CARBON NANOTUBES AND GRAPHENE NANO FIELD-EFFECT TRANSISTOR-BASED BIOSENSORS

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1. Abstract:

Graphene and carbon nanotubes (CNTs) have gained major research interests as signal transducing elements in electrical biosensors for applications in biosensing of a wide range of analytes. This is mostly due to the unique physical, chemical, and electrical properties of the carbon nanomaterials. This review discusses the integration and applications of these carbon allotropes into field-effect transistor-type (FET-type) nanobiosensors. We first discuss the various properties of CNTs and graphene that make them useful and attractive for FET-type sensing, followed by methods for synthesis of CNTs and graphene nanomaterials. Additionally, the underlying sensing mechanisms of CNT- and graphene-based FET-type biosensors and the methods for fabrication of these devices are discussed. Finally, recent reports on CNT- and graphene-based FET biosensors that employed a variety of novel device configurations, fabrication techniques, and assay strategies to achieve sensitive detection of small molecules, metal ions, proteins, and nucleic acids are examined.

2. Introduction:

Serving as analytical devices that integrate biorecognition elements with signal transduction elements to convert molecular interactions to measureable signal, biosensors are important tools for myriad fields and applications [1]. Since the first carbon nanotube field-effect transistor (CNT-FET) devices reported in 1998, carbon nanomaterials, such as carbon nanotubes and graphene, have increasingly garnered considerable interest from the biosensor research community as they offer the potential for improved sensitivity, biocompatibility, portability, and most importantly the convenience of label-free sensing [2]–[4]. Due to the unique chemical, electrical, and physical properties of these carbon allotropes, these nanomaterials have been of particular interest in applications for field-effect transistor-based (FET) biosensors [2], [5], [6].

3. Carbon nanomaterials:

3.1. Carbon Nanotubes:

3.1.1. Properties of CNTs:

The carbon nanotube (CNT), which can be considered a 1-dimensional (1D) allotrope of carbon, can be described as a ribbon of graphene comprising sp^2 hybridized carbon atoms with a hexagonal lattice seamlessly rolled into a cylindrical tube [7], [8]. CNTs can further be categorized

based on the number of graphene layers forming the cylindrical tube. Single-walled carbon nanotubes (SWCNTs) have one single layer of sp^2 carbon atoms forming the nanotube (i.e. one graphene sheet), while multi-walled carbon nanotubes (MWCNTs) consist of two or more layers of graphene sheets that form concentric cylinders [7], [9]. Depending on the synthesis method used, SWCNTs and MWCNTs have diameters that vary between 0.43-2.0 nm and up to more than 10 nm, respectively, with lengths ranging from hundreds of nanometers to hundreds of microns [10], [11]. While MWCNTs have metallic electronic properties, SWCNTs can have either semiconducting or metallic electronic properties depending on their chirality and diameter [12]. Furthermore, SWCNT bandgap energies vary inversely with the diameter.

Despite sharing similar sp^2 chemical properties to 2D graphene sheets, the tube curvature of CNTs and quantum confinement in the circumferential direction result in unique electronic properties that are different from those of graphene sheets. Most relevant to FET-based biosensors are semiconducting SWCNTs which are inherently p-type semiconductors with holes as the main charge carriers. Semiconducting SWCNTs are consequently used as the semiconducting channel between the source (S) electrode and the drain (D) electrode [12], [13].

3.1.2. Carbon Nanotube Synthesis:

There are three major methods for CNT synthesis: arc-discharge [7], laser-ablation (vaporization) [14], and catalyst-assisted vapor chemical deposition (CVD) [11]. Arc discharge synthesis of CNTs requires the use of two graphite electrodes to produce a direct current electric arc discharge in an inert environment, where the CNTs are collected in the soot material [7]. SWCNT synthesis via this method further requires a metal catalyst such as cobalt, whereas MWCNT synthesis do not require a metal catalyst [15]. CNT growth by laser ablation uses intense laser pulses to ablate a carbon target, which contains 0.5 percent atomic concentration of cobalt and nickel, in a tube furnace at 1200°C under inert environment [11]. Both arc discharge and laser ablation methods yield high quantity and high quality CNTs. In CVD growth of CNTs, a catalyst material is heated to high temperatures in a tube furnace while the precursor hydrocarbon gas is flowed through the tube for a duration of time [16]. The hydrocarbons and catalyst material used, as well as the growth temperature are essential parameters to CVD synthesis of CNTs. Typical catalysts for this method are iron, nickel, or cobalt nanoparticles. The catalyst layer is typically patterned on a silicon substrate with an SiO_2 insulating layer [17].

Significant efforts have been underway to optimize these methods to yield defect-free crystalline CNTs and to control for the types of CNTs grown. Since these synthesis methods result in a mixture of metallic and semiconducting CNTs of varying dimensions, that have varying

degrees of amorphous carbon contaminations, and may contain bundles of CNTs, isolation and purification of semiconducting SWCNTs are needed. Various purification methods have been developed as summarized by Ramnani et al. to yield the desired defect-free semiconducting SWCNTs [3]. Thanks to the development effort for scaled-up synthesis of CNTs, commercial sources for purified CNTs are readily available.

3.2. Graphene

3.2.1. Properties of Graphene:

Graphene is described as a planar 2-dimensional monolayer of sp^2 hybridized carbon atoms packed in a honeycomb lattice [6], [18]. It is considered to be the building block for other carbon structures of other dimensionalities [6]. Graphene can be wrapped up into zero-dimensional fullerenes, rolled up into 1D CNTs, and vertically stacked to obtain 3D graphite. Free-standing pristine graphene was first isolated and electrically characterized in 2004. Graphene is a zero-bandgap semiconductor that exhibits ambipolar electric field effect, where charge carriers are continuously tunable between holes and electrons in concentrations, n , ranging up to 10^{13} cm^{-2} with mobilities, μ , above $15,000 \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1}$ in ambient conditions [6], [18], [19]. This ambipolar characteristic suggests graphene's electrical sensitivity to both electron-donating and electron-withdrawing species. Furthermore, graphene's chemical stability allows the material to resist oxidation in solution under low voltage, eliminating the need for an electrical passivation layer. Graphene's high chemical stability, its unique electrical characteristics, its sub-nanoscale thickness, and large surface area suggests the nanomaterial's potential in FET-based biosensing [2].

