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Functionalization of Carbon Nanotubes for Bioelectrochemical Applications

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Abstract—Due to the increasing need to monitor health and environment in real time and to energize small electronic devices, new materials are under extensive investigations. Between others, carbon nanotubes (CNTs) are promising alternatives as building blocks in bioelectrochemical devices due to their unique electrical, mechanical properties, and their high specific surface. Furthermore, their ease and well-established organic functionalization brings new properties to nanostructured electrodes such as specific docking sites for biomolecules. Moreover, CNT films exhibit a high electroactive surface area due to the natural formation of highly porous three-dimensional networks, suitable for the anchoring of a high amount of bioreceptor units, thus leading to improved sensitivities. With some simple functionalization techniques, CNTs can act as almost perfect support for enzyme wiring and is still the privileged material in the field of enzymatic biofuel cells. The most appropriate functionalization techniques for CNTs are summarized leading to enhance the performances of biosensors and biofuel cells.

Keywords— Carbon nanotubes, functionalization, immobilization of biomolecules, enzyme wiring, biosensors, biofuel cells

I. INTRODUCTION

CNTs were proposed in the early two thousands as the material of the twenty first century [1] related to the exceptional electronic and mechanic properties. Even when the interest in this material decreased for some proposed applications, CNTs are still a privileged material for bioelectrochemical applications such as biosensors or biofuel cells. Biosensors are defined by its receptor unit of biological or biomimetic origin. Such biomolecules provide unique specificity towards one single analyte and are therefore of particular interest for the analysis of complex media like body or environmental fluids. Biofuel cells operate as classic fuel cells. A fuel is reduced at a cathode where the involved electrons are transferred from the catalyst to electrode and conducted to the anode where a catalyst used these electrons to reduce an oxidizer, mostly oxygen. The difference of the anodic and cathodic redox potentials determine the cell voltage and together with the catalytic currents, the power output. The difference with biofuel cells is that the catalysts are enzymes (biomolecules) instead of noble metal or alloys. The advantage of such bioconvertors of energy is that

power can be produced at room temperature, neutral pH, and in a non-toxic environment. Enzymatic glucose fuel

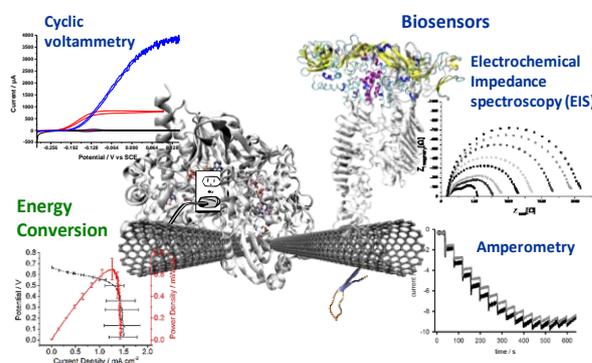


Figure 1: Illustration of biofunctionalized CNTs for various bioelectrochemical applications

cells were considered as alternative power supply for implantable devices such as pacemakers or insulin pumps since glucose and oxygen are both present in extracellular fluids. However, even when the required power demand can be reached, the operational lifetime is by far too short for realistic applications. A more promising use of biofuel cells is for single use and disposable devices such as pregnancy or ovulation tests [2].

The challenge of biosensor and biofuel research is the immobilization strategy without altering the biological activity of the receptor units. Further to the immobilization issue is the “wiring” (establishment of electron transfers) of the enzymes to the electrode material. CNTs provide optimal properties for the immobilization of biomolecules and wiring of enzymes. The efforts in this domain aim the formation and the characterization at molecular scale nano-architecture associating different nano-objects *via* organic or organo-metallic connectors to immobilize redox enzymes or antibodies. For biofuel cell research the efforts focus further to immobilization redox enzyme wiring on nanotubes to enable efficient electron transfer. In this context, different functionalization methods and procedures for CNTs to form bioassemblies for electrochemical applications are discussed.

II. (BIO)FUNCTIONALIZATION OF CNTS

Existing functionalization methods for CNTs comprise covalent, and non-covalent methods. Covalent methods are

addition reactions of nitrenes, carbenes, oxidizers, or radicals which disrupt the aromatic bonds on tube sidewall [3]. In case of oxidizers, the formed oxygenated functions (principally carboxylic acids) on the wall are mostly designated for further functionalization via ester or amide coupling. A well-established and efficient method to graft covalently a great pallet of different chemical functions onto CNTs is the reductive coupling with diazonium salts [4].

