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Review

Label-free indicator-free nucleic acid biosensors using carbon nanotubes

Carbon nanotubes (CNTs) are promising components for electrical biosensors due to their high surface-to-volume ratio and improved electron transfer properties. This review surveys CNT-based label-free indicator-free biosensing strategies that have been demonstrated for the sensitive detection of nucleic acids. After an introduction to CNTs, the fabrication of biosensors and techniques for the immobilization of probe nucleic acids are outlined. Subsequently, two major label-free strategies namely electrochemical transduction and field-effect detection are presented. The focus is on direct detection methods that avoid labels, indicators, intercalating agents, mediators, and even secondary receptors. The review concludes with a comparison between the various biosensors and presents ways of engineering them so that they can be deployed in realistic diagnostic applications.

Keywords: Biosensors / Carbon nanotubes / DNA / Label-free / RNA

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1 Introduction

The fields of genetic engineering, biotechnology, and medical diagnostics are experiencing a revolution since the advent of the polymerase chain reaction (PCR) [1, 2]. The deciphering of the human genome (http://genomics.energy.gov) has given a further impetus to this rapidly emerging field. The focus is currently on understanding the interplay between variations in the genomic composition and the functioning of biological and biochemical processes in many organisms [3, 4]. This not only includes single nucleotide polymorphisms (SNPs) but also other structural variations such as insertions, deletions, inversions, and copy number variants [4, 5]. Through such analyses, major breakthroughs are underway or are expected in understanding the genetic basis of a range of diseases and disorders [6, 7]. For this purpose, a broad range of strategies for nucleic acid diagnostics are being deployed both at a fundamental level for the understanding of gene expression [8] as well at the analytical and diagnostic level to sensitively detect nucleotide sequences that function as biomarkers for specific diseases [9]. The availability of microarrays has led to high-throughput nucleic acid analysis enabling the possibility of such genome-wide information in a broad range of gene expression studies and genotyping investigations [10]. At the heart of such investigations are assays that are designed to detect specific nucleic acid sequences. The drive is toward miniaturized biological assays that promise genomic information at a high throughput and low cost [11].

A broad plethora of possibilities is available for the sensitive detection of nucleic acids, be it DNA or RNA. Many of these techniques that are currently in use in molecular biology and analytical laboratories are based on labeling the target nucleic acid [12]. Furthermore, in situations where the amount of available DNA is low, a series of PCR steps is required to amplify the amount of DNA in order to attain a detectable concentration [1]. For the detection of RNA, an additional reverse transcriptase step (RT-PCR) is necessary to generate complementary cDNA strands, which can be subsequently amplified [7]. In order to avoid labeling, sandwich strategies have also been designed [12]. While all these approaches have been key in delivering a large amount of information about nucleic acids, the multitude of processing steps involved in the detection protocols has been posing an important drawback in bringing such diagnostic technologies closer to point-of-use especially while using such assays in medical diagnostics [13]. The need for many processing steps forces the realization of fluid handling systems on a chip along with the biosensing stages [13]. In spite of these complexities, a number of systems have been demonstrated that have successfully integrated PCR stages along with buffers and other reagents on to mobile or disposable cartridges [14, 15]. However, there is no well-established system yet that is approved and could be deployed in realistic trials, partly due to the inherent complexities of these lab-on-a-chip systems and the high costs involved in the fabrication of these devices.

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Abbreviations: CNT, carbon nanotubes; **EDC**, 1-ethyl-3-(3-dime- thylaminopropyl)carbodiimide hydrochloride; **FET**, field-effect transistor; **MWCNT**, multiwalled carbon nanotubes; **NHS**, *N*-hydroxysuccinimide; **SWCNT**, single-walled carbon nanotubes

Ideally, it would be easier to realize such point-of-use or point-of-care devices if among other aspects, the number of processing steps in the assay were minimized. For this purpose, one important aspect involves the deployment of label-free and indicator-free strategies, where the labeling of the target or the use of indicators or even secondary receptors is avoided. In order to achieve this, new paradigms have emerged especially by using nanostructures [16, 17]. Furthermore, the possibility to avoid PCR will further simplify such lab-on-a-chip systems, where the concerned reagents necessary could be avoided to a large extent. Nanostructure-based assays show promise in this direction by allowing for very low detection limits due to their specialized electrical properties arising mainly due to their high surface-to-volume ratio. Finally, the use of an optical microscope is a major hurdle for the realization of point-of-care nucleic acid devices. This has motivated the search for alternative transduction strategies mainly based on electrical detection [17]. The advantage of electrical detection lies in the fact that the detection circuitry can be integrated in a very compact manner at low costs directly next to the reaction chamber. The well-known glucose strips are the best examples that have been widely successful, still enjoying a comfortable market share [18]. The possibility to have similar strips—for example for the detection of nucleic acids—is expected to revolutionize the field of molecular diagnostics.