3.2.2. Synthesis and Variations in Graphitic properties:

Single layer graphene was first isolated from highly oriented pyrolytic graphite (HOPG) by Novoselov and Geim in 2004 via mechanical exfoliation [18]. This was achieved by repeated peeling of π -stacked graphene layers from the bulk graphite with scotch tape [18]. Mechanical exfoliation provides higher quality graphene with minimal chemical defects, but the method suffers from low yield and lacks scalability. An alternative method for mechanical exfoliation utilizes sonication of graphite in liquid phase (in organic solvent or water) while using ionic surfactants to maintain colloidal stability of the exfoliated graphene sheets to prevent aggregation [20], [21]. Centrifugation of the dispersed graphene solution allows for further separation of single layer and few-layer graphene. Liquid phase mechanical exfoliation of graphite is scalable for higher yield of graphene that have minimal defects. Dispersed graphene solution can be conveniently used to deposit graphene flakes onto desired substrates via spray

coating and drop-casting [22]. However, heat treatment of deposited graphene flakes may be required to ensure complete removal of chemical contaminants such as residual solvents or surfactants.

Epitaxial growth of graphene via thermal decomposition on the (0001) surface of single-crystal Si-6H substrate is another alternative for graphene synthesis that yields graphene with few defects [23], [24]. Graphene synthesis via this method is carried out under ultrahigh vacuum and at high temperatures. Si atoms desorb from the surface of the SiC substrate while carbon atoms remain, undergoing surface rearrangement and re-bonding to form graphene layers [25]. This method yields graphene on a semi-insulating SiC substrate, which eliminates the need for the transfer of graphene onto another insulating substrate, allowing for in situ use. However, the intrinsic thickness of the SiC substrate only allows for top-gating and liquid-gating—and not bottom-gating—of the graphene which will be discussed later in the review [26].

One popular synthesis method for graphene is chemical vapor deposition of carbon precursor gas on metal catalysts such as copper [27] or nickel [27], [28]. CVD grown graphene enjoy minimal defects. In a tube-furnace, hydrocarbons are thermally decomposed to yield carbon atoms which undergo diffusion into the metal surface, whereby upon cooling of the metal-carbon solid solution, the carbon atoms precipitate out and segregate on catalytic metal surfaces, which form graphene. CVD graphene synthesis using copper foil is of particular interest as it yields large-area and high quality graphene films [27]. By controlling synthesis parameters such as the type of metal catalyst, the hydrocarbons, growth temperatures, and growth duration, various qualities of graphene film can be controlled, such as the number of layers and the size of individual graphene crystals. Graphene grown on metal catalyst substrates allow for transfer of the graphene film onto the desired substrate by securing the graphene film with a polymer (e.g. poly(methyl methacrylate) or (PMMA)) support layer followed by etching away the metal layer. Wafer-scale synthesis of graphene using CVD method followed by transfer of graphene films have been previously demonstrated highlighting the method's potential for large-scale synthesis [29].

Graphene can also be synthesized through reduction of graphene oxide. For this, bulk graphite is chemically oxidized via Hummer's method to produce graphite oxide and then exfoliated by sonication to yield graphene oxide flakes [30], [31]. Briefly, graphite is treated with potassium permanganate, sodium nitrate, and concentrated sulfuric acid to introduce polar oxygen-containing functional groups, such as carboxyl, epoxide, hydroxyl, and carbonyl, to introduce hydrophilicity into the layers of the material [30], [32]. These highly oxygenated layers in graphite oxide can be mechanically exfoliated via sonication from the bulk material to form stable colloidal dispersions of single-layer and few-layer graphene oxide (GO) in various organic

solvents and even water. Similar to mechanically exfoliated graphene solutions stabilized in surfactant, GO flakes can be applied onto the desired substrates via spray coating and drop casting. The electrical properties of exfoliated GO, however, are drastically compromised compared to pristine graphene due to the various chemical defects and loss of crystallinity. Restoration of the electrical properties requires reduction to remove chemical oxide groups and sp^3 defects while restoring sp^2 bond structures [32]. GO can be reduced chemically using reducing agents (e.g. hydrazine), electrochemically by directly applying a reducing potential on the GO surface, or thermally at elevated temperatures [3], [33]. Depending on the methods of reduction, the quality of reduced GO (rGO) will vary. Since not all defects are removed in rGO compared to pristine graphene, only partial electrical characteristics are restored depending on the extent and method of reduction. While chemical and structural defects in GO lead to insulator behavior, removal of these defects via reduction restores semiconductor properties to rGO for applications in electronics, especially for FET-based biosensing [34]. Singh et al. reported the tuning of electrical properties and bandgap of rGO by variation of temperatures for thermal reduction of GO [34].

4. Operating principle and sensing mechanism of FET-based biosensor using carbon nanomaterials

Field-effect transistor (FET) devices generally consist of a source, a drain, and a gate terminal where current in the semiconducting channel between the source and drain (I_D) terminals is modulated by the electric field generated by the voltage of the gate (V_G) terminal and the voltage applied between the source and drain (V_{SD}) terminals [35]. The gate voltage, V_G , can be applied via bottom-gating (back-gating) or top-gating. For SWCNT and graphene FET biosensors fabricated on SiO_2/Si substrates, the source and drain electrodes and the semiconducting channels are fabricated on top of the insulating SiO_2 layer. The degenerately doped semiconducting Si layer is then used as the bottom-gate. In FET biosensors in which assays are performed in the liquid solution on top of the device (typically in the presence of some electrolytes), top-gating is achieved through the use of a reference electrode (e.g. Ag/AgCl) submerged into the liquid phase. Top-gating in this environment is also referred to as liquid-gating or electrolyte-gating if electrolytes are present in the solution.