The commonly occurring passivation of the surface related to the formation of a thick layer of polyphenyls is nowadays well-controlled with adjusted electrochemical parameters. Another way of grafting chemical functions onto CNTs is the electropolymerization of pyrrole derivatives. Thin films can be formed which allow a dense coverage of any desired chemical entity [5]. Such CNT modified electrodes offer a high specific surface with an excellent accessibility for the anchoring of proteins via e.g. amide coupling for covalent grafting or so-called affinity interactions as shown in Figure 2. Affinity interactions comprises the formation of supramolecular or metal organic complexes between the complementally modified CNTs and biomolecules. The most famous example is the biotin-avidin-biotin strategy where avidin with its four subunits, each capable to complex one biotin molecule, serves as cross linker between biotinylates CNTs and biotinylated bioreceptors [6]. However, such avidin layers prevent the efficiency of signal capture for impedance and amperometric transduction where an electrochemical probe has to diffuse through a dense avidin layer to the CNT surface. This cross linker can be omitted by profiting from alternative interactions of biotin with β -cyclodextrin or Cu(II)@nitrilotriacetic acid (NTA) functions [7-8]. NTA/Cu²⁺ and β -cyclodextrin can easily be attached to pyrene or pyrrole derivatives in one-step synthesis. Pyrene and its derivatives are the mostly used moiety for non-covalent functionalization of CNTs via π -stacking interactions [9]. One advantage is that the electronic and mechanic properties are entirely preserved. Another is that the functionalization can be performed under ambient conditions by simple dip-coating [10]. The attached

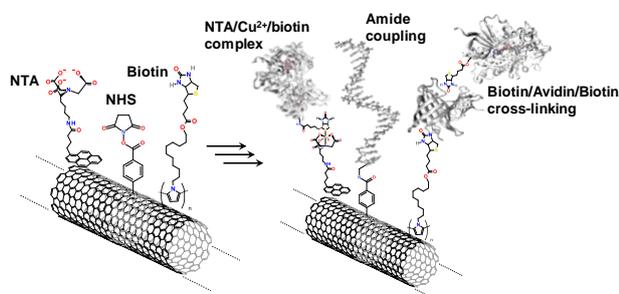


Figure 2: Schematic presentation of the functionalization of CNTs via non-covalent π -stacking interaction with pyrene or electrochemical coating using diazonium salts or pyrrole monomers to graft various chemical functions suitable for the subsequent immobilization of bioreceptor molecules.

pyrene layer can be reinforced via electrochemical polymerization. Such electrochemical coating forming thin polypyrene or polypyrrole films do not introduce any

defects to the CNT walls. The ratio of the Raman G- and D band even decreased which is assigned to a resonance phenomenon of the polymeric π -system induced by the CNT resonance [11].

III. BIOSENSORS BASED ON CNT ELECTRODES

The greatest advantage of CNTs for biosensor application is the random porosity and high specific surface. This allows immobilizing an enhanced amount of biological macromolecules by preserving optimal accessibility for the analyte. A series of CNT based enzymatic biosensors were developed with chronoamperometric and cyclic voltammetric transduction of the recognition events of the analytes like glucose or pollutants [12]. Simple drop casting of a CNT dispersion on an electrode and drying is sufficient to multiply sensitivities compared to flat electrodes. A more sophisticated electrochemical transduction technique is electrochemical impedance spectroscopy (EIS), which is highly sensitive to variations of the surface-solution interface. This allows precise sensing and quantification of immune systems or DNAs down to the femtomolar range without the need of redox labels [13]. The drop casting and drying method as mentioned before leads to slightly different deposit morphologies, which are negligible for amperometric or cyclic voltammetric transduction, but vanish repeatable EIS sensing. This drawback was circumvented by a filtration and transfer process of thin CNTs films on glassy carbon electrodes. Filtration of CNT dispersions forms deposits

