Nanomaterial-based electrochemical biosensors

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The unique properties of nanoscale materials offer excellent prospects for interfacing biological recognition events with electronic signal transduction and for designing a new generation of bioelectronic devices exhibiting novel functions. In this Highlight I address recent research that has led to powerful nanomaterial-based electrical biosensing devices and examine future prospects and challenges. New nanoparticle-based signal amplification and coding strategies for bioaffinity assays are discussed, along with carbon-nanotube molecular wires for achieving efficient electrical communication with redox enzyme and nanowire-based label-free DNA sensors.

1. Why nanomaterials?

The buzzword "nanotechnology" is now around us everywhere. Nanotechnology has recently become one of the most exciting forefront fields in analytical chemistry. Nanotechnology is defined as the creation of functional materials, devices and systems through control of matter at the 1–100 nm scale. A wide variety of nanoscale materials of different sizes, shapes and compositions are now available.[1] The huge interest in nanomaterials is driven by their many desirable properties. In particular, the ability to tailor the size and structure and hence the properties of nanomaterials offers excellent prospects for designing novel sensing systems and enhancing the performance of the bioanalytical assay. The goal of this article is to highlight recent advances in nanomaterials for such electrical sensing devices.

2. Nanoparticles, nanowires and nanotubes

Research efforts on metal and metal semiconductor nanoparticles have flourished in recent years.[2,3] Metal nanoparticles are generally defined as isolable particles between 1 and 50 nm in size, that are prevented from agglomerating by protecting shells. Owing to their small size such nanoparticles have physical, electronic and chemical properties that are different from those of bulk metals. Such properties strongly depend on the number and kind of atoms that make up the particle. Several reviews have addressed the synthesis and properties of nanoparticles.[2,3] Typically, such particles are prepared by chemical reduction of the corresponding transition metal salts in the presence of a stabilizer (capping agent such as citrate or thiol) which binds to their surface to impart high stability and rich linking chemistry and provide the desired charge and solubility properties. Designer particles, including colloidal gold or inorganic nanocrystals have found broad applications in many forms of biological tagging schemes. For example, colloidal quantum dots have been widely used for optical bioassays because their light emitting properties can be broadly tuned through size variation.[4] Recent years have witnessed the development of powerful electrochemical bioassays based on nanoparticle labels and amplification platforms.

One-dimensional (1-D) nanostructures, such as carbon nanotubes (CNT) and semiconductor- or conducting-polymer nanowires, are particularly attractive for bioelectronic detection. Because of the high surface-to-volume ratio and novel electron transport properties of these nanostructures, their electronic conductance is strongly influenced by minor surface perturbations (such as those associated with the binding of macromolecules). Such 1-D materials thus offer the prospect of rapid (real-time) and sensitive label-free bioelectronic detection, and massive redundancy in nanosensor arrays. The extreme smallness of these nanomaterials would allow packing a huge number of sensing elements onto a small footprint of an array device. Metal and conducting polymer nanowires can be readily prepared by a template-directed electrochemical synthesis involving electrodeposition into the pores of a membrane template.[5] Carbon nanotubes (CNT) are particularly exciting 1-D nanomaterials that have generated a considerable interest owing to their unique structure-dependent electronic and mechanical properties.[6] CNT can be divided into single-wall carbon-nanotubes (SWCNT) and multi-wall carbon-nanotubes (MWCNT). SWCNT possess a cylindrical nanostructure (with a high aspect ratio), formed by rolling up a single graphite sheet into a tube. SWCNT can thus be viewed as molecular wires with every atom on the surface. MWCNT comprise of an array of such nanotubes that are concentrically nested like rings of a tree trunk. The remarkable properties of CNT suggest the possibility of developing superior electrochemical sensing devices, ranging from amperometric enzyme electrodes to label-free DNA hybridization biosensors.[7] The tailored electronic conductivity of conducting polymers, coupled with their ease of processing/modification and rich chemistry, make them extremely attractive as 1-D sensing materials. Newly introduced CNT/conducting-polymer nanowire materials,[8] based on incorporating oxidized CNT as the charge-balancing dopants

within electropolymerized wires, should further enhance the sensing capabilities of 1-D materials.