Along the foregoing discussion, this review will focus on the use of nanostructure-based electrical methods that are being developed for the label-free detection of nucleic acids, without the use of indicators or sandwich protocols. We will concentrate here on one kind of nanostructure namely carbon nanotubes (CNTs). They are the smallest commercially available one-dimensional nanostructure with a diameter that is in the same range as that of nucleic acids. Needless to say, CNT-based biosensing strategies are still at a research stage in contrast to well-established fluorescence-based diagnostic assays. Nevertheless, the review is intended to serve as a source to motivate the readers to engineer available strategies in research labs so that they can be mature enough to be applied in realistic diagnostic scenarios.

The review is organized as follows. We start with an introduction about CNTs followed by different methods for the fabrication and assembly of CNT-based nucleic acid biosensors. Most of these biosensors are based on the hybridization of a target sequence to a probe sequence that is immobilized on or in the vicinity of the CNT surface. The methods for immobilizing probe oligonucleotides on the sensor surface are outlined next. Following this, a survey of the two major sensing strategies for label-free nucleic acid detection is presented. *Label-free* refers to the target analyte being analyzed in its native form without being modified by any kind of markers. There have been demonstrations of biosensors using indicators or sandwich approaches, where the target nucleic acid does not require to be labeled. However, these methods require a second binding event such as the binding of an intercalating agent to the duplex or the formation of a sandwich. We will also not discuss such protocols in detail here. Detailed reviews about these aspects are available elsewhere [19–22]. We conclude the review with a comparison of the various sensing strategies and a discussion of the hurdles involved in the use of CNT-based nucleic acid biosensors for realistic applications.

2 Carbon nanotubes

CNTs are long cylinders made of just one atomic layer of sp^2 carbon arranged in a hexagonal lattice [23]. They can be visualized as rolled-up graphene sheets, as shown in Fig. 1 [24]. On the other hand, they are the thinnest wires that can be synthesized in free form. They have a diameter starting from as low as 0.4 nm and up to 3 nm and hence are comparable to the sizes of nucleic acids. They can be grown up to hundreds of microns long. Single-walled CNTs (SWCNTs) are composed of just one cylinder of carbon atoms. They can also occur in the form of concentric cylinders (see Fig. 2), which are then called multiwalled CNTs (MWCNTs).

CNTs can be produced by many different methods. Nowadays, they are readily available commercially in various formulations [19]. Since they can be rolled up in many ways, the production techniques deliver a mixture of different allotropes, which constitutes a major hurdle for many applications. This is in contrast to many chemicals that one can obtain usually in its pure form. There are methods emerging to obtain high-purity nanotubes of just one chemical structure [25]. However, these nanotubes are usually short ($<1\ \mu$) and are available only in the single-walled form (http://www.nanointegris.com). Due to their large curvature, they exhibit increased chemical reactivity [24]. This is also the basis for the construction of a variety of biosensing strategies [26].

Among other nanostructures, CNTs are unique due to their all-carbon structure and the possibility to obtain very high aspect ratios (at least 1000:1 for a 1-μ long 1-nm nanotube) easily. The latter aspect is the main motivating factor for using CNTs in electrical biosensors. Unlike bulk wires, charge variations on the surface of nanostructures cause changes in the electronic

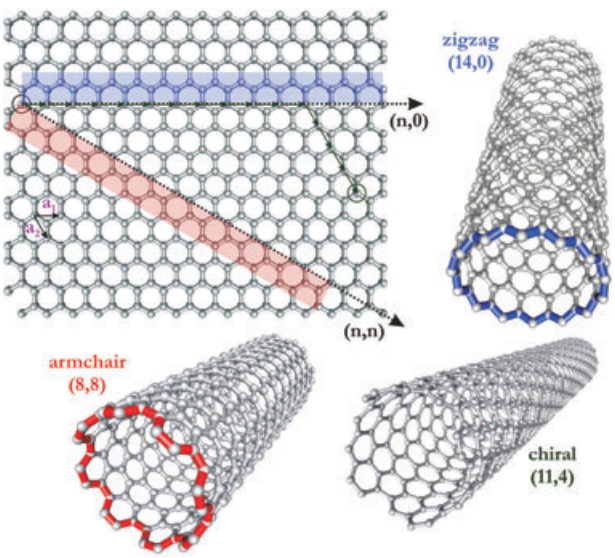

Figure 1. Physical structure of carbon nanotubes. Carbon nanotubes (CNTs) can be imagined as rolled-up graphene sheets. Depending on the way they are rolled up, different types of nanotubes can be formed such as armchair, zigzag, and chiral. Reproduced with permission from Reference [24].

