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Carbon Nanotubes in Biomedicine and Biosensing

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1. Introduction

Since carbon nanotubes (CNTs) were discovered by Iijima in 1991(Iijima, 1991), they have become the subject of many studies because of their unique electrical, optical, thermal, and mechanical properties(Ouyang, Huang, & Lieber, 2002; Thostenson, Ren, & Chou, 2001; Troiani, Miki-Yoshida, Camacho-Bragado, Marques, Rubio, Ascencio, et al., 2003; Wan, Dong, & Xing, 1998). Carbon nanotubes (CNTs) can be visualized as a sheet of carbon atoms rolled up into a tube with a diameter of around tens of nanometers. There are two main types of CNTs, Single-walled (SWCNTs) and multi-walled carbonnanotubes (MWCNTs), the latter being formed by several concentric layers of rolled graphite (Figure 1). In particular, SWCNTs are characterized by a high aspect ratio. Moreover, Their versatile physicochemical features enable the covalent and noncovalent introduction of several biomedicine and biosensing application relevant entities. Thus exploitation of their unique electrical, optical, thermal, and spectroscopic properties in a biological context is hoped to yield great advances in the therapy of disease and detection biomolecules such as DNA, antigen–antibody, cells, and other biomolecules.

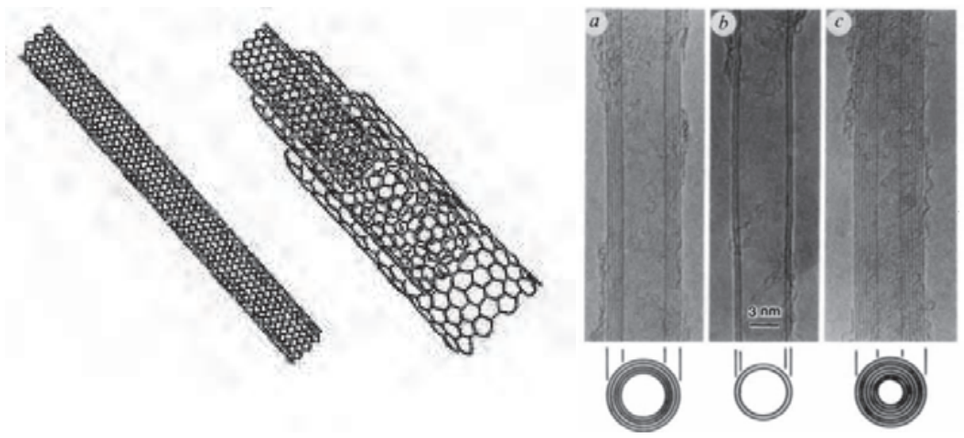


Fig. 1. Schematic of a SWNT and MWNT (left). (Lacerda, Raffa, Prato, Bianco, & Kostarelos, 2007) and TEM of CNTs. a, Tube consisting of five graphitic sheets, diameter 6.7 nm. b, two-sheet , diameter 5.5 nm. c, seven-sheet , diameter 6.5 nm, which has the smallest hollow diameter (2.2 nm) (right). (Iijima, 1991).

Part A. Carbon nanotubes in biomedicine

CNTs have been used as efficient electrochemical and optical sensors, substrates for directed cell growth, supporting materials for the adhesion of liposaccharides to mimic the cell membrane(Bianco & Prato, 2003; Lin, Taylor, Li, Fernando, Qu, Wang, et al., 2004), transfection(Kam, Jessop, Wender, & Dai, 2004; Pantarotto, Briand, Prato, & Bianco, 2004; Pantarotto, Singh, McCarthy, Erhardt, Briand, Prato, et al., 2004), and controlled drug release(Kam, Kim, & Dai, 2004; Kam, Liu, & Dai, 2005; Kam, O'Connell, Wisdom, & Dai, 2005; Lacerda, Raffa, Prato, Bianco, & Kostarelos, 2007). Some researches have shown the ability of single-walled carbon nanotubes (SWNTs) to cross cell membranes and to enhance deliver peptides, proteins, and nucleic acids into cells because of their unique structural properties(Kam & Dai, 2005; Pantarotto, Briand, Prato, & Bianco, 2004; Prato, Kostarelos, & Bianco, 2008). For this reason, carbon nanotubes could serve as an excellent vehicle to administer therapeutic agent providing effective utilization of drug and less elimination by the macrophage.

One key advantage of carbon nanotubes is their ability to translocate through plasma membranes, allowing their use for the delivery of therapeutically active molecules in a manner that resembles cell-penetrating peptides. Moreover, utilization of their unique electrical, optical, thermal, and spectroscopic properties in a biological context is hoped to yield great advances in the detection, monitoring, and therapy of disease.

Advantage	Disadvantage
• Unique mechanical properties offer in vivo stability • Extremely large aspect ratio, offers template for development of multimodal devices • Capacity to readily cross biological barriers; novel delivery systems • Unique electrical and semiconducting properties; constitute advanced components for in vivo devices • Hollow, fibrous, light structure with different flow dynamics properties; advantageous in vivo transport kinetics • Mass production – low cost; attractive for drug development	• Nonbiodegradable • Large available surface area for protein opsonization • As-produced material insoluble in most solvents; need to surface treat preferably by covalent functionalization chemistries to confer aqueous solubility (i.e. biocompatibility) • Bundling; large structures with less than optimum biological behavior • Healthy tissue tolerance and accumulation; unknown parameters that require toxicological profiling of material • Great variety of CNT types; makes standardization and toxicological evaluation cumbersome

Table 1. Advantage and Disadvantage of using CNTs for biomedical applications. (Lacerda, Raffa, Prato, Bianco, & Kostarelos, 2007).

1. Functionalization of CNTs

For biological applications, the improvement of solubility of CNTs in aqueous or organic solvents is a major task. Great efforts have devoted to search cost-effective approaches to functionalize CNTs for attachment of biomolecules as recognition elements. Generally, this procedure can be performed by noncovalent and covalent functionalization strategy.

2. Noncovalent interaction

The noncovalent approach via electrostatic interaction, Van der Waals force, or π–π stacking is a feasible immobilization method for biomolecules. Particularly, it is promising for improving the dispersion proteins of CNTs without destructing of the nanotube structure. Generally, this route can be performed by physical adsorption or entrapment.(Arnold, Guler, Hersam, & Stupp, 2005; Richard, Balavoine, Schultz, Ebbesen, & Mioskowski, 2003)

2.1 Physical adsorption

A variety of proteins can strongly bind to the CNTs exterior surface via physical adsorption. When the ends of the CNTs are open as a result of oxidation treatment, smaller proteins can be inserted into the tubular channel (~5–10 nm in diameter).
The combined treatment of strong acids and cationic polyelectrolytes is known to reduce the CNTs length and enhance the solubility under physiological. After this treatment, cationic polyelectrolytes molecules adsorb on the surface of the nanotubes by van der Waals force to produce the distribution of positive charges, which prevents the aggregation of CNTs.

2.2 Entrapment

Another method for immobilizing biomolecules on CNTs is to entrap them in biocompatible polymer hydrogen and sol–gel. Single strand DNA (ssDNA) can wrap around SWCNTs through aromatic interaction to form a soluble DNA–SWCNT complex, which has been used for construction of effective delivery for gene therapy.
Sol–gel chemistry has paved a versatile path for the immobilization of biomolecules with acceptable stability and good activity retention capacity.

3. Covalent interaction

Since the as-produced CNT contain variable amounts of impurities, such as amorphous carbon and metallic nanoparticles, the initial efforts in their purification focused on the selective oxidation of the impurities with respect to the less reactive CNT. The combined treatment of strong acids and sonication is known to purify the CNTs and generate anionic groups (mainly carboxylate) along the sidewalls and ends of the nanotubes (see figure 2). Also, dangling bonds can react similarly, generating other functions at the sidewalls.(Bahr, Yang, Kosynkin, Bronikowski, Smalley, & Tour, 2001; Williams, Veenhuizen, de la Torre, Eritja, & Dekker, 2002)

4. CNTs for biomedical applications

4.1 CNTs for protein delivery

Various low molecular weight proteins can adsorb spontaneously on the sidewalls of acid-oxidized single-walled carbon nanotubes(Kam & Dai, 2005). The proteins are found to be readily transported inside mammalian cells with nanotubes acting as the transporter via the endocytosis pathway. This research was reported by Dai group. The results shown streptavidin (SA) and cytochrome c (Cyt-c) could easily transport into the cytoplasm of cells by the CNTs and take effect of their physiological action in the cell. Carbon nanotubes could become new class of protein transporters for various in vitro and in vivo delivery applications.