In the following sections I will discuss how the unique properties of nanoparticles, nanowires and nanotubes can enhance the performance of existing electrochemical sensors and can lead to the creation of a new generation of bioelectronic devices.

3. Nanomaterial-derived electrochemical biosensors

Electrochemical sensors offer several distinct advantages. In particular, such devices offer elegant routes for interfacing, at the molecular level, biological recognition events and electronic signal-transduction processes. In addition, electrochemical devices are uniquely qualified for meeting the size, cost, low-volume, and power requirements of decentralized testing and indicate great promise for a wide range of biomedical or environmental applications.[9,10] Nanomaterials can be used in a variety of electrochemical biosensing schemes and the present article is divided accordingly. The organization of nanomaterials into controlled surface architectures is essential for the successful realization of these sensing protocols.

Nanomaterial-based enzyme electrodes

Enzyme electrodes have been widely used for monitoring a wide range of clinically or environmentally important substrates. An extremely important challenge in amperometric enzyme electrodes is the establishment of satisfactory electrical communication between the active site of the enzyme and the electrode surface.[11] The redox center of most oxidoreductases is electrically insulated by a protein shell. Because of this shell, the enzyme cannot be oxidized or reduced at an electrode at any potential. The possibility of direct electron-transfer between enzymes and electrode surfaces could pave the way for superior reagentless biosensing devices, as it obviates the need for co-substrates or mediators and allows efficient transduction of the biorecognition event. "Trees" of aligned CNT in the nanoforest, prepared by self assembly, can act as molecular wires to allow electrical communication between the underlying electrode and redox proteins (covalently attached to the ends of the SWNT).[12,13] Willner's group[14] demonstrated that aligned reconstituted glucose oxidase (GOx) on the edge of SWCNT can be linked to an electrode surface (Fig. 1). Such enzyme reconstitution on the end of CNT represents an extremely efficient approach for 'plugging' an electrode into GOx. Electrons were thus transported along distances higher than 150 nm with the length of the SWCNT controlling the rate of electron transport. An interfacial electron transfer rate constant of 42 s^{-1} was estimated for 50 nm long SWCNT. The catalytic properties of metal nanoparticles have also facilitated the electrical contact of redox centers of proteins with electrode surfaces. For example, gold nanoparticles were shown to be extremely useful as electron relays ("electrical nanoplugs") for the alignment of glucose oxidase on conducting supports and wiring its redox center.[15]

A wide range of enzyme electrodes based on dehydrogenase or oxidase enzymes rely on amperometric monitoring of the liberated NADH or hydrogen peroxide products. The anodic detection of these species at ordinary electrodes is often hampered by the large overvoltage