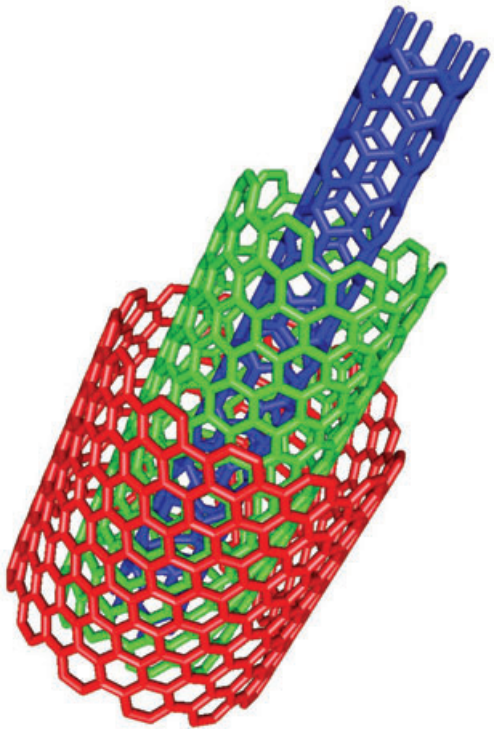

Figure 2. A molecular model of a multiwalled CNT (MWCNT). All colored atoms refer to carbon atoms.

properties of the CNTs. This is judiciously utilized in fabricating biosensors in order to detect DNA-binding events happening on the surface of CNTs. Furthermore, due to the very minimal surface (1–5 μ × 1 nm), they can be loaded with very few receptors (ultimately down to single molecules) and hence show promise for obtaining lowest limits of detection [27]. Moreover, their nanoscopic sizes allow for easy interfacing with electronic circuitry on-chip. These advantages have aroused the tremendous interest in the use of CNTs for applications as nucleic acid biosensors.

3 Design and fabrication

Almost all the electrical biosensors for the detection of nucleic acids are based on hybridization of the target analyte (DNA or RNA) on to a probe nucleic acid. In order to specifically detect the target sequence, the binding event must generate a signal that can be efficiently transduced. The transducer comprises of one or many CNTs on to which the probe nucleic acid has to be fixed by some means. The first step in the realization of the biosensor involves the fabrication of such CNT electrodes. Figure 3 presents some examples of CNT-based electrodes deployed in biosensors. The electrodes can comprise of a single nanotube (see Figs. 3A and B) to arrays of nanotubes on appropriate substrates (Figs. 3C and D). For obtaining individual nanotube electrodes, the CNTs are first dispersed in a surfactant solution (such as sodium dodecyl sulfate or Triton-X-100) and subsequently deposited on a substrate by spotting. Using predefined markers on the substrate, they can be located and electrical contacts are made by lithographic procedures [24, 28, 29]. The nanotubes can also be grown directly across the electrical contacts using chemical vapor deposition [30, 31]. Networks of nanotubes can also be prepared using this strategy (see Fig. 3C) [32–34]. Alternatively, the electrical contacts can be realized in a first step and individual nanotubes trapped from the suspension using AC dielectrophoresis [27]. The substrates are usually made of silicon oxide or glass. For obtaining a high density of nanotubes, they can be directly grown on millimeter-sized metallic substrates (see Fig. 3D) [35]. Such electrodes can even be procured commercially [19].

In all the above cases, the nanotubes form the active component of the biosensing electrode. However, they can also be used as additives to improve the electrical and electrochemical characteristics of other standard bulk electrodes such as glassy carbon electrode (GCE) [36, 37], carbon paste electrode [38, 39], graphite electrode [40], or gold [41] electrode. For example, CNTs can be incorporated into a polymer matrix on the surface of such bulk electrodes (see Fig. 3E) [37–39]. In some cases, CNTs are combined with other nanostructures such as nanoparticles (see Fig. 3F) [42–46]. In general, it has been observed that the addition of nanostructured materials onto bulk electrodes leads to an improvement in the electrochemical properties and hence gives higher sensitivities and lower detection limits [47–49]. This is expected since the nanoscale structure of the electrodes provides for increased surface and intimate contact with the biomolecules that need to be detected. Furthermore, in electrochemical detection methods, the use of nanostructures generally allows for improved charge transfer at the electrode interface [50, 51].

4 Chemical functionalization—immobilization of probe nucleic acid

Once the CNT-based electrode has been designed and fabricated, it has to be rendered sensitive and specific to a target DNA or RNA sequence. In order to achieve this, a probe nucleic acid sequence has to be immobilized on the CNT electrodes. This probe sequence is DNA in most cases, however peptide nucleic acids (PNA) have also been used as probes [30, 52]. Through chemical functionalization of the nanotube surface, the probe nucleic acid is attached either covalently or noncovalently [24]. The covalent coupling is performed using heterobifunctional linkers (see Fig. 4A). In many cases, the CNT surface contains carboxyl groups, and probe DNA with a terminal amino group is used for the functionalization [27, 29, 37, 41, 53–55]. The most common linking strategy involves the use of EDC/NHS (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride/*N*-hydroxysuccinimide), which activates the carboxyl groups on the nanotube surface, allowing for the coupling to the amino group of the DNA resulting in an amide bond. In some cases, the electrodes that form contacts to the nanotubes have been functionalized with probe DNA. This is achieved by using thiolated DNA probes, which form disulfide bonds on to the gold surface [31]. However, in such a situation, the active sensing element is not anymore the nanotube but the contact