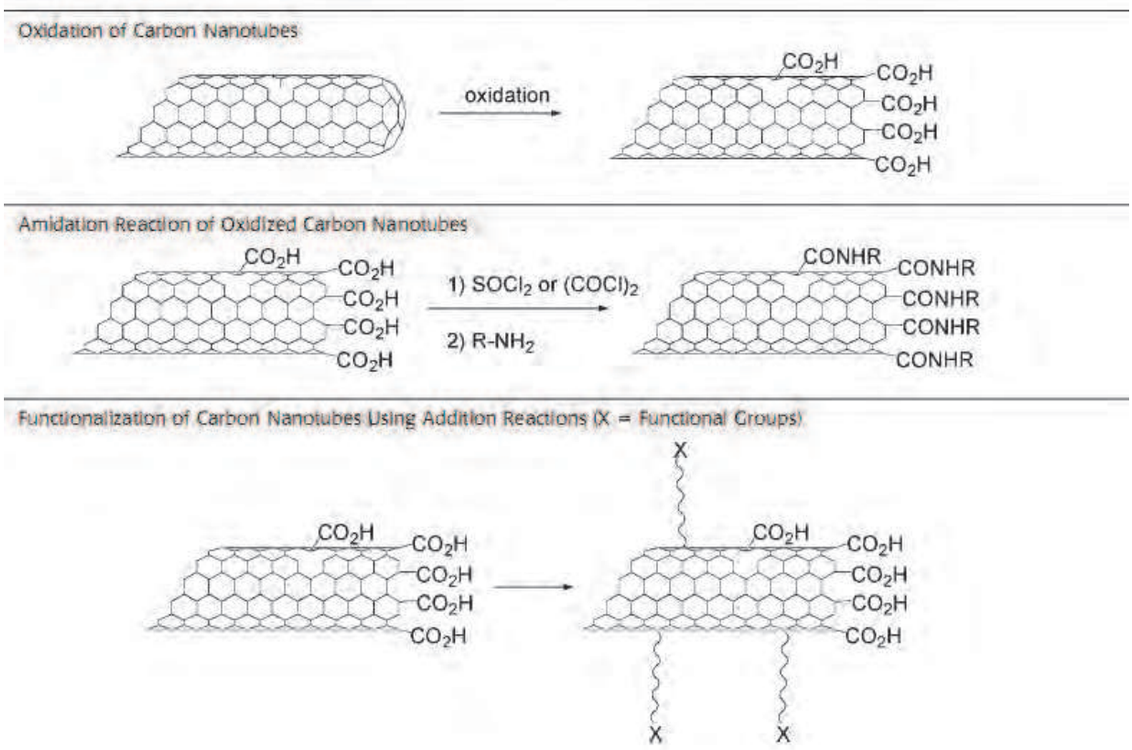


Fig. 2. Oxidation and Functionalization of CarbonNanotubes (Prato, Kostarelos, & Bianco, 2008).

4.2 CNTs for gene delivery

One of the most promising concepts to correct genetic defects or exogenously alter the cellular genetic makeup is gene therapy. Some challenges have existed in gene therapy. Primary concerns are the stability of molecules, the amount of intracellular uptake, their susceptibility to enzyme degradation, and the high impermeability of cell membranes to foreign substances. To overcome this problem, the CNTs are used as vector able to associate with DNA, RNA, or another type of nucleic acid by self-assembly and assist its intracellular translocation. These systems offer several advantages, including easy upscaling, flexibility in terms of the size of nucleic acid to be delivered, and reduced immunogenicity compared with viruses.

The Kostas group reported CNT-mediated gene delivery and expression leading to the production of marker proteins encoded in double-stranded pDNA(Y. Liu, Wu, Zhang, Jiang, He, Chung, et al., 2005; Pantarotto, et al., 2004). The delivery of pDNA and expression of β-galactosidase (marker gene) in Chinese hamster ovary (CHO) cells is five to ten times higher than naked pDNA alone.

The concept of gene delivery systems based on CNTs has also been reported by Liu group(Y. Liu, et al., 2005). They report a noncovalent association of pDNA with PEI–CNTs by electrostatic interaction. They have tested CNT–PEI:pDNA complexes at different charge ratios in different cell lines. The levels of expression of luciferase (marker gene) are much higher for the complexes incorporating CNTs than pDNA alone and about three times higher than PEI alone.

4.3 CNTs for chemical delivery

Recently, Dai group reported that using supramolecular π–π stacking to load a cancer chemotherapy agent doxorubicin (DOX) onto branched polyethylene glycol (PEG) functionalized SWNTs for in vivo drug delivery applications(Z. Liu, Fan, Rakhra, Sherlock, Goodwin, Chen, et al., 2009). It has been found that the surface of PEGylated SWNTs could be efficiently loaded with DOX by supramolecularπ–π stacking. These methods offer several advantages for cancer therapy, including enhanced therapeutic efficacy and a marked reduction in toxicity compared with free DOX.

4.4 CNTs for cancer therapy

More interestingly, CNTs can be used as platforms for multiple derivatization by loading their surface with therapeutic agents (treatment), fluorescent, magnetic, or radionuclide probes (tracking), and active recognition moieties (targeting).

We present a strategy for using SWNTs as intracellular vectors for delivery of ASODNs modified with gold nanoparticles (figure 3). This strategy allows intracellular delivery and localization to enhance the therapeutic efficiency of the ASODNs by the conjugations of SWNTs and GNPs compared with the naked ASODNs in this experiment.

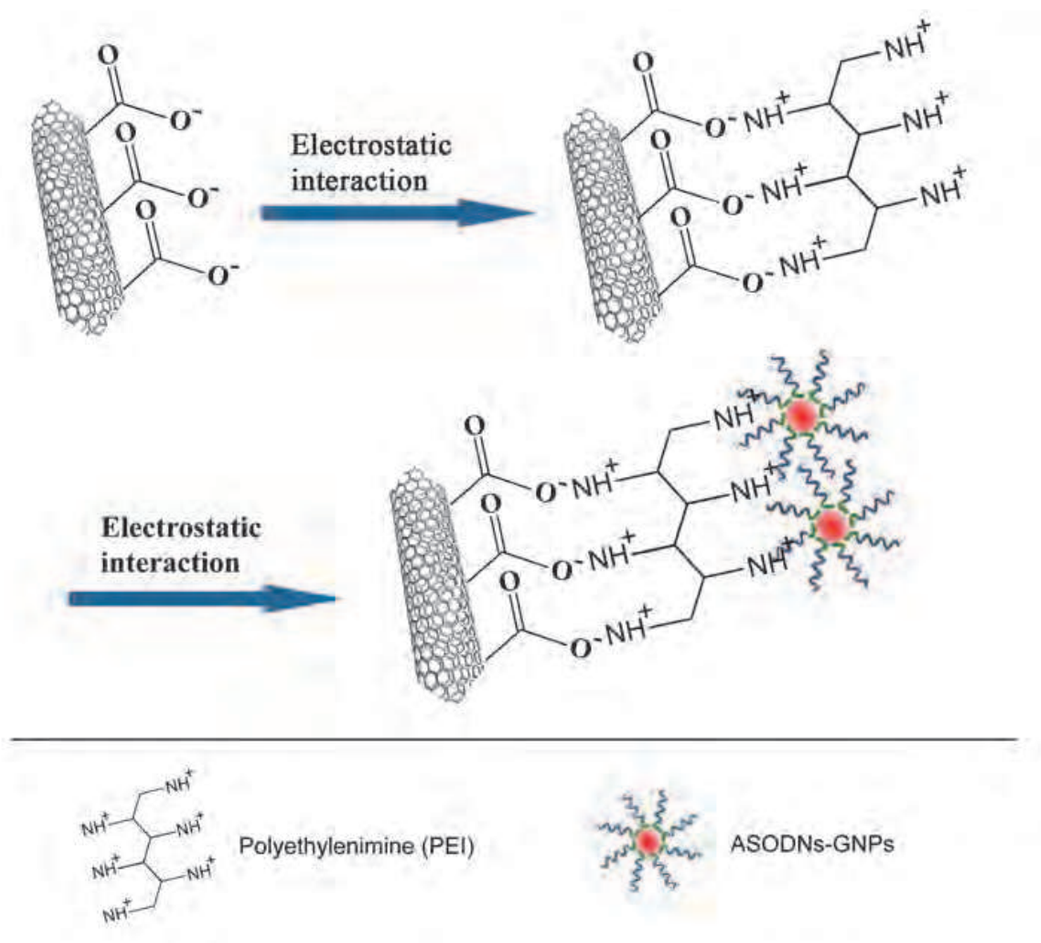


Fig. 3. Preparation and schematic structures of f-SWNTs-PEI-ASODNs-GNPs.

Recently, Jia et al, have explored a novel double functionalization of a carbon nanotube delivery system containing antisense oligodeoxynucleotides (ASODNs) as a therapeutic gene and CdTe quantum dots as fluorescent labeling probes via electrostatically layer-by-layer assembling(N. Jia, Lian, Shen, Wang, Li, & Yang, 2007). With this novel functionalization, it has demonstrated efficient intracellular transporting, strong cell nucleus localization and high delivery efficiency of ASODNs by the PEI –MWNTs carriers. Furthermore, the ASODNs bound to PEI-MWNTs show their effective anticancer activity.
Another strategy to achieve this is used CNTs covalently bound to Pt (IV) to deliver a lethal dose of an anticancer drug and to a noncovalently bound (via a lipid coating of the CNTs) fluorescein to track the system(figure 4)(Feazell, Nakayama-Ratchford, Dai, & Lippard, 2007). Here the toxic effect of the anticancer drug is dependent upon its release and reduction inside the cell, only possible at lower pH environments such as endocytic vesicles, which was exemplified using a testicular carcinoma cell line NTera-2.