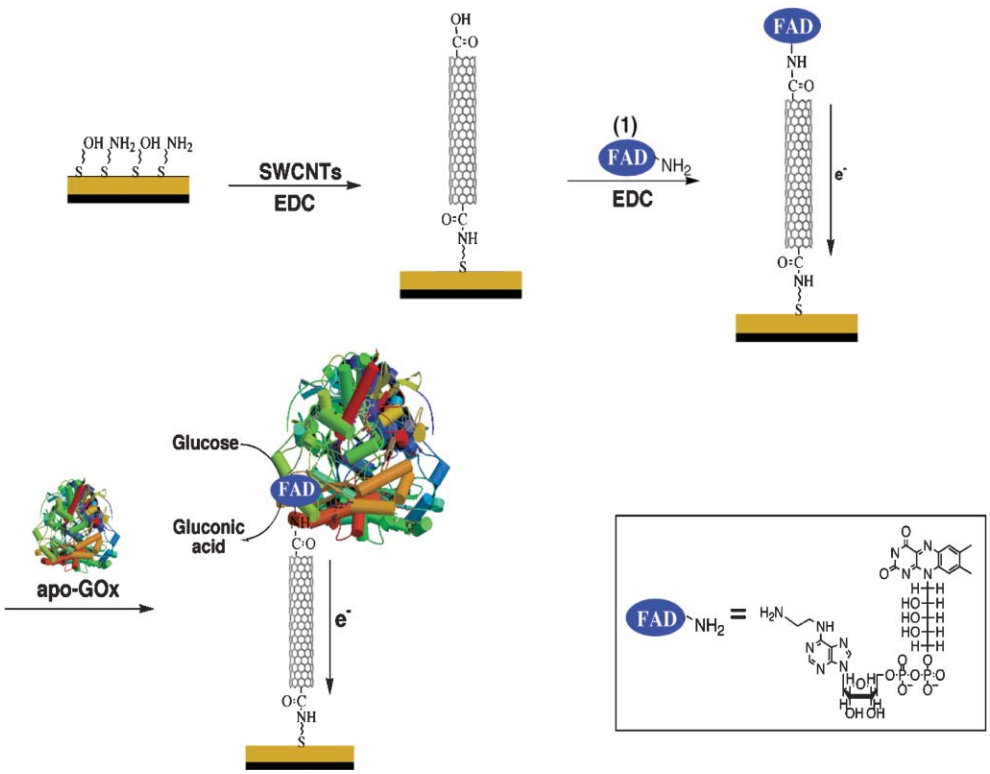

Fig. 1 Assembly of SWCNT electrically contacted glucose oxidase electrode: linking the reconstituted enzyme, on the edge of the FAD-functionalized SWCNT, to the electrode surface. (Based on ref. 14 with permission.)

encountered for their oxidation. The greatly enhanced redox activity of hydrogen peroxide[16] and NADH[17] at CNT-modified electrodes addresses these overvoltage limitations and makes these nanomaterials extremely attractive for numerous oxidase- and dehydrogenase-based amperometric biosensors. The ability of CNT to promote electron transfer reactions is attributed to the presence of edge plane defects at their end caps. Carbon-nanotube-modified electrodes have also been shown to be extremely useful for circumventing surface fouling associated with the oxidation of the liberated NADH product.[17] The deposition of platinum nanoparticles onto CNT has led to further improvements in the detection of the enzymatically-liberated peroxide species.[18] In addition to CNT films, it is possible to use CNT-based inks[19] and pastes[20] for designing screen-printed and biocomposite, respectively, amperometric biosensors. The excellent electrocatalytic properties of metal nanoparticles (compared to bulk metal electrodes) can also benefit amperometric enzyme electrodes. For example, Niwa and coworkers[21] dispersed iridium nanoparticles (2 nm diameter) in graphite-like carbon and used the resulting transducer for improved amperometric biosensing of glutamate.

Tao's group[22] described a conducting-polymer nanosensor for detecting glucose based on a pair of nanoelectrodes, separated with a small (20–60 nm) gap connected by a polyanaline/glucose-oxidase film. The remarkable small dimensions of the new device, coupled with its very fast response and minimal oxygen consumption, makes it attractive for *in-vivo* monitoring of glucose. Another promising and controllable route for preparing conducting-polymer nanowire enzyme sensors involves electrodeposition within the channel between electrodes.[23]

Nanomaterial-based bioaffinity electrochemical sensors

The development of electrical DNA hybridization biosensors has attracted considerable research efforts.[24,25] Such DNA sensing applications require high sensitivity through amplified transduction of the oligonucleotide interaction. Nanoparticle-based amplification schemes have led to improved sensitivity of bioelectronic assays by several orders of magnitude. In 2001 both my group[26] and that of Limoges[27] reported on the use of colloidal gold tags for electronic detection of DNA hybridization. This protocol relies on capturing the nanoparticles to the hybridized target, followed by highly sensitive anodic-stripping electrochemical measurement of the metal tracer. Analogous bioelectronic measurements of proteins based on sandwich immunoassays and gold nanoparticle tracers have also been reported.[28] Electronic DNA hybridization assays have been extended to other metal tracers, including silver[29] or iron.[30] Commonly we rely on the coupling biorecognition element to surfaces of magnetic beads, as it offers an effective minimization of non-specific binding. The hybridization of probe-coated magnetic beads with the metal-tagged targets results in three-dimensional network structures of magnetic beads, crossed-linked together through the DNA and gold nanoparticles. The 'magnetic' collection of such magnetic-bead/DNA/metal-label assembly onto the electrode leads to direct contact of the metal label and the surface and enables solid-state (chronopotentiometric) measurements without dissolving the metal tag.[31] This route could facilitate the creation of magnetically-addressable DNA arrays.

