *et al*. also reveal the influence of pore dimension on biosensing performance by covalently attaching between streptavidin and biotin[94]. The AAO membrane was prepared in different pore sizes and length, followed by the functionalization of streptavidin on the inner surface of the pores, creating a covalent binding site for biotin molecules. Final results indicated that lowering nanochannel diameters (<30 nm) provides better response and sensitivity. However, longer pores (>10 μm) are also not favourable for non-faradic EIS detection due to the higher resistance and long time for diffusion of analyte molecules inside the pores. Hence the optimization of nanochannel dimensions is also one of the critical factors that have significant influence on the performance of nanopore based electrochemical biosensing devices.

Nanogap Electrodes

EIS is an electrical based technique for detecting, quantifying and characterizing systems of interest. The sensing activities are mostly taken place in ionic aqueous environment, where the electric field was applied

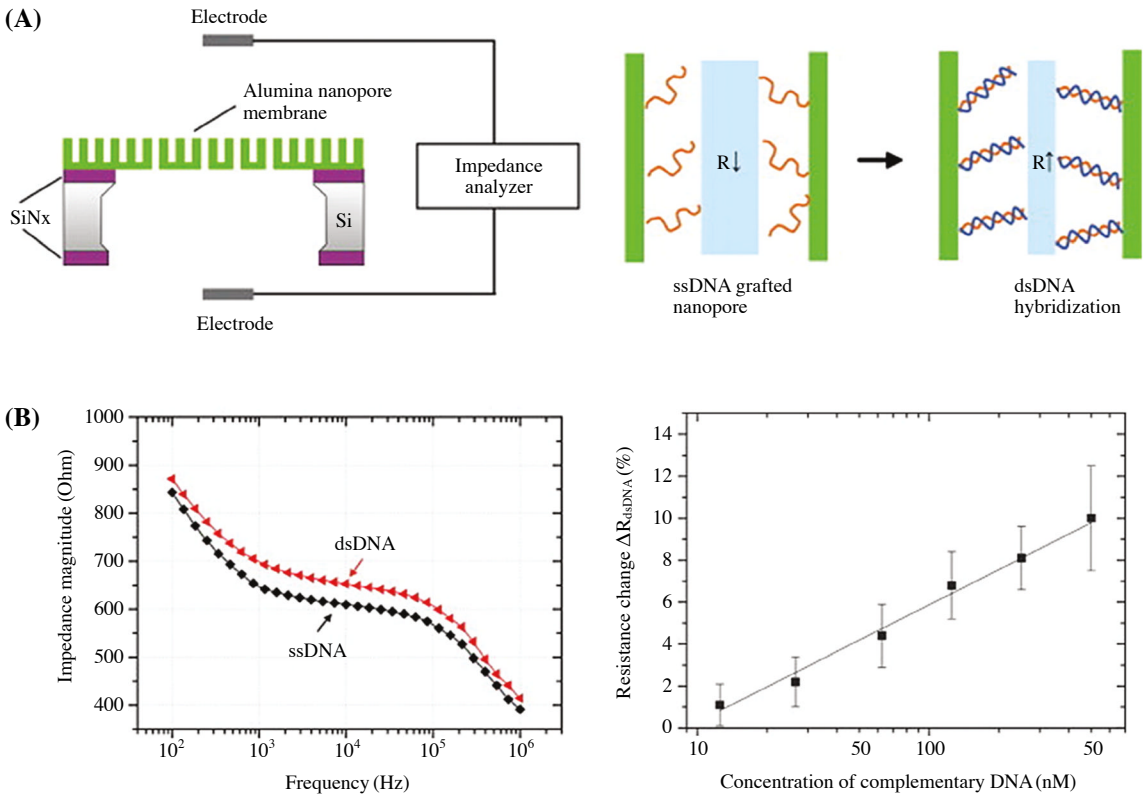


Figure 5. DNA impedimetric detction using AAO nanoporous membranes. (A) (Left) Schematic of a impedimetric DNA sensor. The nanoporous membrane is sandwiched between two half cells where a pair of Ag/AgCl electrodes are inserted and connected to an impedance analyzer; (Right) Schematic of impedance increase due to the blocking of nanopores after dsDNA hybridization. (B) (Left) Impedance change of an ssDNA grafted alumina membrane after hybridization of double-stranded DNA (dsDNA, 25 bases); (Right) Plot of resistance change values during dsDNA hybridization versus the logarithm of complementary DNA concentrations. (Image reproduced from ref. 83 with permission. Copyright 2016, Elsevier).