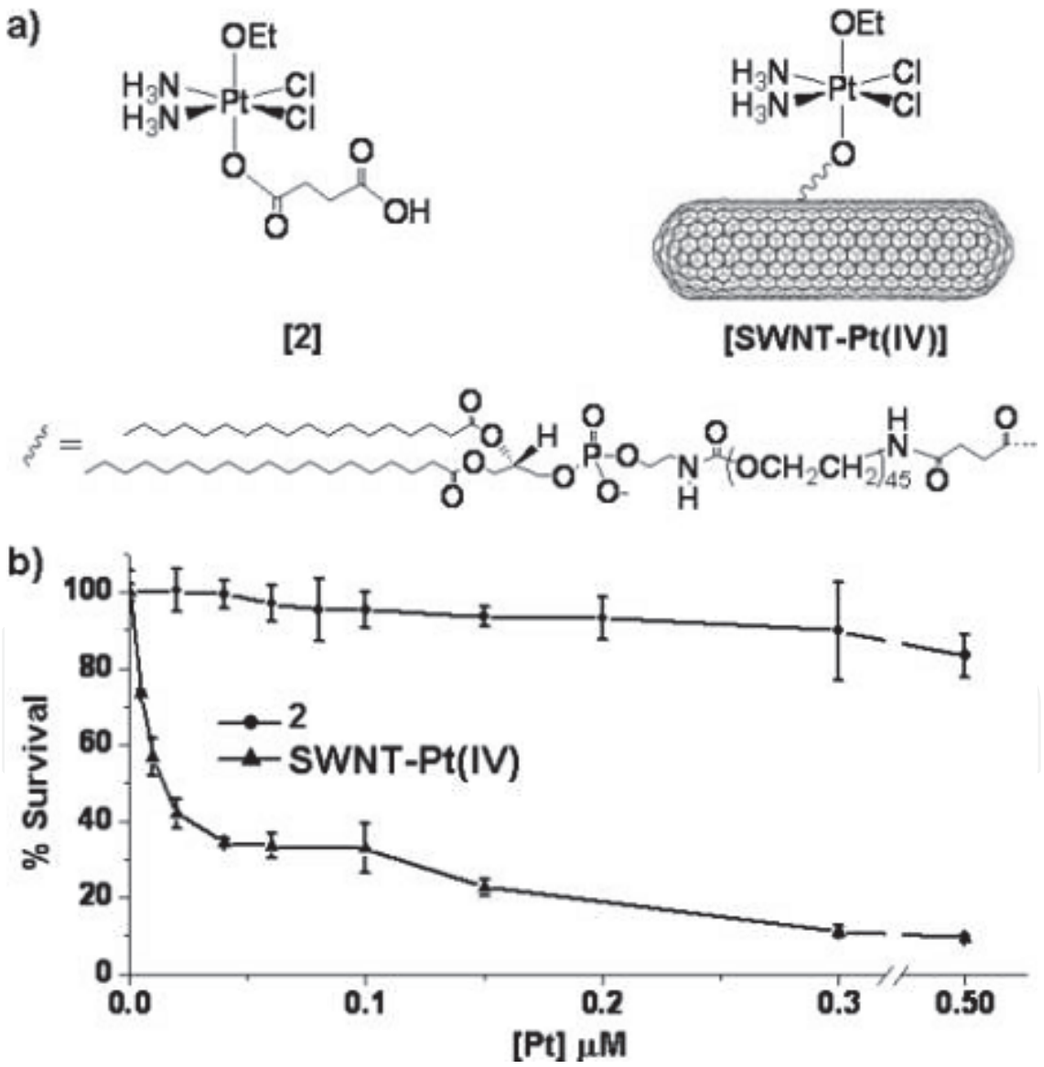


Fig. 4. (a) Preparation and schematic structures of Longboat Delivery Systems. (b) Cytotoxicity of free Pt(IV) and SWNT-tethered Pt(IV) inNTera-2cells.

4.5 CNTs for HIV/AIDS therapy

Recently, Liu et al.(Z. Liu, Winters, Holodniy, & Dai, 2007) have shown the delivery of siRNA molecules conjugated to CNT to human T cells and primary cells. The results Show that nanotubes are capable of siRNA delivery to afford efficient RNAi of CXCR4 and CD4 receptors on human T cells and peripheral blood mononuclear cells (PBMCs).The siRNA sequences used in these studies are able to silence the expression of the cell-surface receptors CD4 and coreceptors CXCR4 necessary for HIV entry and infection of T cells. This work demonstrates that siRNA linked through cleavable disulfide bonds to lipid molecules coating CNTs can be efficiently delivered, leading to knockdown (about 60%) of the CD4 and CXCR4 expression. Furthermore, the siRNA–S–S–lipid coated CNT conjugates greatly improve the silencing in T cells compared with Lipofectamine 2000 and other liposome-based transfection agents. Even though preliminary at this stage, these results indicate the potential use of CNTs for the treatment of HIV.

Part B. Nanotubes in biosensing

Carbon nanotubes (CNTs) have recently emerged as novel electronic and optical biosensing materials for the detection of biomolecules such as DNA, antigen–antibody, cells, and other biomolecules.(W. Cheng, L. Ding, S. J. Ding, Y. B. Yin, & H. X. Ju, 2009; Drouvalakis, Bangsaruntip, Hueber, Kozar, Utz, & Dai, 2008; Hu, Huang, Li, Ling, Liu, Fei, et al., 2008) Among widespread nanoscale building blocks, such as organic or inorganic nanowires and nanodots, CNTs are considered as one of the most versatile because of their superior mechanical and electrical properties and geometrical perfection. DNA analysis plays an ever-increasing role in a number of areas related to human health including diagnosis of infectious diseases, genetic mutations, drug discovery, food security, and warning against biowarfare agents. etc. And thus make electrical DNA hybridization biosensors has attracted considerable research efforts due to their high sensitivity, inherent simplicity and miniaturization, and low cost and power requirements.

1. Optical DNA sensors

Alternatively, an effective sensing platform has been presented via the noncovalent assembly of SWCNTs and dye-labeled ssDNA.(Yang, Tang, Yan, Kang, Kim, Zhu, et al., 2008) Figure 1(a) shows the signaling scheme. When the SWCNTs are added to the dye-labeled ssDNA solution, the ssDNA/SWCNT hybrid structure can be formed, in which the dye molecule is in close proximity to the nanotube, thus quenching the fluorescence of dye molecule.(Nakayama-Ratchford, Bangsaruntip, Sun, Welsher, & Dai, 2007)

The dye-labeled ssDNA can restore the fluorescence signal to an initial state in the presence of the target. Figure 1(b) illustrates no significant variation in the fluorescence intensity of fluorescein derivative (FAM)-labeled oligonucleotides (P1) in the absence of CNTs. In the presence of SWCNT, a dramatic increase of the fluorescence intensity at 528 nm can be observed in the DNA concentration range of 5.0–600 nM, suggesting that the SWCNT/DNA assembly approach is effective in biosensing target DNA (Yang, et al., 2008)Yang, et al., 2008).

Furthermore, a visual sensor has been designed to detect DNA hybridization by measuring the light scattering signal with DNA modified MWCNT as recognition element as shown in figure 2. (Hu, et al., 2008) This sensor can be reused for at least 17 times and is stable for more than 6 months.

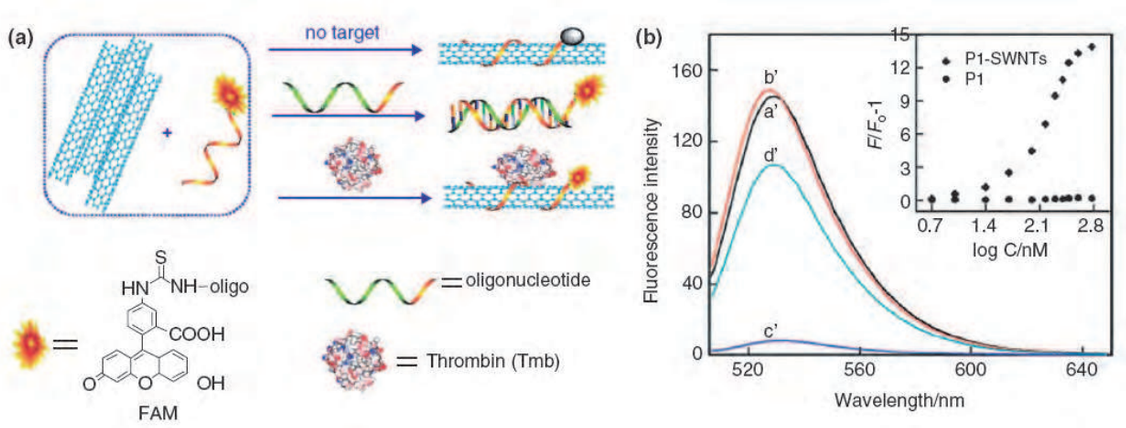


Fig. 1. (a) Scheme for signaling biomolecular interaction by the assembly of single-walled carbon nanotubes (SWCNT) and dye-labeled single strand DNA. (b) Fluorescence emission spectra of 50 nM FAM-labeled oligonucleotides (P1) in (a) phosphate buffer (PBS), (b)300 nM perfect cDNA (T1), (c) SWCNT, and (d_) SWCNT + 300 nM T1. Inset: fluorescence intensity ratio of P1 and P1–SWCNT with *F/F*0 plotted against the logarithm of the concentration of T1. Excitation was at 480 nm, and emission was monitored at 528 nm. (Yang, et al., 2008).