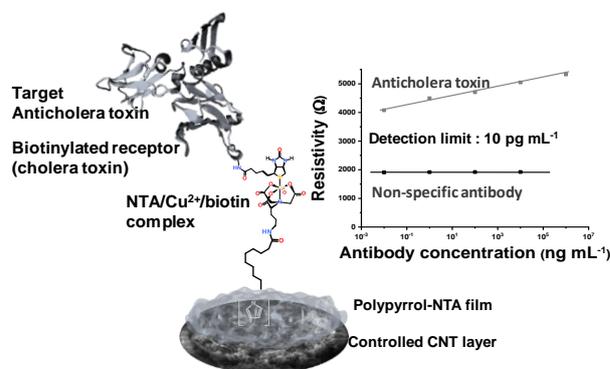


Figure 3: (left) Illustration of the detection principle using a controlled CNT layer modified with an electrogenerated polypyrrol-NTA film for the immobilization of the biotinylated receptor (here cholera toxin) to quantify anticholera toxin. (right) linear range of the calibration curve for the detection of anticholera toxin using the specific (grey) and non-specific (black) antibody.

with identical surface morphologies, which are suitable for EIS transduction. A high performing EIS sensor toward anti-cholera toxin could be fabricated based on this concept by coating the CNT layer with polypyrrole-NTA/Cu²⁺ and immobilizing biotinylated cholera toxin, the receptor unit, on this electrode [14]. More impressive than the improved sensitivity and detection limit, a linear concentration range of more than eight orders of magnitude could be reached (Figure 3).

IV. CNT BIOELECTRODES FOR BIOFUEL CELLS

Beside reliable immobilization of enzymes on CNTs, the electrical wiring for high electron transfer kinetics is

essential. The bent CNT walls already facilitates the access to the enzymes' active centre but the functionalization with appropriate chemical groups can clearly improve the electron transfer. The choice of the chemical function depends on the natural operation of the enzyme. For instance, at the cathode, multicopper enzymes like laccase or bilirubin oxidase are used for the reduction of oxygen to water. There are two copper redox centres. A trinuclear cluster, called T2/T3 centre catalysed the reduction of oxygen, a mononuclear centre (T1) oxidizes its natural substrate. In case of laccase, it oxidises phenolic compounds to ortho hydroxy phenols. To capture these aromatic compounds, a hydrophobic domain is situated closely to the T1 centre. Inspired by this natural process, similar compounds like pyrene, anthraquinone, or paphthalene were attached to the CNT surface. These molecules form stable inclusion complexes with the hydrophobic domain of laccase leading to its oriented immobilization bringing closely the T1 centre close to the CNT surface [15]. Another strategy has to be applied for the oriented immobilization of bilirubin oxidase. The T1 centre oxidises bilirubin, a metabolite of hemin. To mimic bilirubin, protoporphyrin IX i.e. hemin without iron, the cyclic form of bilirubin was chosen. Porphyrins form strong π stacking interaction with CNTs and provokes the oriented immobilization of bilirubin oxidase [16]. The respective oriented immobilization strategy for laccase and bilirubin oxidase lead to so called direct electron transfers from the CNT electrode to the enzyme.

More challenging is the wiring of anodic biocatalysts especially when glucose-oxidizing enzymes operating with flavin adenine dinucleotide (FAD) as co-factor are used. FAD is deeply embedded in the protein structure and no site-specific orientation strategy could be found up to date. In these cases, an electron shuttle, called redox mediator has to be used to transfer the electrons from the enzyme to the electrode. For the choice of the optimal mediator, a compromise between redox potential and electron transfer kinetics has to be accepted. In fact, the higher the difference between the redox potentials of the FAD unit and the

mediator, the higher is the driving force for the electron transfers leading to higher electrocatalytic currents. However, such increased potential differences directly lowers the cell voltage and the global power output. Low potential difference lead to more optimal cell voltages but to lower current densities. Quinones have demonstrated to be excellent electron shuttles for FAD dependent enzymes

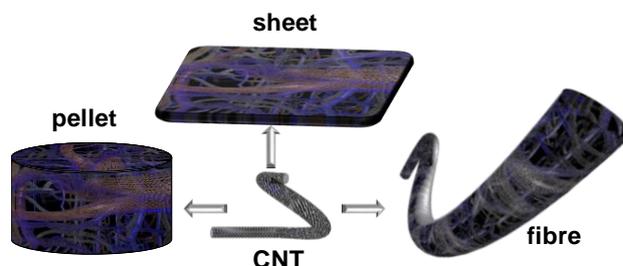


Figure 5: Most common shapes for free-standing CNT electrodes.

in terms of the availability of a wide range of redox potentials, and stabilities. Particular interesting is the example of the electro-oxidation of pyrene in buffer solution forming redox active pyrenediones. The redox potential is perfectly situated for high catalytic currents and satisfying cell voltage [17]. A further advantage is that electro-oxidized pyrene derivatives with activated esters for covalent enzyme immobilization show almost the same electron transfer behaviours [18] as illustrated in Figure 4.