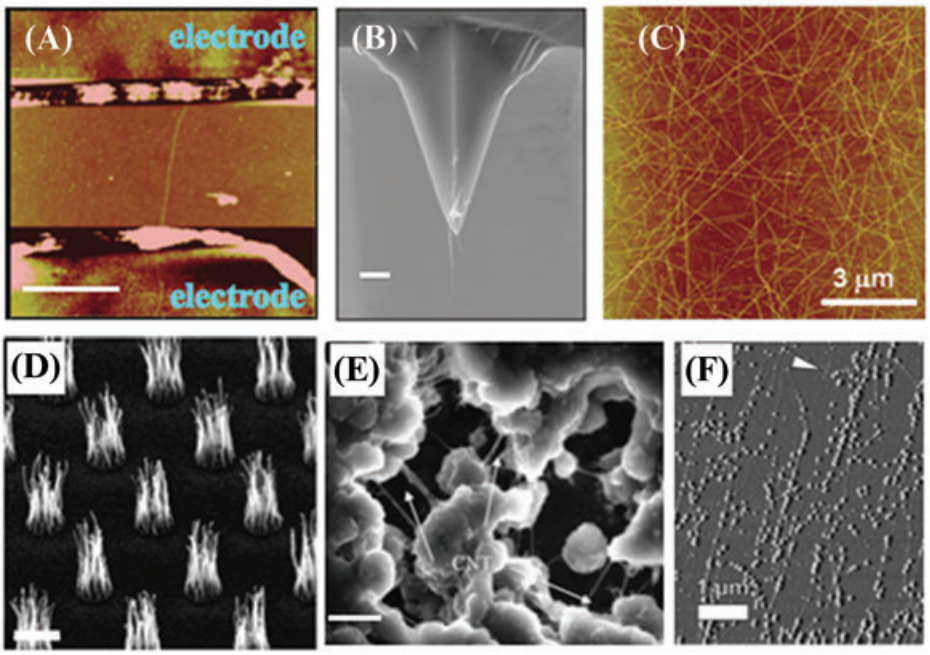

Figure 3. CNT-based electrodes for biosensing. (A) Individual single-walled CNT (SWCNT) contacted by two electrodes, from Reference [28]. (B) Individual MWCNT attached to a tip electrode. (C) SWCNT networks on silicon oxide substrate, from Reference [32]. (D) CNT electrode array, from Reference [35]. (E) Composite electrode with nanotubes integrated into a polymer matrix, from Reference [39]. (F) SWCNT network decorated with nanoparticles, from Reference [44]. The scale bars are 2 µm in (A), (B), (D), and (E), 3 µm in (C), and 1 µm in (F).

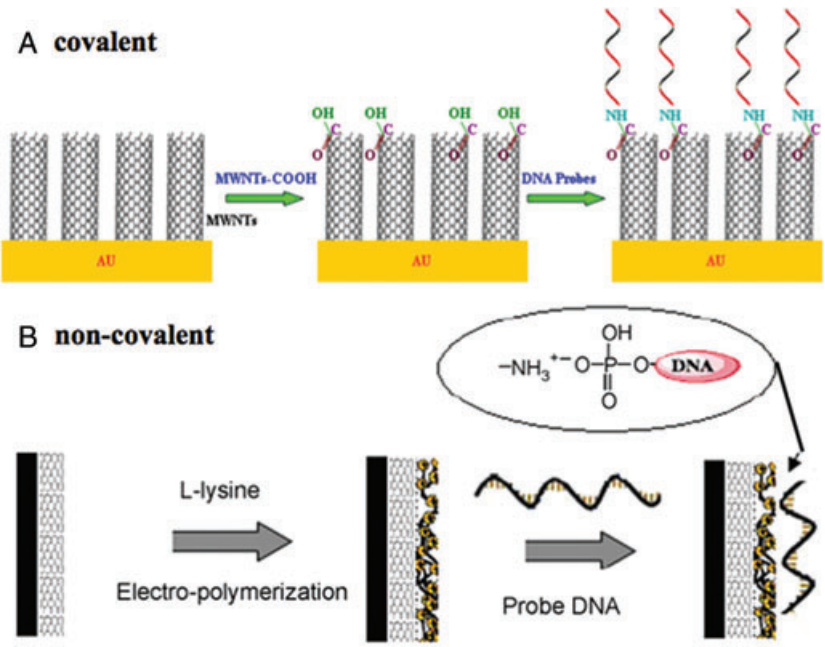

Figure 4. Examples showing immobilization of probe DNA to the CNT surface. (A) Covalent coupling to oxidized MWCNTs, from Reference [55]. (B) Noncovalent immobilization to an electropolymerized layer, from Reference [39].

between the electrode and the CNT, which usually results in a lower sensitivity (see discussion later).