Several amplification processes can be used for dramatically enhancing the sensitivity of particle-based bioelectronic assays. For example, the metal nanoparticle tags can act as catalytic sites for the electroless deposition of other metals. Treatment of gold-linked DNA-hybrid assembly with silver ion in the presence of hydroquinone thus results in catalytic deposition of silver on the gold tracer (acting as catalyst), leading to a dramatic (>100 fold) signal amplification.[32] Instead of enlarging spherical nanoparticle tags, it is possible to enhance the sensitivity by using long nanorod tracers.[33] We also described a triple-amplification bioassay, coupling the carrier-sphere amplifying units (loaded with numerous gold nanoparticles tags) with the 'built-in' preconcentration of the electrochemical stripping detection and a catalytic enlargement of the multiple gold-particle tags[34] (Fig. 2). The success of these and other nanoparticle-based amplification strategies depends on our ability to maintain a low background response (through proper attention to the surface-blocking chemistry and wash conditions).

Inorganic nanocrystals offer an electrodiverse population of electrical tags as needed for designing electronic coding. We demonstrated the use of different inorganic-nanocrystal tracers for a multi-target electronic detection of DNA[35] or proteins.[36] Three encoding nanoparticles (zinc sulfide, cadmium sulfide and lead sulfide) have thus been used to differentiate the signals of three protein targets in connection with a sandwich immunoassay and stripping voltammetry of the corresponding metals (Fig. 3). Each binding thus yields a

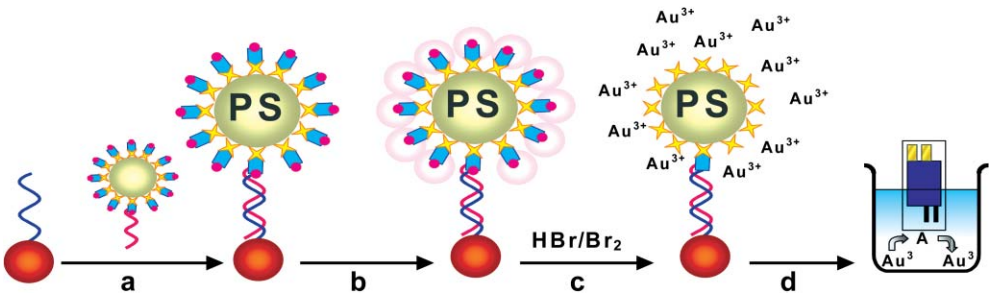

Fig. 2 Amplified bioelectronic detection of DNA hybridization, using polymeric beads carrying multiple gold nanoparticle tracers, catalytic enlargement of the gold particles and a stripping voltammetric signal transduction. (Based on ref. 34 with permission.)

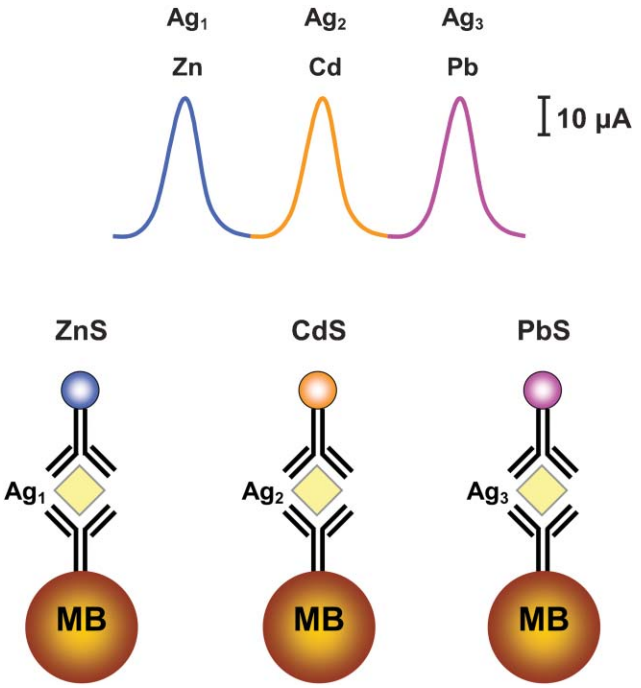

Fig. 3 Multi-antigen sandwich immunoassay protocol based on different inorganic-colloid (quantum dots) nanocrystal tracers. (Based on ref. 36 with permission.)