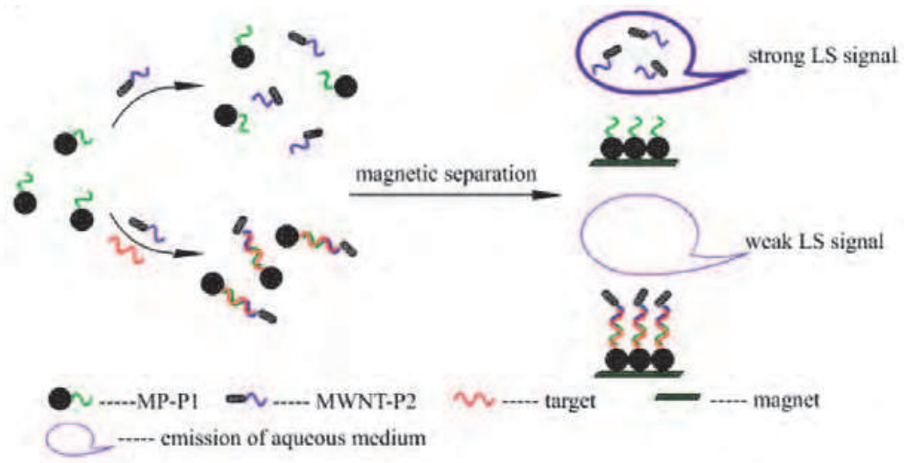


Fig. 2. The DNA Detection with Magnetic Particles-Carbon Nanotubes Coupled Sandwich Probe.

2. Antigen–antibody immunoreactions

There are two different types of detection patterns for CNT-based immunosensors: label-free immunosensors and immunosensors that employ labels and mediators. The label-free immunosensor shows a convenient fabricating and detection procedure. Several label-free peptide-coated CNTs based immunosensors has been proposed for the direct assay of human serum sample using square wave stripping voltammetry (Ly & Cho, 2009), quartz crystal microbalance measurements, and differential pulse voltammetry (DPV) (Okuno, Maehashi, Kerman, Takamura, Matsumoto, & Tamiya, 2007). Based on CNT-FET, a label-

free protein biosensor was also prepared for monitoring of a prostate cancer marker (Kim, Lee, Lee, Hong, & Sim, 2009). As one of the most popular tracer labels, enzymes, including ALP (Aziz, Park, Jon, & Yang, 2007), HRP (Wang, Liu, & Jan, 2004), and GOD(Lai, Yan, & Ju, 2009) have been immobilized on CNTs for enhancing the enzymatic signal. Typically, a novel immunosensor array was constructed by coating layer-by-layer colloidal Prussian blue (PB), gold nanoparticles (AuNPs), and capturing antibodies on screen-printed carbon electrodes (Figure 3) and coupling with a new tracer nanoparticle probe labeled antibody (Ab2) that was prepared by one-pot assembly of GOD and the antibodies on AuNPs attached CNTs (Lai, Yan, & Ju, 2009).

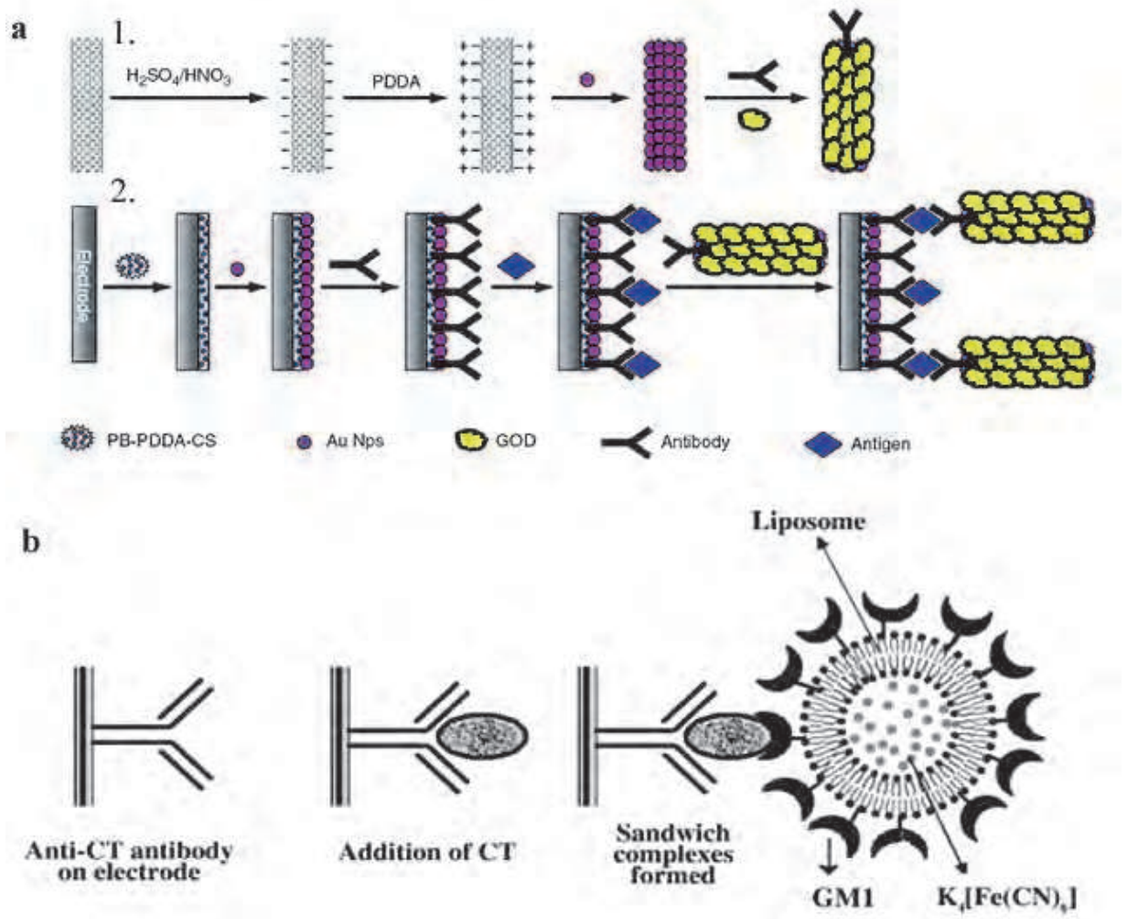


Fig. 3. Schematic representation of (a1) preparation procedure of glucose oxidase (GOD)–Au Nps/carbon nanotubes (CNTs)-Ab2 tracer and (a2) preparation of immunosensors and sandwich-type electrochemical immunoassay (Lai, Yan, & Ju, 2009). c. Schematic outlines of Immunosensor for Cholera Toxin.

The immobilized PB could not only eliminate the electrochemical cross talk but also avoid the interference of dissolved oxygen. Using carcinoembryonic antigen and α-fetoprotein as model analytes, the simultaneous multiplexed immunoassay method showed the linear ranges of three orders of magnitude with the detection limits down to 1.4 and 2.2 pg mL−1, respectively (Lai, Yan, & Ju, 2009). This assay approach showed a great potential in clinical

applications and detection of low-abundant proteins. In addition, a sensitive method for the detection of cholera toxin (CT) using an electrochemical immunosensor with liposomic magnification has been proposed as shown in Figure 3c (Viswanathan, Wu, Huang, & Ho, 2006). The sensing interface consists of monoclonal antibody against the B subunit of CT that is linked to poly (3, 4-ethylenedioxythiophene) coated on Nafion-supported MWCNT caste film on a glassy carbon electrode. The sandwich assay provides the amplification route for the detection of CT ranging from 10^{-14} to 10^{-7} g mL^{-1} with a detection limit of 10^{-15} g mL^{-1}. In the same group, a disposable electrochemical immunosensor for carcinoembryonic antigen using ferrocene liposome and MWCNT modified screen-printed carbon electrode was also developed (Viswanathan, Rani, Vijay Anand, & Ho, 2009).