Noncovalent strategies are more prevalent for the immobilization of probe DNA on to the surface of CNTs. The simplest method is to incubate the CNT-based electrode in a probe DNA solution (unmodified or amino terminated) and rely on hydrophobic or electrostatic interactions for the attachment of the oligonucleotides on to the CNT surface [30,33,40,56,57] (see Fig. 4B). Although this technique is simple, it has the disadvantage that the probe sequence is deposited nonspecifically on the entire electrode surface leading to lower sensitivities [47]. This can be avoided to some extent by protecting the device surface (usually silicon oxide, excluding the CNTs) through a blocking step [33]. Another approach involves the use of an organic monomer such as pyrrole that can be electropolymerized on the CNT surface in the presence of the probe DNA [36]. The probe DNA is thereby embedded into the polymer matrix. Alternatively, the CNTs can be coated in a first step with polymers containing carboxyl groups

or amino functionalities. In the former case, amino-terminated probe DNA is coupled covalently using the EDC/NHS coupling strategy [27, 28], while in the latter unmodified probes are electrostatically adsorbed on to the CNT surface [39]. Noncovalent coupling of molecules on to CNTs does not generally reduce the favorable electronic properties of CNTs. On the other hand, covalent strategies reduce the conductivity of the functionalized CNTs [58].

5 Label-free biosensing strategies

Toward the label-free detection of nucleic acids using CNT-based biosensors, two transduction principles have been mainly demonstrated namely electrochemical and field-effect detection. Although the latter is a kind of electrochemical (potentiometric) detection [59], we will handle them separately here. Other nonportable methods that are promising for label-free biosensing such as quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) have been seldom utilized for CNT-based nucleic acid detection. In contrast to various fluorescence-based detection strategies, which require RT-PCR for the detection of RNA, electrical methods do not require this step and RNA can be directly detected on probe DNA functionalized electrodes [30].

5.1 Electrochemical transduction

Electrochemical transduction principles mainly utilize the detection of oxidation and/or reduction reactions occurring at the biosensing electrode [60]. Most of these strategies involve Faradaic or non-Faradaic processes at the electrode/solution interface leading to the generation of a current or the build-up of an interfacial voltage. They are termed amperometric and potentiometric detection. Alternatively, the voltage can be scanned and the current measured such as in voltammetric methods [59]. Finally, there is also the possibility of measuring the impedance between the active electrode and a second electrode in solution. This method is called electrochemical impedance spectroscopy (EIS) [48]. The fundamental advantage of using CNTs in electrochemical transduction methods is the possibility of increased electron transfer rates leading to higher sensitivity [19, 21]. However, it is debated that impurities in the nanotube raw material (such as metal nanoparticles) or the architecture of the electrode is responsible for the increased sensitivity [61].

The various detection principles are based on how the binding of the target nucleic acid on the probe DNA is detected through the CNT electrode. The simplest way is to directly detect the duplex strand. For this purpose, guanine or adenine bases can be directly oxidized [62]. This generates a current and can be detected using voltammetric techniques [53, 54, 56, 63]. This is a true label-free electrochemical method for detecting the binding of target without involving any kind of additive (label, marker, intercalating agent, or indicator). However, the disadvantage here is that the probe as well as the target is irreversibly oxidized leading to destruction of the nucleic acid sequences. As a result of this, the sensor is not reusable and requires a repeated attachment of probe DNA sequences. Furthermore, the sensor signal is strongly dependent on the proportion of guanine or adenine bases in the nucleic acids. Table 1 presents a comparison of various CNT-based DNA biosensors utilizing direct oxidation of guanine.

In order to avoid the destruction of target and analyte sequences, a redox active molecule in solution can be used as an indicator. This is based on the principle that upon binding of the target the possibility to induce charge transfer to the redox indicator is reduced [48]. This increases the "charge transfer resistance," which serves as a sensor signal for the amount of target analyte bound to the probe [48]. The detection here is mainly performed using impedimetric techniques in order to obtain a good signal-to-noise ratio [37, 39, 41]. The sensitivity and the detection limit are usually improved when using indicators. The impedimetric method can be used to detect hybridization events even without an electroactive indicator in a true label-free manner [36].

An alternative strategy is to use a redox active molecule that has the capability to intercalate with the duplex strand after hybridization. This is analogous to the use of widely prevalent ethidium bromide that can stain nucleic acids in gel electrophoresis. Commonly used electroactive intercalators include methylene blue [46, 64, 65], adriamycin [51, 66, 67], daunomycin [68, 69], Luteolin copper(II) [45], and even ethidium bromide [70]. The indicator can be subsequently oxidized and the current generated is proportional indirectly to the concentration of target DNA. Other labeled detection strategies utilize a similar redox active molecule attached to the target or further formation of sandwiches to detect the hybridization event. Detailed reviews on such electrochemical transduction methods can be found elsewhere in the literature [20–22, 49].