distinct voltammetric peak, whose position and size reflect the identity and level, respectively, of the corresponding antigen. The concept can be scaled up and multiplexed by using a parallel high-throughput automated microwell operation, with each microcavity capable of carrying out multiple measurements. Libraries of electrical codes have been created by encapsulating different predetermined levels of multiple inorganic nanocrystals into polymeric carrier beads or depositing various metal tracers onto the pores of a host membrane.[37] The resulting voltammetric signatures reflect the predetermined proportions of the corresponding metals in such 'identification' nanomaterials.

Nanoparticle-induced changes in the conductivity across a microelectrode gap can also be exploited for highly sensitive and selective electronic detection of DNA hybridization.[38,39] The capture of the nanoparticle-tagged DNA targets by probes confined to the gap between the two microelectrodes, and a subsequent silver enlargement, results in a conductive metal layer across the gap, and leads to a measurable conductivity signal (Fig. 4). Target DNA concentrations down to 500 fmol can thus be detected with excellent point-mutation selectivity. This low-cost, simple scheme offers the potential of parallel readout of multiple electrode arrays. One-dimensional nanowires can also be used for bridging two closely-spaced electrodes for label-free DNA detection. For example, a p-type silicon nanowire—functionalized with PNA probes—has been shown to be extremely useful for real-time label-free conductometric monitoring of the hybridization event.[40] This relies on the binding of the negatively-charged DNA target that leads to an increase in conductance, reflecting the increased surface charge.

Similar improvements have been reported in connection to nanowires and CNT functionalized with other receptor molecules. For example, Patolsky *et al.*[41] reported recently on the use of nanowire devices for direct real-time electronic detection of single virus molecules. Measurements made with nanowires modified with antibodies for influenza A showed distinct and reversible conductivity changes upon binding and unbinding of single viruses. Conducting-polymer nanowire biosensors have also been shown to be attractive for label-free bioaffinity sensing. For example, Ramanathan *et al.*[42] demonstrated the real-time monitoring of nanomolar concentrations of biotin at an avidin-embedded polypyrrole nanowire. Similarly, non-covalent functionalization of CNT was shown to be useful for label-free conductivity measurements of antibodies associated with human autoimmune diseases.[43] Non-specific binding on the CNT was overcome by immobilizing polyethylene oxide chains.

Carbon nanotubes can also lead to ultrasensitive bioelectronic detection of DNA hybridization.[44–47] For example, CNT can be used as carriers for several thousands enzyme tags and for accumulating the α-naphthol product of the enzymatic reaction (Fig. 5). Such a CNT-derived double-step amplification pathway (of both the recognition and transduction events) allows the detection of DNA down to the 1.3 zmol level and indicates great promise for PCR-free DNA analysis.

The ability of CNT to facilitate the adsorptive accumulation of the guanine nucleobase can lead to a dramatic amplification of label-free electrical detection protocols, based on the intrinsic

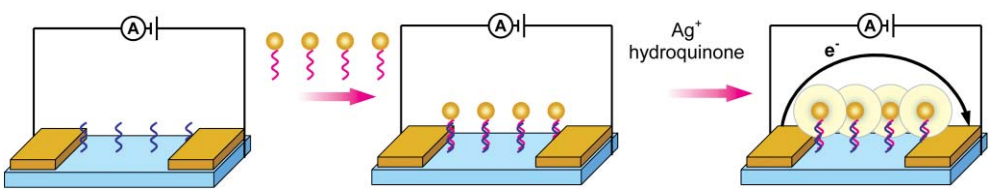

Fig. 4 Conductivity detection of nanoparticle-based microelectrodes arrays. The capture of the nanoparticle-tagged DNA targets by probes confined to the gap, and a subsequent silver enlargement, electrically short the gap and lead to a measurable conductivity signal. (Based on ref. 38 with permission.)