3. Sensing of cells

To achieve biocompatible interactions between CNTs and living cells, a strategy to functionalize CNTs with biomolecules such as peptide as shown in Figure 4 (W. Cheng, L. Ding, S. Ding, Y. Yin, & H. Ju, 2009) (Cheng, Ding, Lei, Ding, & Ju, 2008)and monosaccharides was presented (Sudibya, Ma, Dong, Ng, Li, Liu, et al., 2009). A novel

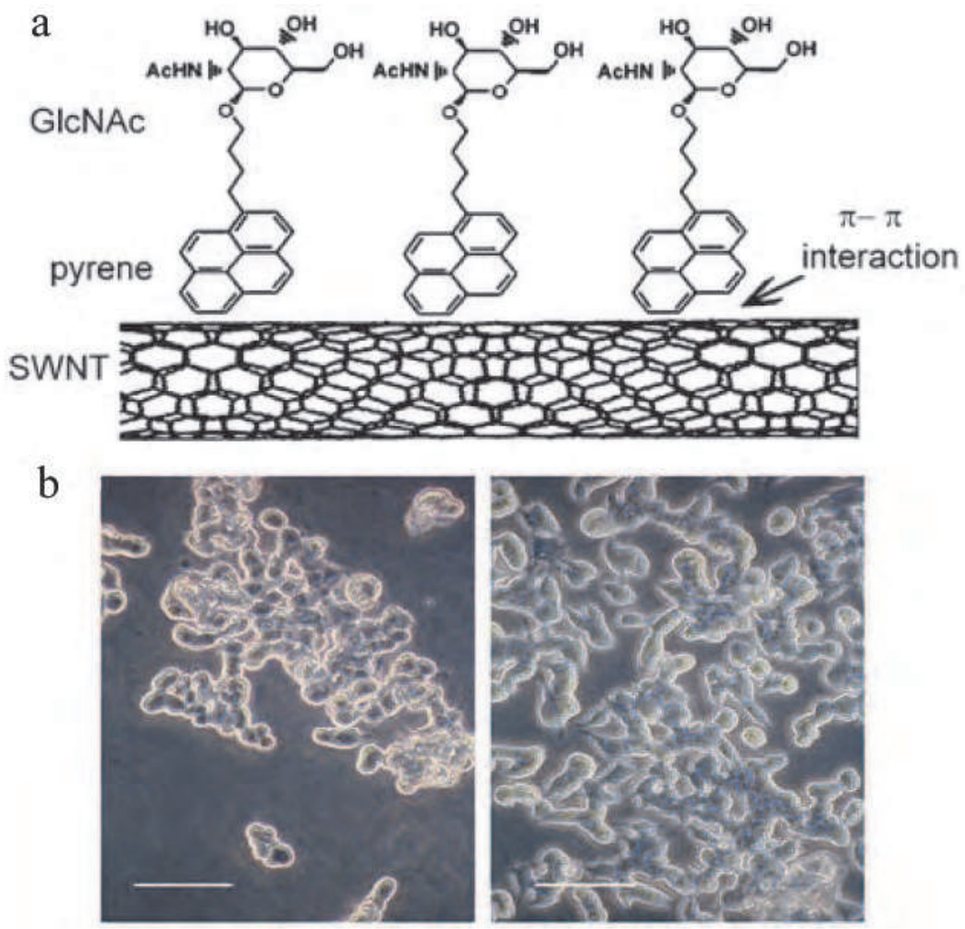


Fig. 4. a. Illustration of GlcNAc–pyrene functionalization of SWNTs. b. Phase-contrast images of PC12 cells cultured on a bare SWNT-net (left) and GlcNAc–SWNTs (right).

electrochemical cytosensing strategy was designed based on the specific recognition of integrin receptors on cell surface to arginine–glycine–aspartic acid–serine (RGDS)-functionalized SWCNT (Figure 4) (Cheng, Ding, Lei, Ding, & Ju, 2008). The conjugated RGDS showed a predominant ability to capture cells on the electrode surface by the specific combination of RGD domains with integrin receptors. On the basis of the dual signal amplification of SWCNT and enzymatic catalysis, the cytosensor could respond down to 620 cells mL^{-1} of BGC cells with a linear calibration range from 1.0×10^3 to 1.0×10^7 cells mL^{-1}. Furthermore, the mannosyl group on a single living intact BGC cell was evaluated to be 5.3×10^7 molecules of mannose. The same group further prepared a cytosensor array for multiplex evaluation of both the glycan expression profile on an intact cell surface and the dynamic changes in the glycome during drug treatment.(W. Cheng, L. Ding, S. Ding, Y. Yin, & H. Ju, 2009) The further functionalization of the metal-cluster-decoration CNTs with Tween 20 could suppress non-specific binding and enabled label-free and selective detection of *A. anophagefferens (Ishikawa, Stauffer, Caron, & Zhou, 2009)*.

4. Detection of other biomolecules

4.1 NADH

The electrochemical oxidation of NADH at the electrode surface has received considerable interest due to the need to develop amperometric biosensors for substrates of NAD^+ dependent dehydrogenases. Dihydronicotinamide adenine dinucleotide (NADH) and its oxidized form, nicotinamide adenine dinucleotide (NAD^+), are the key central charge carriers in living cells. However, the oxidation of NADH at a conventional solid electrode surface is highly irreversible with considerable overpotentials, which limits the selectivity of the determination in a real sample. CNTs have been devoted to decreasing the high overpotential for NADH oxidation on carbon paste electrodes (Blackburn,R.S. & Burkinshaw,S.M 2007) and microelectrodes (Wang, Deo, Poulin, & Mangey, 2003). By integrating the hydrophilic ion-conducting matrix of CHITn with electron mediator toluidine blue O and CNTs, the produced NADH sensor shows very low oxidation overpotential and good analytical performance (Zhang & Gorski, 2005).

4.2 Glucose

The detection of glucose in blood is one of the most frequent performances for human healthy, since some diseases are related to the blood glucose concentration. However, the direct electron transfer for oxidation of $FADH_2$ or reduction of FAD(Shan, Yang, Song, Han, Ivaska, & Niu, 2009) is hard to realize at conventional electrodes, because the FAD is deeply seated in a cavity and not easily accessible for conduction of electrons from the electrode surface. Thus, many CNTsbased nanohybrids, such as MWCNT/AuNPs/ionic liquid (F. Jia, Shan, Li, & Niu, 2008), SWCNT/GOD/Nafion (Lyons & Keeley, 2008), polyaniline (PANI)-coated Fe_3O_4 nanoparticle/MWCNT (Zhun Liu, Wang, Xie, & Chen, 2008), and palladium/SWCNT (Meng, Jin, Yang, Lu, Zhang, & Cai, 2009), have been explored to immobilize GOD for glucose biosensing. More interestingly, Willner's group demonstrated that aligned reconstituted GOD on the edge of SWCNT as conductive nanoneedles can be linked to an electrode surface for fast glucose response (G. Liu & Lin, 2006).

4.3 Organophosphate pesticides

The rapid detection of these toxic agents in the environment and public places has become increasingly important for homeland security and health protection. The flow injection

amperometric biosensor for OPs has been developed by assembling AChE on CNTs-modified GCE. Under optimal conditions, the biosensor has been used to measure paraoxon as low as 0.4 pM with a 6-min inhibition time (G. Liu & Lin, 2006).

4.4 H_2O_2

H_2O_2 is a product of the enzymatic reactions between most oxidases and their substrates. This detection is very interesting for the development of biosensors for oxidase substrates. The earlier work on the electrocatalytic action of CNTs toward H_2O_2 was reported at an apparently decreased overvoltage using the CNTs/Nafion-coated electrode. With the introduction of MWCNT, the polyaniline-PB/MWCNT hybrid system showed the synergy between the PANI-PB and MWCNT, which amplified the sensitivity greatly (Zou, Sun, & Xu, 2007).

5. Near-IR fluorescent based CNTs biosensor

Generally, the change modes of SWCNT NIR can be modulated to uniquely fingerprint agents by either the emission band intensity or wavelength. CNTs have been found to be useful optical materials with high photostability and efficiency for sensing applications because of their NIR fluorescence properties from 900 to 1600 nm. Other than optical detection, SWCNTs as sensing elements have a particular advantage due to the fact that all atoms are surface atoms causing the nanotube to be especially sensitive to surface adsorption events (Strano & Jin, 2008) (Barone, Parker, & Strano, 2005).

5.1 Sensing with change of emission intensity

Quenching of SWCNT fluorescence by means of oxidative charge transfer reactions with small redox-active organic dye molecules has been demonstrated by suspending in SDS solution and biotin–avidin test system. The NIR optical properties of SWCNT have attracted particular attention for nanobiosensors based on the redox chemistry. At the most sensitive band of 1270 nm, the detection limit for H_2O_2 is found to be 8.8, 0.86, and 0.28 ppm by three different methods based on the concentrationdependent rate constant, pectral intensity change, and signal-to-noise ratio (Tu, Pehrsson, & Zhao, 2007). Another NIR optical protein assay based on aptamers wrapped on the sidewall of SWCNT was designed. After the target protein (thrombin) was added into the SWCNT–aptamer solution, the NIR absorption at 1142 nm decreased linearly upon the increasing concentration from 0.2 to 6.3 nM. This signal provides a label-free and separation-free optical method for aptamer-based protein assays (H. Chen, Yu, Jiang, Zhang, Liu, & Kong, 2009).

5.2 Sensing with shift of emission wavelength

The shift of emission wavelength has also been a usefulway to make sensing in addition to emission intensity-based sensing, When cations adsorb onto the negatively charged backbone of DNA, DNA oligonucleotides transform from the native, righthanded B form to the left-handed Z form, which modulates the dielectric environment of SWCNT and decreases their NIR emission energy up to 15 meV. The change of the emission wavelength results in an effective ion sensor, especially formercuric ions. These NIR ion sensors can operate in strongly scattering or absorbing mediator to detect mercuric ions in whole blood, black ink, and living mammalian cells and tissues (D. A. Heller, Jeng, Yeung, Martinez, Moll, Gastala, et al., 2006).

5.3 Single-molecule detection

Nanoscale sensing elements offer promise for single molecule detection through NIR fluorescence in physically or biologically constrained environments. A single-molecule detection of H_2O_2 has been demonstrated by stepwise NIR photoluminescence quenching of surface-tethered DNA–SWCNT complexes (Figure 5(a)).