Both options for back-gating and top-gating (or liquid-gating) operations allow for the observation of the ambipolar electric-field effect of a graphene FET. The back-gating operation requires measurements to be performed without any liquid on the device as well as a gate-voltage range greater than ± 20 V to observe the full ambipolar electric field-effect [5], [36]. Top-gating operation, however, allows for the use of phosphate buffer and require a much smaller gate-

voltage range between ± 0.1 V; therefore, transconductance, calculated as the slope of the I_D - V_G curve on either side of the Dirac point, is more than two orders of magnitude greater for liquid-gating operations compared to back-gating [5]. Thus, compared to back-gating, there are several advantages to liquid-gating, which allows for the direct electrical measurements of the FET biosensor without requiring removal of the solution in which analyte detection is taking place and smaller gate-voltages [5].

Depending on the type of semiconducting channel material used, modulation of the gate voltage can generate an electric field which controls the number of charge carriers (holes and electrons) in the channel, thereby affecting the conductance/resistance of the channel. Thus, biosensors employing an FET architecture and operation differ from the conventional electrochemical biosensors' three-electrode setup and operation, which consists of a working electrode, a reference electrode and a counter electrode [1], [37]. Specifically, conventional three-electrode setup is utilized for measurement modes such as amperometry and potentiometry which require electroactive species or biocatalytic elements to generate electrical signals, respectively [1], [35]. FET-based biosensors, on the other hand, yield electrical responses upon affinity-based binding or adsorption of charged biomolecules with the semiconducting (transducer) channel which result in modulation of the number of charge carriers in the channel, thus changing the observed conductance or resistance of the channel. This characteristic highlights the potential of this biosensor architecture for label-free and affinity-based sensing. The electric field generated by charged biomolecules—in the case where SWCNT or graphene nanomaterials are used as semiconducting channels—near the interface of the semiconducting material provides gating effects on the channel that are similar to effects from applying a voltage potential to the gate terminal.

A chemiresistor-type biosensor is a variation of the FET-biosensor configuration, which omits the gate electrode, allowing the modulation of device conductance by same mechanisms as in traditional FET-based biosensors [38], [39]. Electrical characterization of chemiresistor-type biosensor is performed via I_D - V_S (or simply I - V) measurements from which resistance can be calculated. Changes in conductance or resistance of the device indicate physical and chemical alterations of the surface properties of the carbon nanomaterials [40].

In both SWCNT and graphene FET-based biosensors, the single-layer surface atoms of the carbon nanomaterials are directly exposed to the environment, such that small alterations at the interface result in large changes in electrical properties of the nanomaterials [2]. This confers high sensitivity for biosensing. Upon intermolecular interactions with the biomolecules near the interface of the carbon nanomaterials and the environment, one or more of the following

mechanisms cause the observed changes in electrical properties (i.e. measured electrical responses) of SWCNT and graphene nanomaterials in FET biosensors: (1) surface charge-induced gating effect or electrostatic gating, (2) charge transfer between the biomolecules and the carbon nanomaterials, (3) charge scattering across the carbon nanomaterials, and (4) Schottky barrier modification occurring between the carbon nanomaterials and metal electrodes [2], [5], [41]–[43].

Through series of controlled experiments, Heller et al. verified that the change in conductance of semiconducting SWCNTs in FET biosensors primarily occurs via the two mechanisms, electrostatic gating and Schottky barrier modulation (Figure 1) [42]. Electrostatic gating occurs upon adsorption of charged biomolecules on the bulk of SWCNTs which induces doping in the SWCNTs causing shifts in Fermi level that is observed as the horizontal shifts of the I_D - V_G transfer curve along the V_G axis (Figure 1A). Adsorption of charged biomolecules at the contact regions modulate the metal work function which modulates the width of the Schottky barriers, and in turn affects the current across the nanotube and metal contact [41], [42], [44]. When analyzing device characteristics, Schottky barrier effects can be observed by the asymmetric change in slope (i.e. conductance) of the p- and n- branches of the transfer curve since the Schottky barrier height changes in opposite directions for hole (p) and electron (n) transport (Figure 1B) [42]. Byon and Choi have taken advantage of the Schottky barrier effect in SWCNT-FET biosensor by maximizing the Schottky contact area to increase the sensitivity of their devices for protein detection in the picomolar (pM) range [43]. Metal contacts were fabricated to extend the contact regions of semiconducting SWCNTs and source-drain electrodes.

Figure 1: Example of modulation of SWCNT-FET transfer characteristic due to strong electrostatic gating (A) and strong Schottky barrier effect (B). Adapted from [42].

On the other hand, for a graphene FET device, current is primarily limited by diffusive transport in the graphene channel instead of Schottky barriers at the metal-semiconductor

contacts [44]. Therefore, the gate influences conductance through changing the quantity (doping) and mobility of charge carriers. Graphically, on an I_D - V_G curve (a.k.a. transfer curve), of a graphene FET biosensor, the minimum of conductance corresponds to a minimum of charge carriers near the neutrality point, or the Dirac point (Figure 2A) [5], [44]. To the left of the neutrality point, the majority carriers are holes, while to the right, the major charge carriers are electrons. The shift in Fermi level is observed by the lateral shift of the transfer curve—and consequently the shift in the neutrality point—along the V_G axis (Figure 2B) [42], [44].

The mechanism of electrostatic gating effect can be observed for the case where the semiconducting SWCNT or graphene channel experiences a negative surface charge from a biomolecule or ion near, but external to the semiconducting channel at the liquid interface. The negative surface charge attracts mobile positive charges to the semiconductor-liquid interface, making the interfacial microenvironment charge-neutral. The attracted mobile positive charges consist of both positive ions from the liquid phase (in the electrical double layer) and the positive charges (holes) in the semiconductor. Screening of the original negative surface charge from the biomolecule occurs due to the mobilization of positive ions in the electrical double layer, which compete to reduce the fraction of positive charges in the SWCNT/graphene surface. The positive charges induced in the SWCNT or graphene essentially p-dopes the material—increasing the number of hole carriers—which is graphically observed in the shift of the I_{DS} - V_G transfer curve towards positive gate voltages [5], [44].