5.2 Field-effect transduction

Label-free DNA sensors using SWCNTs have also been demonstrated in a field-effect transistor (FET) configuration [71, 72]. In such biosensors, the electrical properties of the nanotubes play a major role. Due to the nanoscale size and the high surface-to-volume ratio, the electrical resistance of nanotubes is extremely sensitive to changes in charge variations in their surroundings. Based on this property, the binding of target DNA on to a probe DNA functionalized surface can be sensitively detected by measuring the current through the nanotube. In order to provide a reference potential in the system, a gate electrode is required [47, 71]. Hence, the devices are constructed in an FET configuration, wherein the nanotube is connected between a source and a drain electrode lying on a silicon/siliconoxide substrate. The silicon substrate constitutes the gate electrode (back gate, see Fig. 5A). Alternatively, the measurement can be done directly in liquids, where an electrochemical FET configuration is utilized, as shown in Fig. 5B. Here, a reference electrode that is in contact with the solution acts as the gate [73]. The arrangement in liquids is very similar to that of ion-selective FETs (ISFETs and pH electrodes). By varying the potential at the gate electrode, the conductance of the nanotube can be varied. Changes in the electrostatic environment of the nanotube (e.g., when a target DNA

Table 1. Label-free indicator-free carbon nanotube (CNT) based electrochemical biosensors for the detection of DNA.

Biosensor architecture	Detection mode	Probe attachment	Analyte or target	Imm. time[a] /response time	Detection limit	Concentration range	Reference
GCE–MWCNT	Guanine oxidation (VA)	Separate[b] hybridization	60-bp synthetic DNA	–/–[b]	2.7 nM	2.7 nM–13.5 nM	[63]
GCE–MWCNTs	EIS	Electropolymerization of pyrrole in the presence of DNA	24-bp synthetic DNA	5 min/30 min	10 nM	30 nM–10 μM	[36]
Pencil graphite–MWCNT	Guanine oxidation (DPV)	Incubation[c] in amino-DNA	20-bp synthetic DNA	1 h/20 min	16 nM	50 nM–250 nM	[40]
GCE–SWCNT	Guanine oxidation (DPV)	EDC/NHS coupling with amino-DNA (to COOH groups of CNT)	18-bp synthetic DNA	12 h/45 min	20 nM	20 nM–110 nM	[54]
Al/Ni–MWCNT	Guanine oxidation (DPV)	EDC/NHS coupling with amino-DNA (to COOH groups of CNT)	24-bp synthetic DNA	17 h/30 min.	0.5 μM	0.5 μM–10 μM	[53]
Pencil graphite–SWCNT	Guanine oxidation (DPV)	Incubation[c] in amino-DNA	20-bp synthetic DNA	NR/NR	4 μM	4 μM–32 μM	[56]

[a] Imm. time = time for immobilization of probe DNA on the sensor surface.
[b] In this case, the hybridization was carried out separately using microspheres functionalized with the probe. After hybridization, the duplex was removed and denatured to obtain individual nucleic acid bases. The detection was performed on this digested solution.
[c] Incubation implies that the sample was left in a solution of probe DNA for a long time usually several hours. The probe DNA is not chemically coupled to the CNT surface.
NR = not reported; Al = aluminium; Ni = nickel; GCE = glassy carbon electrode; VA = voltammetry; DPV = differential pulse voltammetry; EIS = electrochemical impedance spectroscopy.

binds to an immobilized probe) lead to shifts in the field-effect characteristics of the device (such as resistance or threshold voltage). The resistance changes or threshold voltage shifts function as the sensor signal [49, 72].

Table 2 summarizes the analytical parameters of label-free DNA and RNA biosensors based on CNT-FETs demonstrated until now. Initial DNA biosensors based on CNTs utilized the back-gated configuration for sensing of DNA [31, 33, 57]. In many cases, the probe DNA was attached by incubation of the FET devices in the nucleotide solution [30, 33, 34, 57]. Sensitivities down to the picomolar range [33] have been reported using this technique. The main disadvantage in using a back-gated configuration is the appearance of hysteresis in the field-effect transfer curves [33, 34, 57]. The liquid-gated configuration has been successful in overcoming this hurdle [73]. Moreover, it has been observed that the specific binding on to CNTs is very limited when using nonspecific incubation protocols [29]. Furthermore, the back-gated devices were used for sensing without passivating the source and drain electrodes (see Table 2). In such a situation, the probe DNA accumulates also on the electrodes, which reduces sensitivity. This is apparent from Table 2, where the use of passivated electrodes in combination with a liquid-gated measurement technique yields an improvement in detection limit by up to four orders of magnitude (see Fig. 6) [27]. The attomolar sensitivity was made possible here due to two further improvements namely the use of an improved AC detection technique and a specialized electrochemical functionalization scheme for the immobilization of probe DNA [27]. It is worth mentioning here that the detection limit of 100 aM achieved by this CNT-DNA-sensor is the best reported until now using any direct label-free detection technique based on nanomaterials without involving indicators or reporters or sandwich protocols [47, 71].