to generate a desired electric current passing through a bulk solution and to record the physicochemical changes occurring on the electrode surface. However, impedance spectroscopy in ionic solutions is impeded by the forming of charged layer called electric double layer as a result of the unequal distribution of cations and anions in the vicinity of the charged surface[95]. This cause a major problem for the detection when the applied electric field does not appear on the significant portion of the target medium. One strategy to eliminate the influence of double layer effect is increasing the Debye length by decreasing the ionic strength of the solution. However, it leads to the need of additional separation and purification steps for medium replacement. Another strategy of dealing with this problem is decreasing the electrode separation distance (Figure 6) to warrant the electric field uniformity within the target medium, which inspires researchers to the study of nanogap electrodes[96].

The term nanogap refers to the arrangement of two electrodes with the separation distance no more than 300 nm, which represents the practical upper limit of the characteristic thickness of the electrical double layer. This limitation creates an ideal detection region for DNA's[97], protein[98] and other biological molecules[99], which are all in nanometer-scale. Rational structure design of electrode is also diverse, based on the desired geometry of detection region as well as the accompanied measurement technique. 1-D nanogap with point-type gap junctions is typically used for single molecular detection by applying AC voltages to produce resistive measurements. 2-D nanogap with band-type gap junctions and 3-D nanogap with surface-type gap junctions are mostly used for the extrapolation of biological parameters (e.g. binding efficiency of biomolecules) from complex impedance response via AC measurement techniques (e.g. dielectric spectroscopy, EIS). Balakrishnan *et al*. demonstrated the 1-D polysilicon nanogap electrode with (3-aminopropyl) triethoxysilane (APTES) and glucose oxidase (GOx) surface

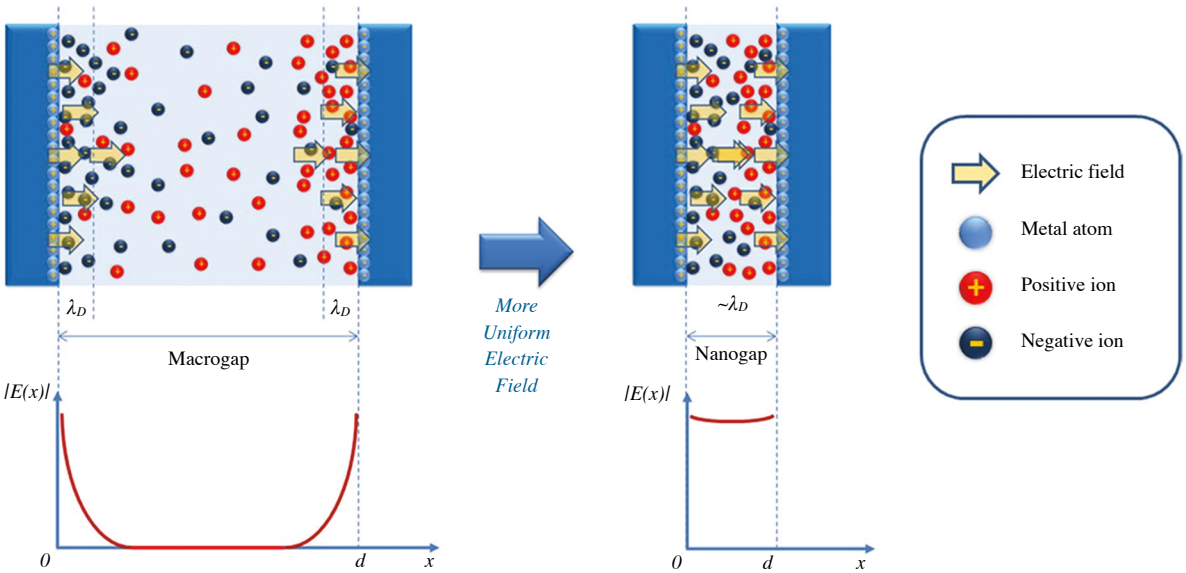


Figure 6. Electric field intensity between the two electrodes in a solution of macrogap electrodes and nanogap electrodes (Image reproduced from ref. 99 with permission. Copyright 2014, Spinger).