The resulting bioanodes showed clearly improved electrocatalytic performances and operational stabilities than other currently used quinone-based mediators like naphthaquinone or phenanthraquinone.

V. Shaping of CNTs

Beside the great physical, chemical, and electrochemical properties of CNT for bioanalytics and energy conversion, a further beneficial property is the possibility to shape bulk CNT materials to form pellets, fibres, or sheets without the need of binders or cross-linkers (Figure 5) [19].

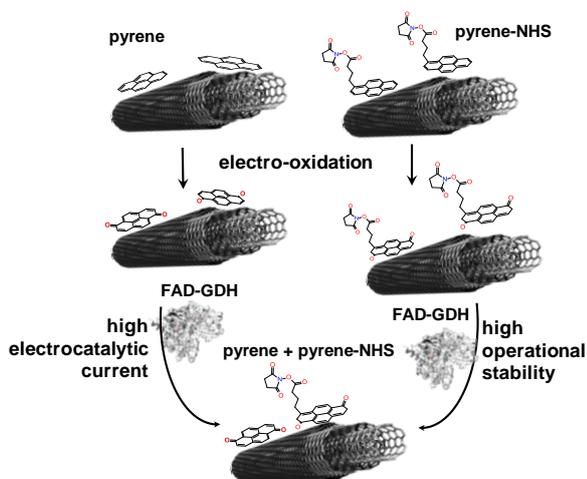


Figure 4: Electro-oxidation of the pyrene units attached to the CNT sidewalls. (left) pure pyrene, (right) pyrene-NHS. The first provides high electrocatalytic current while the second showed improved operational stabilities. A mixture of both pyrenes allowed obtaining best of both worlds.

Elegant methods to form CNT fibres are based on wet spinning technique [20-21], or by drilling of CNT yarns from vertically aligned CNT carpets [22] with diameters ranging from several micrometres down to few hundreds of nanometres. For the wet spinning method, an aqueous CNT dispersion is injected in a coagulation solution under constant flow conditions which forms the fibres. Initially, the coagulation solution contained polyvinyl alcohol (PVA) where particularly strong fibres could be obtained. Without this polymer, the coagulation is provoked by monomeric alcohols like ethanol or glycerol leading to more brittle but still processable fibres. Mano and co-workers adapted this process to introduce enzymes and mediators in the fibres forming a high performing micro sized biofuel cell [23].

Stronger and greatly processable fibres are obtained using the yarn drilling method. The Baughman team realized fibred bioelectrodes using a twist based yarn spinning process [24] where the obtained enzyme-yarn provided an exceptional mechanical robustness that they could be woven into textiles with a sewing machine.

Pellets can easily be formed just by compression of a CNT paste in a stencil of desired shape and thickness. Macroscopic electrode could thus be formed with a huge amount of trapped enzymes and mediators [25]. This provides high power outputs and clearly enhanced long term stabilities with operational lifetimes up to one year [26]. Such formed CNT pellet bioelectrodes were capable, when implanted in a rat, to convert sufficient energy out of the rat's body fluids to run a digital thermometer for several minutes and to flash a LED five times for 0.5 seconds [27].

Beside these examples of free-standing CNT electrodes where only few research teams explore, the most commonly used shape is a sheet, called buckypaper. They are obtained at lab scale by filtration of CNT dispersions. Buckypapers are even nowadays mass produced and commercialized. The overall availability of such sheets is most likely the reason that they are most extensively used and studied for bioelectrochemical applications and in particular for biofuel cell research. Most prominent examples are summarize in reference [28].

VI. CONCLUSION

CNTs provide excellent properties for (bio)-electrochemical applications. They became an emerging material related to established functionalization techniques which are easy to perform with very satisfying repeatability. They are now in the shadow of graphene-based materials but the development of mass production processes leading to the availability of low cost CNTs with identic properties independent of the batches, kept the interest in this material for some applications and therein biosensor and biofuel cells.

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