Figure 2: Schematic for (A) graphene FET transfer characteristic with the band structures for each regime and (B) changes in transfer characteristic due electrostatic gating by charged analytes. Adapted from [5].

Charge screening effect from the double layer capacitance offsets the surface potential—from a charged analyte or biomolecule—experienced by the SWCNT or graphene by neutralizing the surface potential of the surface charge at the semiconductor-liquid interface. Therefore, a

solution with higher ionic strength causes a compression of the electrical double layer, leading to a reduction in the Debye length, and reduces the field effect of the surface charge of the biomolecule, which reduces the overall sensitivity of the device [5], [44].

Thus, based on the above sensing mechanisms, detection of analytes by SWCNTs and graphene FET biosensors can be achieved through monitoring the device resistance/conductance, threshold gate voltage shift or transconductance.

5. Fabrication and Functionalization of SWCNT and Graphene FET biosensors:

As previously described, SWCNT and graphene-based FET biosensor configuration have source and drain terminals that are bridged by a semiconducting channel, which consists of the semiconducting carbon nanomaterials. Although device dimensions may vary from one study to another, typical electrode and channel dimensions can range between few millimeters to micrometers and even nanometers. Microfabrication and nanofabrication techniques, such as photolithography and electron beam lithography, are typically used for patterning the source and drain metal electrodes onto a substrate, typically SiO₂/Si wafer [2], [3]. The back-gate voltage can be applied directly to the semiconducting Si layer of the wafer whereas a top-gate voltage requires the additional use of a third reference electrode—usually an Ag/AgCl reference electrode as shown in [44], [45].

Diverse methods used for synthesizing and preparing SWCNTs, graphene, and rGO yields these products in various forms, such as colloidal suspensions, solid powders, or thin films which dictate the methods that can be used to integrate these carbon nanomaterials into FET biosensors. Nevertheless, in one fabrication approach, carbon nanomaterials are first deposited or grown directly onto the substrate of the FET device, followed by patterning of metal electrodes [26], [43], [46], [47]. For example, CNTs can be directly grown onto SiO₂/Si substrates using CVD synthesis method [17], [43]. Also, graphene grown directly on SiC substrates can be immediately patterned with metal electrodes [26]. In top-down fabrication, deposition of CNTs, graphene, or reduced/unreduced graphene oxide dispersed in a colloidal suspension can be performed via direct application of the solution onto the substrate using methods such as drop casting, spray coating, spin coating, and AC dielectrophoresis [3]. A simple method for preparing dense CNT networks or GO films onto the device surface is the use of (3-aminopropyl)triethoxysilane (APTES) to functionalize SiO₂ surfaces prior to deposition of the carbon nanomaterials [48]–[50]. Modification of the substrate surface with APTES (or similar derivatives) enhanced electrostatic attraction of CNTs and GO flakes onto the aminated substrate surface. Prior to deposition onto the desired substrate, solid powders of carbon nanomaterials can be re-suspended into various organic

solvents as well as surfactant-containing solutions, usually assisted by sonication [32], [51]. This method reproducibly produces a uniformly distributed high density network semiconducting channel.

CVD grown graphene film can be transferred onto the desired substrate by first depositing a supporting polymer coating—usually poly(methyl methacrylate)—to protect the graphene film from damage and disintegration during the transfer step. The metal catalyst is removed from underneath the protected graphene film by chemical etchants. For graphene grown on copper foils, ferric chloride (FeCl_3) solution is used for etching away the copper foil [27], [29].

5.1. Functionalization of CNTs and graphene with bioreceptor molecules.

In order to achieve specific detection of analyte molecules, the carbon nanomaterial transducer element requires functionalization with the biorecognition elements (e.g. antibodies, enzymes, aptamers, and other binding peptides, etc.). Conjugation of CNT and graphene with the biorecognition molecules is achieved through various chemical means that can be categorized as direct covalent conjugation to the carbon nanostructure or through non-covalent conjugation to the nanostructure as illustrated in Figure 3 [52], [53].

Covalent chemical functionalization of CNTs can be categorized into two strategies: (1) End-cap and defect-site chemistry, and (2) functionalization of pristine CNT side-wall [53]. End-cap and defect-site chemistry takes advantage of the more reactive end-caps of CNTs and localized defects in the nanotube lattice—containing oxygenated groups such as carboxyl groups. Covalent chemical functionalization of the pristine CNT side-walls utilizes a multitude of chemistries such as ozonation, fluorination, and oxidation to yield a variety of new functional groups, such as amines (Figure 3B). Carboxyl and amine groups are common chemical targets for further conjugation of biorecognition molecules via EDC/NHS and aldehyde-activated coupling chemistries, respectively.

Similarly, covalent chemical functionalization of pristine graphene and graphene oxide has been extensively studied and comprehensively reviewed by Georgakilas et al. [54], [55]. Covalent functionalization of pristine graphene capitalizes on the reactivity of $\text{C}=\text{C}$ bonds to free radicals and dienophile to introduce new organic functional groups that can be used for further covalent conjugation with biomolecules (Figure 3C). Since GO and rGO already contain oxygenated groups, such as carboxyl and epoxide groups, further chemical functionalization and conjugation of biomolecules can be performed using common bioconjugation chemistries like EDC/NHS conjugation method.