modification as a highly sensitive and non-invasive label-free glucose sensor[100]. Gap size was defined less than 100 nm to overlap double layer effect as well as reduce ohmic drop between the electrodes. Glucose detection was performed via both amperometric and impedimetric assessment to achieve the extremely wide linear ranging from 5 μM to 50 mM with low LOD at 0.6 μM. In another scheme, Singh *et al*. developed an integrative model of nanogap interdigitated electrode (IDE) array with assisted GNPs to enhance the sensitivity of affinity - based detection. With GNPs as signal enhancement factor, the transducer sensitivity was reported to be increased by 350% over that of label-free detection without nanoparticles[101].

Currently, the development of nanogap biosensors is still in the research phase, with no commercial device being available to satisfy the requirement of high throughput, selectivity and reproducibility due to the technical limitations in mass fabrication. However, the perspective of a highly sensitive, small volumes, label-free, low power consumption and all-electrical biosensing device is still alluring. Due to the special electrode geometry which help to overlap double layer effect, nanogap biosensors are able to directly detect specific proteins in serum or blood, offering novel and beneficial systems for the purpose of point-of-care and early disease detection.

Conclusion and Perspective

As demands continue to expand in biosensor technology, the use of labels in traditional detection assays has been criticized as inappropriate for the producing undesirable and unanticipated interactions that can compromise sensing performance and lead to false conclusions. Critics also argue that labeled technologies typically require certain skills to operate, that limits the commercial production of point-of-care devices for simple, rapid and economical testing near the site of patients. At this point in time, the development of label-free sensing technology is growing exponentially to overwhelm the drawback of labeled systems, opening up a promising future for a portable, low-cost, high-throughput, and highly sensitive analytical devices for both experimental analysis and point-of-care testing. Together with the advancements in nanotechnology and nanoscience, more attention is being paid to applying nanomaterials that allow the introduction of novel signal transduction technologies in biosensors.

Herein, we particularly focused on the applications of nanomaterials as signal enhancers for label-free impedimetric biosensing systems. A review covered a wide range of research fields, which utilized various types of nanoscale materials and structures to not only improve the electronic properties, increasing the effective electrode surface for transferring electrochemical signal but also produce detectable signals for direct detection of targets. The materials are diverse in size, shape, dimension, aspect ratio, compositions, as well as physical and chemical properties, which offers specific sensing strategies for the analyte of interest

detection. The synergy of multifunctional materials, specific recognition elements, and high performance electrochemical methods has improved the selectivity, stability, and reproducibility, thus promoting the development of sensors for biological applications and bioassays. Due to the vast number of different nanomaterials all with their own specific properties, only few recent and prominent studies could be mentioned to emphasize the principal advantages of such materials.

It is necessary to remark that there is a growing trend of using nanocomposite materials as the electrode modification factor to amplify the electrochemical signal. The efficient combination of different nanoscale materials with well conductive polymers may open up a new avenue for utilizing novel nanocomposites as enhanced elements for constructing high performance impedimetric sensing platforms. Matrix structure of nanocomposite and its thickness must be optimized to provide a larger binding area for dopant and desired analyte, while still facilitate the ion diffusion within the matrices. On other approach, nanogap electrodes are promising platforms which enable the detection of specific proteins directly in serum or blood by eliminating the double layer effect and ohmic drop, forfeiting the cost and complexity associated with conventional antigen-antibody assays.

In conclusion, the development of nanomaterials-assisted impedimetric biosensors is still in research phase that require the continuous efforts in developing novel materials for various targets, as well as the enormous potentials provided by these biosensors.

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