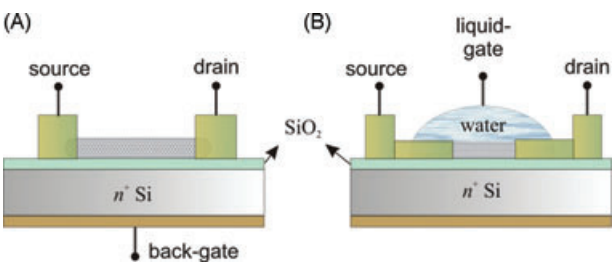

Figure 5. Schematic of field-effect based biosensing (A) in the dry state (B) directly in buffer. From Reference [73].

The ability to detect such a low amount of target DNA without the use of any kind of labeling or sandwich approaches is an important step toward direct detection of nucleic acids in realistic biological samples, possibly without the use of PCR. A first step in this direction has also been demonstrated by attaining this low detection limit in a heterogeneous sample comprising of different noncomplementary sequences [27]. Using PNA as probes and utilizing a liquid-gated configuration with passivated electrodes, RNA could be directly detected in the femtomolar range [30]. CNT-based label-free biosensors have also been demonstrated

Table 2. Label-free CNT-based field-effect biosensors for the detection of DNA and RNA.

Biosensor architecture	Detection mode	Passivation[a)]	Probe attachment	Analyte	Imm. time/ response time	Detection limit	Concentration range	Reference
Pt–SWCNT bundles–Pt	In buffer (liquid gate)	Yes	EDC/NHS coupling with amino DNA (electrochemical)	24-bp synthetic DNA	1 h/30 min	100 aM	100 aM–1 pM	[27]
Au–SWCNT–Au	In buffer (liquid gate)	Yes	Incubation[b)] in amino terminated PNA	12-bp synthetic RNA	NR/few min.	500 fM	500 fM–5nM	[30]
Ti–SWCNT network–Ti	Dry (back gate)	No	Incubation[b)] in unmodified DNA	12-bp and 51-bp synthetic DNA	1 h/1 h	1 pM	1 pM–100 nM	[33]
Au–SWCNT network–Au	In buffer	No	Incubation[b)] in thiolated DNA	15-bp and 30-bp synthetic DNA	22 h/20 min.	100 nM	NI	[31]
Au–SWCNT network–Au	Dry (back gate)	No	Incubation[b)] in amino-terminated DNA	12-bp synthetic DNA	16 h/1 h	500 nM	NI	[34, 57]
Co–SWCNT–Co	Dry (no gate)	No	EDC/NHS coupling with amino DNA (to COOH groups of CNT)	20-bp synthetic DNA	12 h/12 h	500 nM	NI	[29]
Pd–SWCNT–Pd	Dry (back gate)	No	EDC/NHS coupling with amino DNA (to synthetic polymer on CNT)	20-bp synthetic DNA	8 h/2 h	16.2 μM	NI	[28]

[a)] If electrodes are passivated, the nanotubes exclusively constitute the active area. Otherwise, the electrodes are also decorated with probe nucleic acids.
[b)] Incubation implies that the sample was left in a solution of probe DNA for a long time usually several hours. The probe DNA is not chemically coupled to the CNT surface.
NI = not investigated. Implies that only one or two concentrations were experimented; NR = not reported; Au = gold; Ti = titanium; Co = cobalt; Pd = palladium; Pt = platinum; Cr = chromium; SNP = single nucleotide polymorphism.