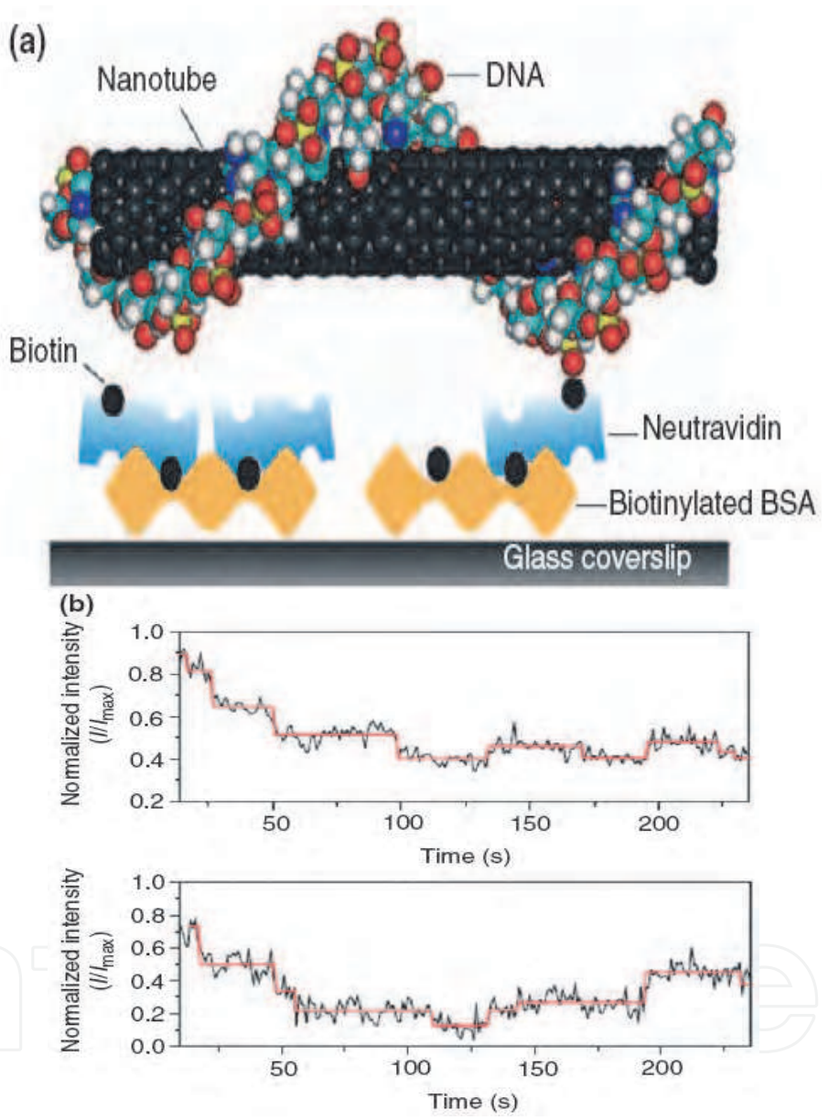


Fig. 5. Single-molecule H_2O_2 detection: (a) Schematic of biotinylated DNA–single-walled carbon nanotubes (SWCNT) binding to a glass surface with bovine serum albumin–biotin and Neutravidin. (b) Fitted traces from a movie showing single-step SWCNT emission quenching upon perfusion of H_2O_2. ((D. A. Heller, Jin, Martinez, Patel, Miller, Yeung, et al., 2009)).

The time trace of SWCNT quenching was obtained by measuring the intensity of four-pixel spots in movies recorded at one frame per second (Figure 5(b)), resulting in multiple traces that exhibited single-step attenuation upon perfusion of H_2O_2. These measurements

demonstrated singlemolecule detection of H_2O_2 and provided promise for new classes of biosensors with the single-molecular level of sensitivity.

6. SWCNT-based field-effect biosensor

Currently, four possible mechanisms have been proposed to account for the observed changes in the SWCNT conductance: electrostatic gating (I. Heller, Männi k, Lemay, & Dekker, 2008) (Gui, Li, Zhang, Xu, Dong, Ho, et al., 2007), Schottky barrier effect (R. J. Chen, Choi, Bangsaruntip, Yenilmez, Tang, Wang, et al., 2004), change in gate coupling (Besteman, Lee, Wiertz, Heering, & Dekker, 2003), and carrier mobility change (Hecht, Ramirez, Briman, Artukovic, Chichak, Stoddart, et al., 2006), among which the electrostatic gating and Schottky barrier effect are dominant in the SWCNTbased FET biosensing device (I. Heller, Janssens, Mannik, Minot, Lemay, & Dekker, 2007). The label-free CNTs-based filed-effect sensor offers a new approach for a new generation of DNA biosensing. For example, a synthetic polymer is well adsorbed to the walls of CNTs and carries activated succinimidyl ester groups to fix the NH_2-ssDNA probes for constructing a large array of CNTs-FETs. Furthermore, a simple and generic protocol for label-free detection of DNA hybridization is demonstrated by random sequencing of 15 and 30 mer oligonucleotides. DNA hybridization on gold lectrodes, instead of on SWCNT sidewalls, is mainly responsible for the acute electrical conductance change due to the modulation of energy level alignment between SWCNT and gold contact. Aptamer is artificial oligonucleotides (DNA or RNA) that can bind to a wide variety of entities with high selectivity, specificity, and affinity, equal to or often superior to those of antibodies. The first SWCNT-FET-based biosensor comprising aptamer was proposed by Lee's group (So, Won, Kim, Kim, Ryu, Na, et al., 2005). Briefly, aptamer immobilization was performed by modifying the side wall of the CNTs with carbodiimidazole-activated Tween 20 through hydrophobic interaction, and covalently attaching the 3-amine group of the thrombin aptamer (Figure 6(a)). The conductance dropped sharply upon addition of 1.5 μmol thrombin. The sensitivity became saturated around protein concentration of 300 nM, where the linear response regime of the sensor was expected to occur within the 0–100 nM range (Figure 6(b)). The addition of elastase did not affect the conductance of the thrombin aptamer functionalized SWCNT-FET. Again, adding thrombin to the thrombin aptamer functionalized SWCNT-FET surface caused a sharp decrease in conductance (Figure 6(c)), thereby demonstrating the selectivity of the immobilized thrombin aptamers.

The aptamer modified SWCNT-FETs are another promising sensor for the development of label-free protein detection. SWCNT-FET are also promising devices for the specific recognition of proteins. The first biosensor based on an individual SWCNT was reported by Dekker's group (Besteman, Lee, Wiertz, Heering, & Dekker, 2003). GOD was attached to the sidewalls of a semiconductive CNT by a bifunctional reagent with a pyrene group. GOD-coated semiconducting SWCNTs acted as sensitive pH sensors due to the strong pH-dependent conductance of GOD immobilized SWCNT. Moreover, change of conductance of GOD coated semiconducting SWCNT upon addition of glucose indicated that an enzyme-activity sensor could be constructed at the single-molecule level of an individual SWCNT. In the presence of redox mediators such as K3Fe(CN)6/K4Fe(CN)6 and K2IrCl6/K3IrCl6, the SWCNT-FETs were shown to linearly detect the enzyme activity of the blue copper oxidase, laccase, varied over two orders of magnitude of enzyme concentration in the picomolar range (Boussaad, Diner, & Fan, 2008).

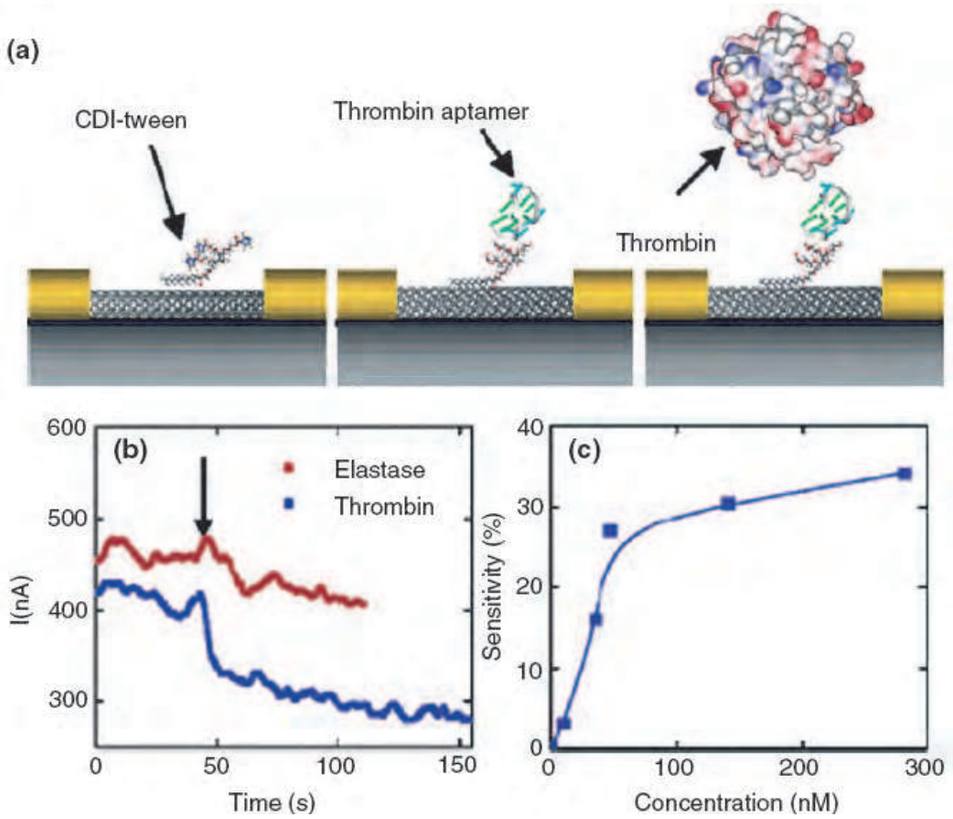


Fig. 6. (a) Binding of thrombin on an single-walled carbon nanotubes-field-effect transistor (SWCNT-FET)-based aptamer sensor. (b) The sensitivity of SWCNT-FET aptamer sensor as a function of thrombin concentration. (c) The sensitivity of SWCNT-FET aptamer sensor as a function of thrombin concentration (So, et al., 2005).