One major disadvantage of covalent functionalization of CNTs and graphene is the alteration of electronic structure and properties of the nanotubes due to disruption of carbon sp^2 bonds [53],

[54]. Thus, non-covalent conjugation strategies provide attractive alternatives. Non-covalent conjugation of biomolecules onto CNT and graphene sp^2 carbon surfaces typically uses bi-functional linker molecules, such as 1-pyrenebutanoic acid succinimidyl ester (PBASE). The π -electron-rich pyrenyl group π -stacks with the carbon nanostructure while the succinimidyl ester group specifically reacts with amine groups on the biorecognition molecule to form an amide bond (Figure 3A). Alternatively, non-covalent conjugation of biomolecules onto CNT and graphene is performed by simple physical adsorption of the biomolecules onto the surface of the carbon nanomaterials [56], [57]. After functionalizing the carbon nanomaterials with the desired biorecognition molecules, unreacted chemical moieties and carbon surfaces are chemically quenched and physically blocked—with surfactants, amphipathic polymers, or nonspecific protein, respectively [3]. Chemical quenching and blocking minimize non-specific binding during sensing procedure. In biosensing applications, the preferred method for conjugation of SWCNT and graphene surfaces with biorecognition molecules is through the non-covalent functionalization approach as discussed in the following examples of recent development of CNT and graphene FET-based biosensors.

6. Carbon Nanomaterial-based Nano FET Biosensors

6.1. Specific detection of proteins and DNA

SWCNT and graphene-based FET biosensors for detection of proteins and nucleic acid-based analytes have been demonstrated using a wide range of detection strategies as well as device configuration and fabrication strategies as summarized in Table 1 [5], [45], [50], [58]–[61]. In one example, a solution-gated rGO-based FET biosensor was developed for detection of the prostate cancer biomarker, prostate-specific antigen complexed with α_1 -antichymotrypsin (PSA-ACT) [50]. Using rGO functionalized (via PBASE linkers) with monoclonal antibodies against PSA-ACT, the device was able to quantitatively detect concentrations of PSA-ACT antigens in human serum in the femtomolar range.

There is significant effort invested into developing methods for improving performance of these biosensors, such as sensitivity and real-time detection capability. For example, to enhance sensitivity of electrical detection of PSA-ACT with monoclonal antibodies against PSA-ACT as the bioreceptor element, Kim et al. employed a functionalization strategy that minimized the effects of charge screening by the electrical double layer. Specifically, by increasing the spacing between antibody receptors, the charged antigens are able to more easily approach the SWCNT surface within the distance of the Debye length to affect the conductance of the SWCNT. Mixtures at different molar ratios of PBASE linkers and 1-pyrenebutanol (PB) spacer molecules were

investigated to demonstrate the effects of density of antibody functionalization on device sensitivity shown in Figure 4. Thus, devices using a 1:3 ratio of PBASE linker to PB spacers for antibody functionalization were found to achieve a much lower LOD of 1.0 ng/mL compared to devices that used only PBASE linkers (LOD ~ 500 ng/mL).

Figure 3: Functionalization strategies for SWCNT and graphene: (A) Noncovalent functionalization of SWCNT sidewalls using PBASE linkers, adapted from [57]; (B) Covalent functionalization chemistries for pristine SWCNT sidewalls, adapted from [53]; (C) Covalent chemical functionalization of pristine graphene sheet using 1,3 dipolar cycloaddition of azomethine ylide, adapted from [54].

While antibodies are popular protein-based biorecognition molecules, aptamers—a class of nucleic acid-based synthetic biorecognition molecules—offer another alternative for specific analyte detection. Upon binding of larger analytes to the smaller-sized aptamer functionalized on the surface of carbon nanomaterials, the effects of charge screening can be reduced as the analytes are physically closer to the transducer surface within the distance of the Debye length [62]. Another similar strategy to allow bound analytes to approach the transducer surface within the Debye length is to use antibody fragments, such as immunoglobulin Fabs or F(ab')₂ fragments. Antibodies, specifically of immunoglobulin-G (IgG) isotype, can be enzymatically cleaved using papain to yield the univalent antigen-binding Fab fragments which are approximately 3-5 nm in the largest dimension compared to the whole IgG molecule which are 10-15 nm. This strategy was demonstrated on a solution-gated graphene-based FET biosensor using Fabs receptor molecules to detect for heat-shock proteins (HSPs) [59]. The biosensor used mechanically exfoliated single-layer graphene (via repeated peeling of graphite) as the transducer. The Fabs were functionalized onto graphene using PBASE linkers. The biosensor achieved an LOD of 100 pM for the HSP analyte with a dynamic range of hundreds of pM to hundreds of nM.

Figure 4: (A) Schematic diagram of PBASE linkers and PB spacers non-covalently attached to SWCNT surface, where amines on the gold nanoparticle only react with PBASE linkers. (B) Calibration curve for immuno-detection of PSA-ACT complexes using CNT-FET devices modified with 1:3 ratio of PBASE to PB. Adapted from [58].

Detection of nucleic acid-based analytes, such as DNAs and microRNAs, has also been shown to be feasible by various groups using CNT and graphene-based FET biosensors, albeit with some variations in assay strategy and sensing mechanisms in comparison to detection of proteins due to the chemical and structural differences between these analytes. More specifically, DNA detection via sequence-specific hybridization is a common assay strategy for ensuring specificity in

biosensing. A general design for FET biosensors that utilized a DNA hybridization strategy is to functionalize the complementary DNA/RNA strand onto the transducer surface, whereby complementary binding of the target strand leads to a detectable electrical response. Demonstrating this detection scheme, Star et al. developed a label-free SWCNT-based FET biosensor for the detection of complementary DNA (cDNA) strands capable of discriminating single-nucleotide polymorphisms (SNP) [61]. Single-stranded DNA (ssDNA) molecules were non-covalently attached to the SWCNT sidewalls simply by allowing the ssDNA to wrap around individual SWCNTs via π -stacking interactions between the nucleotide bases and SWCNT sidewalls. Complementary DNA strands can subsequently be hybridized with the immobilized ssDNA on SWCNT sidewalls, which were observed in changes of the device conductance (inverse of resistance). Hybridization experiments with fully complementary 12-mer oligonucleotides resulted in decreased conductance while completely mismatched DNA oligonucleotides resulted in significantly smaller changes in conductance. Similarly, in SNP discrimination experiments, the biosensor showed significant decrease in conductance only for fully matched DNA target strands. The biosensor was able to detect 100 pM of the target DNA in the presence of 10^4 -fold molar excess of nonhomologous DNA. The authors further employed another chemical strategy, where counterions such as Mg^{2+} were used during incubation steps, which enhanced the efficiency of DNA hybridization, and consequently conferred a 1000-fold increase (from 1 nM to 1 pM) in sensitivity to the biosensor system [61]. Similarly, DNA detection by hybridization of complementary oligonucleotides have also been shown in other CNT-based FET biosensors, with variations in device architecture, functionalization strategy, and performance characteristics [63], [64].