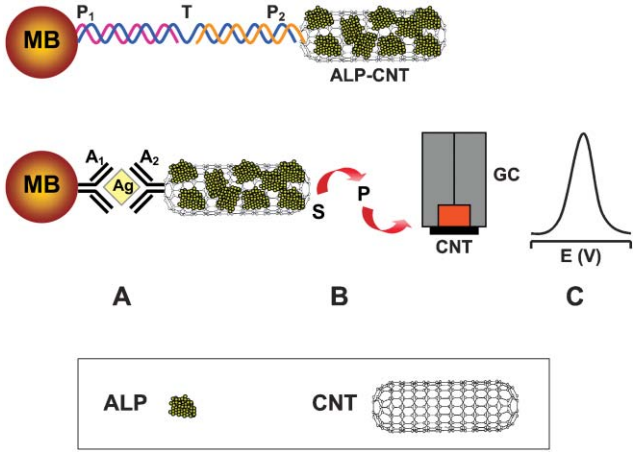

Fig. 5 Ultrasensitive bioassays of proteins and nucleic acids based on the amplification features of carbon-nanotube carriers and modified electrodes. (Based on ref. 44 with permission.)

electroactivity of DNA.[45] The coupling of a CNT nanoelectrode array with the $Ru(bpy)_3^{+2}$-mediated guanine oxidation has facilitated the detection of subattmoles of DNA targets.[46,47] Such a CNT array was also applied for label-free detection of DNA PCR amplicons, and offered the detection of less than 1000 target amplicons.

4. Conclusions and future prospects

The emergence of nanotechnology is opening new horizons for electrochemical biosensors. Recent years have witnessed the development of a variety of nanomaterial-based bioelectronic devices exhibiting novel functions. The use of nanomaterials in such sensing devices has taken off rapidly and will surely continue to expand. Nanoparticles, nanowires and nanotubes have already made a major impact on the field of electrochemical biosensors, ranging from glucose enzyme electrodes to genoelectronic sensors.

What does the future hold for this technology? The unique properties of nanoscale materials suggest that future interdisciplinary research could lead to a new generation of electrochemical biosensors. We are currently exploring nanoparticle-based protocols for electronic detection of proteins. The use of nanoparticle tags for detecting and coding proteins is in its infancy, but the lessons learned in DNA detection should provide useful starting points. The monitoring of protein and protein interactions presents a greater challenge than that of nucleic acids, owing to the absence of (PCR-like) amplification technologies, the complexity of proteins, and their stronger non-specific binding to solid supports. Nanoparticles comprising of mixed (recognition/shielding) monolayers are desired to fully utilize the potential of protein-nanoparticle hybrids. Such addition of protein analysis to the arsenal of particle-based bioassays represents an important step in the direction of making particle bioelectronics a universal biodetection platform. Multiple electrode protein and DNA arrays based on nanoparticle-amplification platforms are thus expected in the near future.

One-dimensional nanostructures are extremely attractive for a wide range of bioelectronic sensing applications. The ability to modify nanowires and nanotubes with biological recognition elements imparts high selectivity onto devices based on 1-D nanomaterials. While several novel sensing concepts based on 1-D nanowires have been presented, incorporating these materials into routine functional devices remains a challenge. The successful bioelectronic utility of 1-D nanostructures requires new nanofabrication capabilities and proper attention to the interconnection challenge, involving reproducible positioning of nanowires and nanotubes between closely-spaced microelectrodes. Such attention to the nanotechnology/microtechnology interface is essential for assembling nanosensors into functional integrated devices. Proper attention should be given also to the interface of these devices with the real world (*i.e.*, to sample delivery issues). Ultimately, such activity will lead to powerful sensor arrays for parallel real-time monitoring of multiple analytes. The creation of such biosensor arrays requires new methods for confining different biomolecules onto closely-spaced 1-D nanostructures.

A wide range of newly introduced nanomaterials is expected to expand the realm of nanomaterial-based biosensors. Such nanomaterials-based electrochemical devices are expected to have a major impact upon clinical diagnostics, environmental monitoring, security surveillance, or for ensuring our food safety. It is only a matter of time before such protocols are used for routine diagnostic applications.

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