7. Electrochemical sensors

Electrochemical DNA sensors can convert the hybridization event into an electrochemical signal. DNA sensing approaches include the intrinsic electroactivity of DNA, electrochemistry of DNA-specific redox indicators, electrochemistry of enzymes, and conducting polymers. The direct electrochemical oxidation of guanine or adenine residues of ssDNA leads to an indicator-free DNA biosensor. For example, Wang's group used CNTs for dramatically amplifying alkaline phosphatase (ALP) enzyme-based bioaffinity electrical sensing of DNA with a remarkably low detection limit of around 1 fg mL^{-1} (54 aM).(Wang, Liu, & Jan, 2004)
Professor Kotov and collaborator (Professor Xu) had demonstrated that CNT/cotton threads can be used to detect albumin, the key protein of blood, with high sensitivity and selectivity(Shim, Chen, Doty, Xu, & Kotov, 2008). In this method, cotton yarn has been coated with CNTs and polyelectrolytes. This method provides a fast, simple, robust, low-cost, and readily scalable process for making e-textiles, reminiscent of layer-by-layer assembly processes used before. The resulting CNT/cotton yarns showed high electrical conductivities as well as some functionality due to biological modification of inter-nanotube tunneling junctions. When the CNT/cotton yarn incorporated anti-albumin, it became an e-textile biosensor that quantitatively and selectively detected albumin, the essential protein in blood. The same sensing approach can easily be extended to many other proteins and

biomolecules. Single-walled and multi-walled carbon nanotubes (SWNTs, MWNTs) were dispersed in dilute Nafion™-ethanol or poly(sodium 4-styrene sulfonate) (PSS)-water solutions. A general commodity cotton thread (1.5 mm in diameter) was dipped in the prepared CNT dispersions and dried (Figure 7ab). After several repetitive dips, reminiscent of the layer-by-layer assembly process, the cotton thread became conductive with a resistivity as low as 20 Ω/cm. As a demonstration of the conductivity, we easily powered an LED device connected to a battery by the prepared threads (Figure 7c).

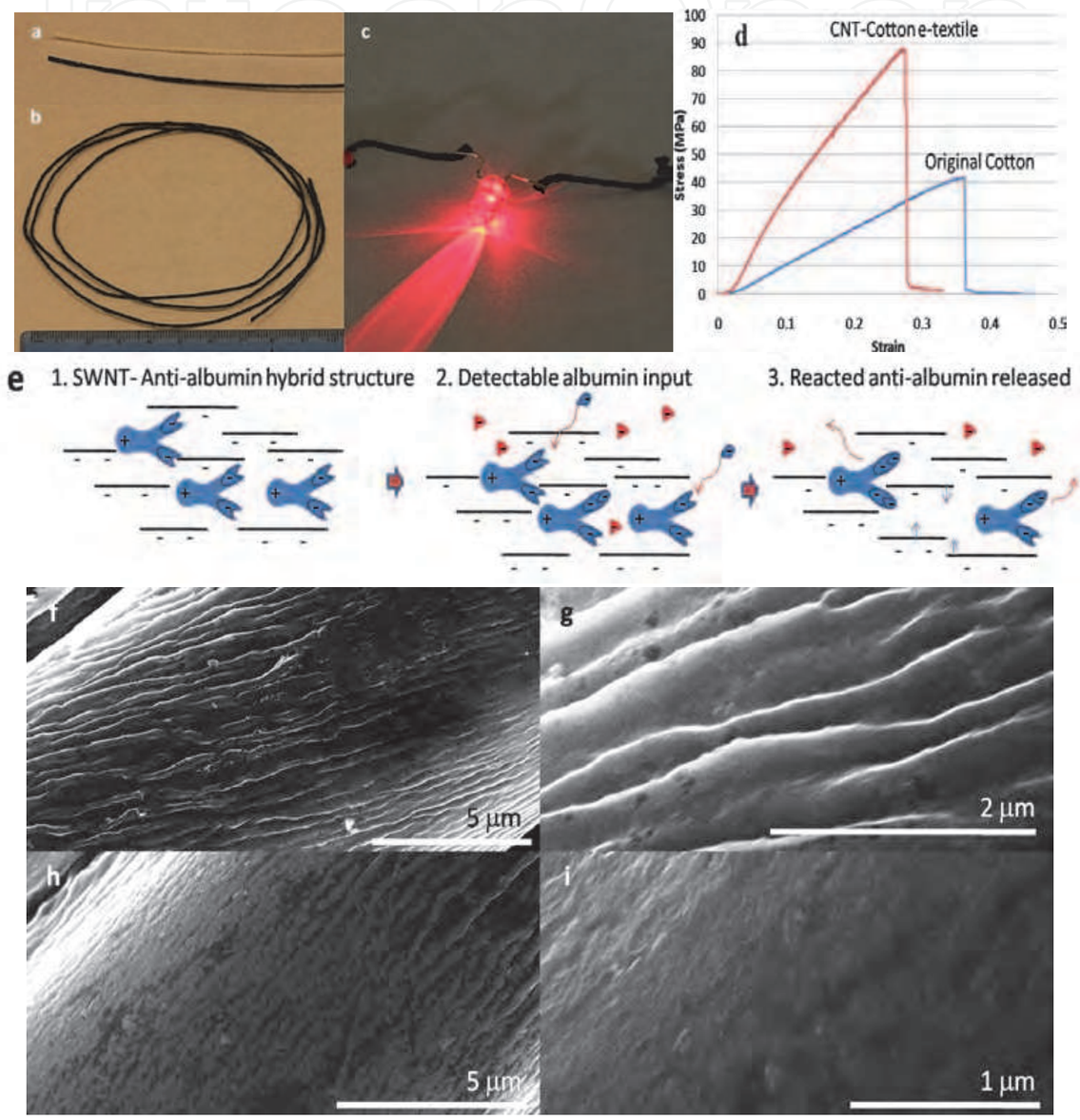


Fig. 7. Photographs of SWNT-cotton yarn. (a) Comparison of the original and surface modified yarn. (b) 1 μ m-long piece as made. (c) Demonstration of LED emission with the current passing through the yarn. (d) Stress-strain curves for the CNT-cotton yarn and the original cotton thread. (e) Suggested detection mechanism of antibody-antigen reaction. SEM images before (f,g) and after (h,i) the antibody/antigen reaction.

The incorporation of CNTs into the cotton yarn was much more efficient than their adsorption into carbon fibers, which was tried elsewhere. This could be a result of the efficient interaction of polyelectrolytes with cotton and other natural polysaccharide- and cellulose-based materials, such as paper, which is well known in industry. Additionally, the flexibility of the CNTs allowed them to conform to the surface of the cotton fibers. Both SWNTs and MWNTs stabilized in Nafion™ seamlessly cover. In comparison with other electronic textiles, fabrics, and threads, the resistivity of the yarn in Figure 7ab is two orders of magnitude lower than the resistivity of comparable CNT-dyed textiles (7.8 kΩ/cm). Furthermore, the reported resistivity of 1 cm-long yarn drawn from CNT forests is at best, if converted to the scale used here, in the range of a few kΩ/cm.

The strength of the CNT/cotton yarn is more than 2 times higher than that of the original cotton thread due to a reduction of the overall diameter, densification and stronger adhesion of the fibers to each other by the polymer material. Even though the cotton yarn became slightly harder after being coated with SWNTs, it is still very flexible and soft, both of which are important for the wearability of electronic fabric. Single exposure of the produced yarn to different solvents imitating washing did not appreciably affect the electrical properties.

The low electrical resistance of CNT/cotton yarn allows for convenient sensing applications which may not require any additional electronics or converters. It also reduces the power necessary for sensing. PSS is more hydrophilic than Nafion™, and, thus, CNT-Nafion™ is more advantageous for dry-state sensing while CNT-PSS will be more advantageous in humid conditions. For intelligent fabric demonstrations, the CNT-Nafion™ yarn was tested as a humidity sensor in a dry state while CNT-PSS yarn served as a wet-state bio-sensor platform. As the humidity was raised, the resistance increased. This is most likely a result of reversible hygroscopic swelling of both Nafion™ and cotton, which readily disrupts the electron transport between CNTs. The change in the resistance was almost instantaneous, and the signal was strong even in the very dry conditions of 20% humidity. Sensitivity to humidity changes also gives a good indication of the so-called “breathability” of the material, which is also an important parameter for smart fabrics.