FET biosensors for specific and label-free detection of DNA hybridization have been developed using both RGO and CVD graphene [65]–[68]. Using chemically reduced GO as the semiconducting channel in a solution-gated FET biosensor architecture, Stein et al. showed that real-time detection of DNA hybridization was feasible with their device [65]. The aminated ssDNA probe strand was functionalized onto RGO surfaces by covalent conjugation method. The GO surface was first aminated with ethylenediamine (EDA) prior to chemical reduction. After chemical reduction of GO using hydrazine vapor, the amine functional groups from the EDA-functionalized RGO was further reacted with glutaraldehyde (a homo-bifunctional linker) to introduce reactive aldehyde groups for further conjugation with aminated-ssDNA probe strands. Real-time detection was enabled by integrating the biosensor chip into a flow cell which allowed for controlled injections of samples at specific times. The biosensing system comprised two FET biosensors—one was functionalized with the complementary ssDNA probe for sensing while the other was functionalized with non-complementary ssDNAs for use as a reference. This approach allowed for

a signal processing step of subtracting the electrical signals between the sensing and reference devices to remove background noise caused by nonspecific binding of non-complementary DNA onto RGO surfaces. The final biosensor achieved an estimated LOD of ~2 nM for target DNA strands.

In another study, Zheng et al. developed an FET biosensor using CVD grown single layer graphene (SLG) as the transducer to detect specific DNA hybridization [67]. Using a PBASE linker, the SLG channel was non-covalently functionalized with peptide nucleic acid (PNA), the probe molecule. An advantage in using PNA as a probe molecule versus DNA is minimization of electrostatic repulsion between two hybridized strands because the negatively charged deoxyribose phosphate backbone in DNA is replaced by the charge neutral peptide backbone in PNA [68]. Hybridization of the PNA with complementary target DNA produced a left-shift of the Dirac point of the device characteristic, which was suggested to be caused by n-doping of graphene by the electron-rich nucleobases of the bound DNA strand. This sensing mechanism for DNA and nucleic acid-based analytes have been previously reported, as well. Non-complementary and one-base-mismatched DNA (at various concentrations) tested on the biosensor yielded negligible and significantly lower changes in transfer characteristics of the device, respectively, compared to incubations with complementary target DNA. The label-free SLG-FET biosensor system achieved a detection limit of 10 fM.

MicroRNAs (miRNAs) are emerging as a useful class of biomarkers in disease diagnostics. Efforts have been made to develop carbon nanomaterial-based FET-type biosensors for detection of these types of biomarkers that would provide viable and highly sensitive alternatives to traditional molecular diagnostic modalities such as reverse transcriptase-polymerase chain reaction [69]. For example, Ramnani et al. devised an SWCNT-based chemiresistor in which a combined assay strategy was employed for quantitative detection of miRNA-122a, a 21 nucleotide-long single-stranded (ss)-RNA biomarker linked to lipid metabolism, homeostasis, and hepatitis C viral replication in the liver [69]. This combined assay strategy first made use of sequence-specific hybridization of the target ss-miRNA-122a strand with a complementary probe strand at an elevated temperature of 37°C. Next, the duplexed miRNA was incubated with SWCNTs functionalized/modified with p19 RNA-binding protein. The 19kDa RNA-binding protein, p19 from Carnation Italian ringspot virus, has specific affinity to 21-23 nucleotide-long double-stranded RNA in a size-dependent and sequence-independent manner. Thus, device conductance/resistance was proportionately modulated upon binding of hybridized miRNA-122a strands to the p19-functionalized SWCNT channel. The SWCNT-based chemiresistor system achieved linear detection of miRNA-122a in the 1 attomolar (aM) to 10 fM range, with a responsivity of 0.42 per decade of

molar concentration of miRNA-122a even in the presence of excess yeast RNA at 10 μg . This biosensor demonstrates the feasibility for a hybrid approach for label-free and sequence-specific detection of small nucleotide strands using nucleotide-based probes and protein-based bioreceptors specific to the hybridized target-probe strands.

Table 1: Recent reports on FET-based biosensors using carbon nanomaterials for detection of protein-based molecules, nucleic acid-based molecules, and pathogens.