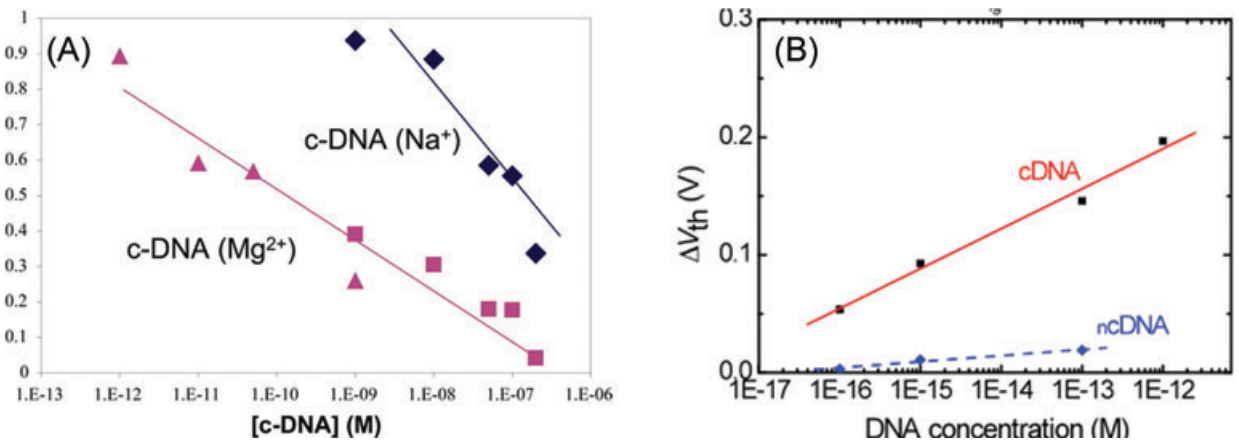

Figure 6. Calibration curves of SWCNT-DNA-sensors based on field-effect detection. (A) Back-gated SWCNT-network-sensors, from Reference [33]. (B) Liquid-gated SWCNT-bundle-sensors, from Reference [27].

for detecting SNPs by using the *Escherichia coli* MutS protein as the receptor [32].

6 Discussion

In this section, we present a comparison between the electrochemical and field-effect biosensing strategies. Both methods have their own pros and cons. Electrochemical methods are ideally suited in order to realize low-cost biosensors. Since they work mostly at a microscale (the main advantage of using CNTs is as an additive to improve the electrochemical properties), they can be easily fabricated in a reproducible manner in large amounts. On the other hand, CNT-based FET biosensors require high-tech processing capabilities that are typical in the microelectronics industry, and hence may turn out to be more expensive than their

electrochemical counterparts. The preparation of electrodes here is more rigorous; however using photolithographic patterning techniques, one can reduce the costs of production when making biosensors at a large scale [28, 74, 75]. Another related aspect is the necessity to position the CNTs at the desired locations. This is indeed possible using techniques such as dielectrophoresis; however, this requires still a sample-by-sample treatment. A prudent way to avoid this problem is the use of CNT networks [33] however, the sensitivity is lower due to the increased surface area of the networks. Once such problems are overcome by engineering the fabrication processes, we would have the ability to directly detect nucleic acids at ultra-low concentrations.

A second hurdle of using CNTs in FETs is the variability in the electronic and chemical properties from one tube to another. For both the FET and electrochemical detection, this variability limits the possibility of calibration. The nonhomogeneity of the electronic properties leads to device-to-device variation in the sensor characteristics making calibration of such sensors difficult. This has been hampering the use of such sensors in field trials. A few strategies have been proposed to overcome this variation by the design of appropriate calibration algorithms [76]. Efforts are also underway to synthesize monodisperse CNTs or to separate them according to their electronic structure after production [25]. Synthesized pure tubes are however quite expensive, which may lead to very high costs during large-scale manufacture.

A third issue that is also related to calibration concerns the immobilization of probe DNA on the surface of CNTs. Incubation in probe DNA has been the widely used method for this purpose. However, unlike microarray techniques where the surface is homogeneous and activated, CNT network surfaces are sparsely covered or appear in three dimension inside polymer matrices. As a result, the loading of the CNT surfaces with probe DNA molecules can be seldom uniform. The covalent and electrochemical methods may serve as a solution to this problem. Since only those tubes that are in contact with the underlying electrodes are functionalized, the loading of DNA on to CNTs is expected to be more uniform from one device to the other.

A fourth concern relates to the use of the chosen measurement strategy. In the case of electrochemical CNT-DNA-biosensors, the detection mostly involves a reaction, and hence they are prone to interferents that are electroactive. When working with real biological samples such as serum, the presence of other molecules that can be oxidized or reduced will also lead to an electrochemical signal thus creating a high proportion of false negatives. This makes preprocessing of complex matrices a prerequisite before performing electrochemical detection. In the case of field-effect biosensors, there is a very high probability of signal drifts over a few measurement cycles, mostly due to nonspecific binding. Furthermore, since the detection is possible only within the electrical double layer on the surface of the CNTs, sequences longer than a few tens of base pairs are difficult to be detected.

7 Conclusions

In this review, we have summarized the various CNT-based label-free biosensing strategies for the detection of nucleic acids. We presented the available results in the literature from a technological point of view pointing out the key aspects in the properties of CNTs, followed by fabrication approaches and biosensing strategies. We have compared the two major label-free electrical biosensing techniques utilizing CNTs for the detection of nucleic acids. Although there are a number of other label-free sensing paradigms available such as QCM and SPR, research in the recent years have focused mainly on electrical detection strategies when deploying CNTs. This is also due to the fact that portable devices can be easily fabricated for electrical detectors, which is hardly realizable for other transduction principles.

Practical Application

The review outlines carbon nanotube-based label-free biosensors for the detection of nucleic acids. Although many of the reported biosensors are still at a research stage, the possibility to detect DNA down to the attomolar range without using labels, indicators or secondary receptors is expected to open new avenues for the direct diagnosis of nucleic acids in biological samples.

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