Analyte Type	Carbon Nanomaterial & Device Configuration	Target Analyte(s)	Bioreceptor(s)	Limit of Detection	Sensitivity	References
Protein-Based Molecules	SWCNT Chemiresistor	Protective Antigen toxin, Anthrax	ssDNA Aptamer	1 nM	0.11 nM^{-1}	[70]
		Salivary α -amylase	IgG anti- α -amylase	6 $\mu\text{g/mL}$	$2.4 \times 10^{-4} \text{ mL}/\mu\text{g}$	[51]
		Cardiac Troponin I (cTnI)	IgG anti-cTnI	0.001 ng/mL	0.02 /log[ng/mL]	[71]
		Cardiac Myoglobin (cMb)	IgG anti-cMb	24.2 pg/mL	11.1 /log[$\mu\text{g/mL}$]	[72]
	SWCNT-FET	Prostate specific antigen/ α 1-antichymotrypsin (PSA-ACT) complex	IgG anti-PSA-ACT complex	1.0 ng/mL	Not Reported	[58]
		H63D mutation in the HFE gene	ssDNA probe	1 pM	Not Reported	[61]
		Immunoglobulin E (IgE)	DNA Aptamer anti-IgE	250 pM	Not Reported	[62]
	Chemically reduced GO-FET	Prostate specific antigen/ α 1-antichymotrypsin (PSA-ACT) complex	IgG anti PSA-ACT complex	100 fg/ml (~1.1 fM)	Not Reported	[50]
	Thermally reduced GO-FET/Chemiresistor	Human IgG	Gold-labeled goat IgG anti-Human IgG	0.2 ng/mL	Not Reported	[33]
	Exfoliated graphene-FET	Heat-shock proteins (HSPs)	Fab anti-HSP	100 pM	Not Reported	[59]
		Bovine serum albumin (BSA)	None (i.e. nonspecific adsorption)	0.3 nM	Not Reported	[60]
Nucleic Acid-Based Molecules	SWCNT-FET	12-mer target ssRNA	Aminated 12-mer PNA	Not Reported	Not Reported	[63]
	Single-SWCNT FET	10-mer target ssDNA	10-mer ssDNA probe	Single molecule of ssDNA target	Not Reported	[64]
	SWCNT Chemiresistor	microRNA-122a	p19 protein	1 aM	0.41 /log[M]	[69]
	Chemically reduced GO-FET	24-mer target ssDNA	24-mer aminated ssDNA probe	2 nM	Not Reported	[65]
		22-mer target ssDNA	22-mer peptide nucleic acid probe	100 fM	Not Reported	[68]
	Thermally reduced and Pt-decorated GO-FET	22-mer target ssDNA	Thiolated 33-mer ssDNA probe	2.4 nM	Not Reported	[66]
	CVD graphene-FET	22-mer target ssDNA	22-mer peptide nucleic acid probe	10 fM	Not Reported	[67]
Pathogens	SWCNT Chemiresistor	E. coli	IgG Anti-E. coli O157:H7	10^3 CFU/mL	0.124 mL/CFU	[73]

		<i>T7 bacteriophage</i>	<i>IgG anti-T7</i>	10^3 PFU/mL	0.142 mL/PFU	
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6.2. Small molecule detection

Small biomolecules, metabolites, and biologically relevant metal ions pose several inherent challenges to conventional FET-based detection methods. Sensitive detection of small biomolecules at low concentrations via CNT or graphene-based FET method is challenging due to the reduced electric field-effect of the smaller-size and fewer-charge analyte on the carbon nanomaterial transducer element. When compared to larger protein-based analytes, the reduced size of the smaller biomolecules suggests fewer surface charges available on each molecule which reduces the electrostatic gating effect on the semiconducting channel by each analyte's upon binding to an immobilized bioreceptor. For small, uncharged analytes, this reduction in gating effect is even more pronounced. Small analytes that only provide a single binding site or epitope for affinity-based assays pose an additional physical restriction to signal amplification via sandwich-type assay formats. Nevertheless, a novel strategy for overcoming these challenges was demonstrated in several studies using SWCNT-based FET biosensors [74]–[76]. This strategy applies the principle of competitive displacement of biorecognition molecules (e.g. antibodies) bound to transducer-immobilized analytes by unbounded analytes in the sample. In this format, the biosensor the semiconducting transducer channel of the transducer is functionalized with the small-molecule antigens, followed by specific binding of the immobilized antigens with the corresponding biorecognition molecules. Upon incubation of the biosensor with the sample (containing the unbounded antigens) the specifically bound significantly large biorecognition molecule is displaced. The proportional displacement of the significantly larger biorecognition molecules by the smaller analytes in the sample leads to larger physical and, consequently larger electrical changes, at the transducer-solution interface.

Cella et al. first demonstrated this competitive displacement strategy in the development of a label-free SWCNT-based FET affinity sensor with a chemiresistor configuration for the detection of a monosaccharide, glucose (MW of 180.16 Da) in human plasma [74]. The biosensor consisted of dielectrophoretically aligned semiconducting SWCNTs deposited between 3- μ m spaced gold source and drain electrodes as the electrical transducer element (Figure 5A). The SWCNTs were non-covalently functionalized with hydrophobic dextran polysaccharide derivatives, which are chemical analogs of glucose, followed by blocking with Tween-20 surfactants. The bioreceptor used was Concanavalin A (Con A), a metalloprotein with four carbohydrate binding sites. ConA reversibly binds to glucose and dextran, although the protein has lower affinity to dextran than to glucose. The binding of ConA to the carbohydrates, and specifically to dextrans functionalized on SWCNTs, leads to conformational changes to the protein which consequently increases the isoelectric point of the protein and increases positive charge accumulation. Sensing

experiments were conducted by first incubating the dextran-functionalized SWCNT chemiresistor with ConA to form dextran-ConA complexes at the SWCNT surfaces, which led to a large increase in device resistance. Then, various concentrations of glucose was incubated with the device followed by washing the displaced ConA and excess analytes from the device, and performing I_D - V_{DS} measurements to determine the corresponding resistance/conductance signals. With increasing concentrations of glucose, the biosensors showed decreasing resistance, as expected with the displacement of ConA receptors from the SWCNT surface that, when previously bound, led to increased device resistance (Figure 5B). The biosensor achieved a detection limit of 1 picomolar (pM), a sensitivity of 0.039 per pM glucose, and showed high binding selectivity for glucose when tested against galactose, another monosaccharide. This biosensing strategy demonstrated feasibility of small-molecule detection and even detection of uncharged molecule [74].

Figure 5: Schematic (A) for SWCNT chemiresistor and glucose detection results (B) in human plasma, adapted from [74].

Electrical detection of the metal ions Ca^{2+} , Ni^{2+} , Mg^{2+} , Hg^{2+} , and Cd^{2+} , was demonstrated on a liquid-gated FET biosensor system using micro-patterned rGO channels functionalized with analyte-specific protein-based receptors by Sudibya et al. [77]. Calmodulin (CaM), a Ca^{2+} -binding protein, was functionalized onto rGO via a PBASE linker to confer specificity for Ca^{2+} and Mg^{2+} detection, albeit CaM has lower affinity to Mg^{2+} than Ca^{2+} . The CaM-modified rGO FET biosensor was able to quantitatively detect both Ca^{2+} (LOD $\sim 1 \mu\text{M}$, signal-to-noise ratio (SNR) ~ 20 to 30) and Mg^{2+} while unmodified rGO FET devices did not detect either metal ions, confirming the specificity due to CaM receptors. Similarly, for specific detection of Hg^{2+} and Cd^{2+} metal ions, the metallothionein type II protein (MT-II) receptor was functionalized onto rGO. Sensing experiments showed that rGO FET biosensors functionalized with MT-II achieved specific and sensitive detection of both Hg^{2+} (LOD $\sim 1 \text{ nM}$, SNR of 25 to 30) and Cd^{2+} (LOD 1 nM, SNR of 15 to 20). Electrical

measurements of the sensors in p-type region ($V_G = -600$ mV) and n-type region ($V_G = 600$ mV) for all four metal ions were expected to show similar trends due to electrostatic gating effects since the metal ions Ca^{2+} , Mg^{2+} , Hg^{2+} , and Cd^{2+} were all equally charged. However, the electrical responses for detection of Ca^{2+} and Mg^{2+} on CaM-modified biosensors compared to detection Hg^{2+} and Cd^{2+} with MT-II-modified biosensors showed electrical responses of opposite polarity. The authors explained that Ca^{2+} and Mg^{2+} binding to CaM-functionalized rGO surface is primarily attributed to the direct field effect induced by the bound metal ions, which n-dopes rGO. Conversely, they proposed that Hg^{2+} and Cd^{2+} heavy metals binding to MT-II-functionalized rGO primarily induces conformational changes in the highly negatively charged MT-II receptor such that surface interactions between MT-II molecules and rGO increases, leading to the increased p-doping effect [77]. Sensitive detection of other small biomolecules and metal ions using CNT- and graphene-based FET biosensors have also been demonstrated as exemplified in these recent reports listed in Table 2.

Table 2: Recent reports on FET-based biosensors using carbon nanomaterials for small molecules and metal ions.

Analyte Type	Carbon Nanomaterial & Device Configuration	Target Analyte(s)	Bioreceptor(s)	Limit of Detection	Sensitivity	References
Small Molecules	SWCNT Chemiresistor	MCLR	IgG anti-MCLR	0.6 ng/mL	$0.175 / \log[\text{g/L}]$	[76]
		ATP	ssDNA aptamer	1 pM	1.3 nM^{-1}	[78]
		Glucose	Concanavalin A	1 pM	0.039 pM^{-1}	[74]
		Cortisol	IgG anti-cortisol	1 pg/mL	13.97 mL/ng	[40]
Metal and Heavy Metal Ions	SWCNT Chemiresistor	Hg^{2+}	ssDNA aptamer	100 nM	$6.72 \times 10^{-3} \text{ nM}^{-1}$	[79]
		Ni^{2+}	Hexa-histidine	Not Reported	Not Reported	[79]
	Chemically reduced GO-FET	Ca^{2+} , Mg^{2+} , Hg^{2+} , and Cd^{2+}	<ul style="list-style-type: none"> Calmodulin (CaM) for detection of Ca^{2+} and Mg^{2+} Metallothionein type II protein (MT-II) for detection of Hg^{2+} and Cd^{2+} 	<ul style="list-style-type: none"> Ca^{2+}: $\sim 1 \mu\text{M}$ Mg^{2+}: Not Reported Hg^{2+}: $\sim 1 \text{ nM}$ Cd^{2+}: $\sim 1 \text{ nM}$ 	Not Reported	[77]

7. Conclusion

Considerable efforts have been invested into development of carbon nanomaterial-based FET-type biosensor systems, as seen in the previously discussed examples. Design and fabrication of FET biosensors using these carbon nanomaterials are dependent on the specific physical, chemical, and electrical properties of the materials as well as the synthesis methods of the materials. Detection strategies for these biosensors further depend on the type of analytes,

testing environment, and bioreceptor molecules used. While the different sensing mechanisms of CNTs and graphene-based FET biosensors have been generally discussed in this review, it is interesting to note that the sensing mechanisms that lead to the changes in electrical responses and device characteristics vary upon the types of analytes detected, biorecognition molecules used, and device configurations. In some cases, integration of the biosensing elements into a fluid control system can provide additional functionalities, such as real-time detection, and enhance other sensor performance characteristics, such as reproducibility. Thus, incorporating fluidic or microfluidic elements into a biosensor for lab-on-chip applications is an attractive design strategy to leverage the label-free sensing capability and sensitivity of FET biosensors using carbon nanomaterials.

A desirable characteristic for any biosensor system is portability which is achievable for FET-based biosensors composed of nanometer- to millimeter-sized features. Furthermore, since signal outputs of FET-based biosensors are directly measured as electrical current, measuring instruments for these devices can be less cumbersome, less expensive, and more portable compared to measuring instruments for other types of transducer platforms, such as optical biosensors which require optical components and photodetectors. Demonstrated by the examples in this review, label-free sensing capability is another major advantage of FET-based biosensors using carbon nanomaterials over some optical biosensor platforms which may require fluorescent labels or other photoactive probes.

While carbon nanomaterials, like CVD graphene and SWNTs, have increased in commercial availability, FET-type biosensors using these carbon nanomaterials have yet to reach the same level of commercial availability, especially for analyte-specific applications. For commercial application of these biosensors, scalability of manufacturing these devices is an important design factor. Thus, highly scalable synthesis methods, such as CVD synthesis, of these carbon nanomaterials, coupled with micro- and nanofabrication technologies adopted from the semiconductor industry make the prospects of commercialization of carbon nanomaterial-based FET biosensors more promising.

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9. References

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