Another example of an integrated, functional biosensor was demonstrated using SWNT-PSS yarn. the choose of the antigen/antibody reaction between human serum albumin (HSA) and its respective immunoglobulin (IgG) anti-HSA for the model system that can be generalized to many other relevant antigen/antibody systems of interest. PSS is known as an excellent stabilizer of proteins and can be used to form a layer-by-layer film with IgG antibodies. After the adding of anti-HSA directly to the SWNT-PSS solution and coated the cotton yarn as before, the CNT-IgG/cotton yarn was frozen and then dried under vacuum in order to minimize antibody denaturation. This cycle was repeated three times before use. For sensing experiments, two different albumin proteins were used; human serum albumin (HSA, 67 kDa) and bovine serum albumin (BSA, 66 kDa). Each experiment involved the measurements of conductivity of yarns being in contact with a 500 μl aqueous volume of water. 50 μl aliquots of bovine and human albumins at different concentration were added to this starting volume. Detection of the antigen with CNT-IgG/cotton yarn was very sensitive and selective. The presence of analyte around the CNT-IgG/cotton yarn was indicated by an increase in conductivity (Figure 8ab). The detectable concentration of HSA was as low as 119 nM (Figure 8a, ×100), producing a signal drop of 2980 Ω, which is a 2.5% change from the baseline. As a reference, the HSA concentration in our blood ranges from 446 μM to 746 μM. The presence of analyte around the CNT-IgG/cotton yarn was indicated

by an increase in conductivity. The detectable concentration of HSA was as low as 119 nM. As a reference, the HSA concentration in our blood ranges from 446 nM to 746nM. The high sensitivity obtained in these experiments is comparable or exceeds that of sensing devices based on surface modified cantilevers similar to those used in AFM. At the same time, the selectivity of the SWNT-cotton yarn sensor was also high.

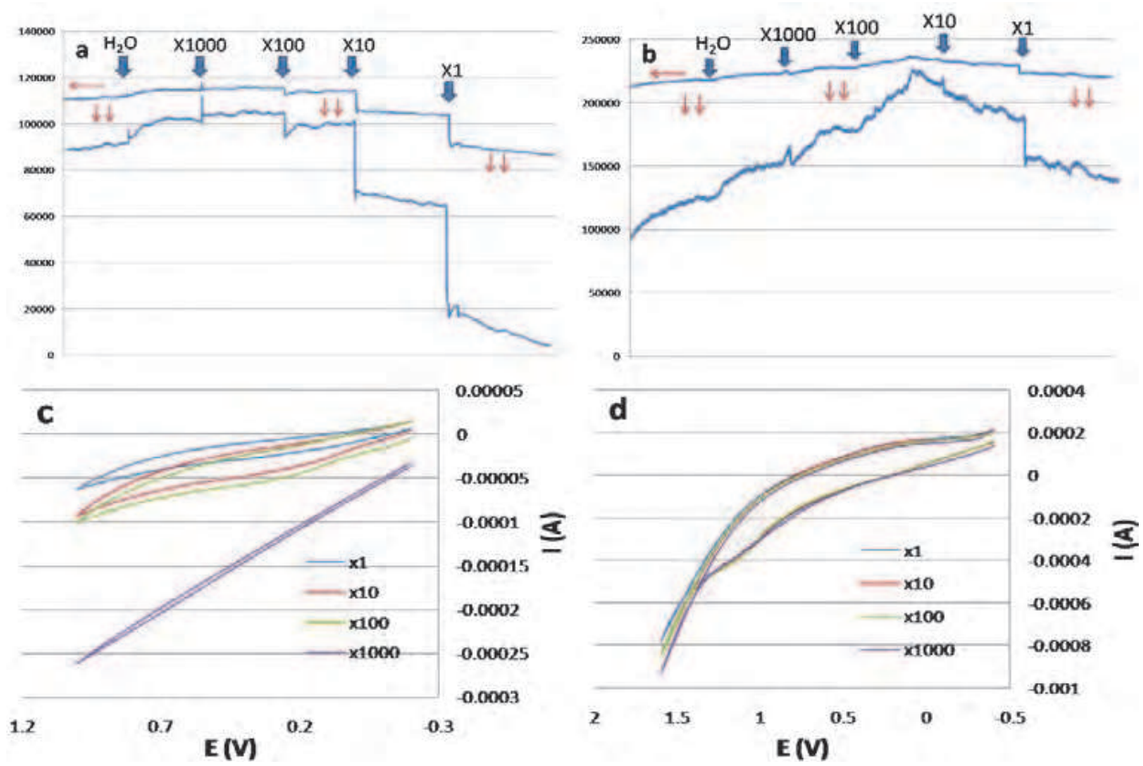


Fig. 8. Demonstration of the biosensing functionality of SWNT-modified yarn using a generic antibody-antigen reaction. (a) Effect of the concentration of HSA (11.9μM at ×1 dilution) and (b) BSA (30 μM at ×1 dilution) on conductivity of a CNT-PSS-anti-HSA coated yarn. (c, d) Cyclic voltammetry measurements of HSA (11.9 μM at ×1 dilution) on (c) a CNT-PSS-anti-HSA coated yarn and (d) a CNT-PSS yarn.

The signal transduction mechanism is believed to involve the release or significant rearrangement of IgGs from the CNT/cotton yarn. Negatively-charged HSA reacts with anti-albumin, which is followed by the process of expulsion from the SWNT-cotton matrix by the negatively charged polyelectrolyte, such as PSS. As a result, more extensive SWNT contacts are formed producing a more conductive network, resulting in the drop of the resistance. Because the contact resistance between SWNTs is affected by changes in the tunneling junction as small as a few angstroms, the removal or rearrangement of large protein macromolecules with diameters of a few nanometer results in a very substantial change in resistivity as one can see above from exceptional sensitivity obtained. SEM observations and cyclic voltammetry (CV) measurements corroborate the suggested signal transduction mechanism. SEM images show substantial restructuring upon exposure to the target protein. Before the biosensing reaction, the SWNT-PSS-anti-HSA coating displays a wavy morphology (Figure 7fg), which likely originates from the drying of frozen SWNT-

PSS-anti-HSA yarn under vacuum. After HSA detection, the wavy structures have disappeared; flat coatings with clearly visible SWNT networks can be seen. It is evident that after reaction with HSA, the SWNTs formed a more compact phase and, thus, more efficient percolation routes. These observations were further validated by CV measurements in which the anti-HSA coated smart yarn was set as a working electrode. CV data indicate a clear increase of conductivity of the smart fabric upon the less diluted antigen proteins (Figure 8c) in solution confirming the partial removal of the insulating spacing between the SWNTs. This effect is clearly absent when no antibody was incorporated between the nanotubes (Figure 8d). This finding also correlates well with the general sensing scheme outlined above. The suggested signal transduction mechanism implies one-time sensing upon complete removal of the antibodies, or cumulative sensing of the protein until it has been completely removed. From a fundamental standpoint, it would be interesting to engineer a coating with reversible sensing functionality. From a practical standpoint, however, which must consider (1) the limited life-time of antibodies and (2) the actual circumstances that can result in the appearance of blood, the multiple use of this sensor is unlikely. So, the reversible sensor to HAS might be interesting from academic point of view but its practicality is questionable.

Based on previously reported the SWNT coated cotton yarns to detect proteins in solutions, it would be fundamentally interesting as well as practically important to establish whether the similar method of analysis can be applied to the environmental needs and food safety. With this idea in mind, we have prepared and characterized the SWNT coated paper as the sensor for MC-LR toxin in the water. We attribute it to greater flexibility of SWNTs and their stronger adherence to paper originating in strong non-covalent cooperative interactions between the polyelectrolytes and cellulose. It is also probably relevant to mention that, even under high electrical current, no detachment of CNTs from the SWNT-modified paper electrode was observed.

Regular filter paper strips were dip-coated with the SWNT and the dip-dry cycles were repeated until the desirable electrical parameters of the sensor were obtained. The number of the cycles is treated as the number of SWNT layers deposited. The deposition of SWNTs can be observed by the change in color from white to black (Figure 9b). SEM images of the SWNT-coated paper indeed indicate the typical paper morphology, presence of the finely integrated nanotubes, and excellent physical integrity of the material. (Figure 9cd) As expected the conductivity of the produced material increases with increasing the SWNT contents and the number of layers of SWNT/PSS dispersion deposited (Figure 9b). The gradual increase of conductivity is quite important because in perspective the conductivity of the paper electrode needs to be within a specific range of values depending on the parameters of electrical circuit being used in order to get the best noise-to-signal ratio and the detection linearity for sensing in aqueous environments.

For sensing, we employed the standard three-electrode electrochemical station to measure changes in electrical properties of the SWNT-paper strips, which were used as work electrodes. Pt wire and the saturated Hg_2Cl_2 were used as a counter and referenced electrodes, respectively. The standard electrochemical set-up gives more accurate results than a simple clamping of the SWNT-paper material between two electrodes due to interfacial potential drops at electrode-SWNT interfaces of different nature including the Schottky barrier. Different concentrations of the MC-LR were obtained by dilution of a stock solution of 0.156 nmol/L, 0.313 nmol/L, 0.625 nmol/L, 1.25 nmol/L, 2.5 nmol/L, 5 nmol/L, 10 nmol/L, 20 nmol/L, 40 nmol/L, 100 nmol/L. Aliquots of this solution were added into

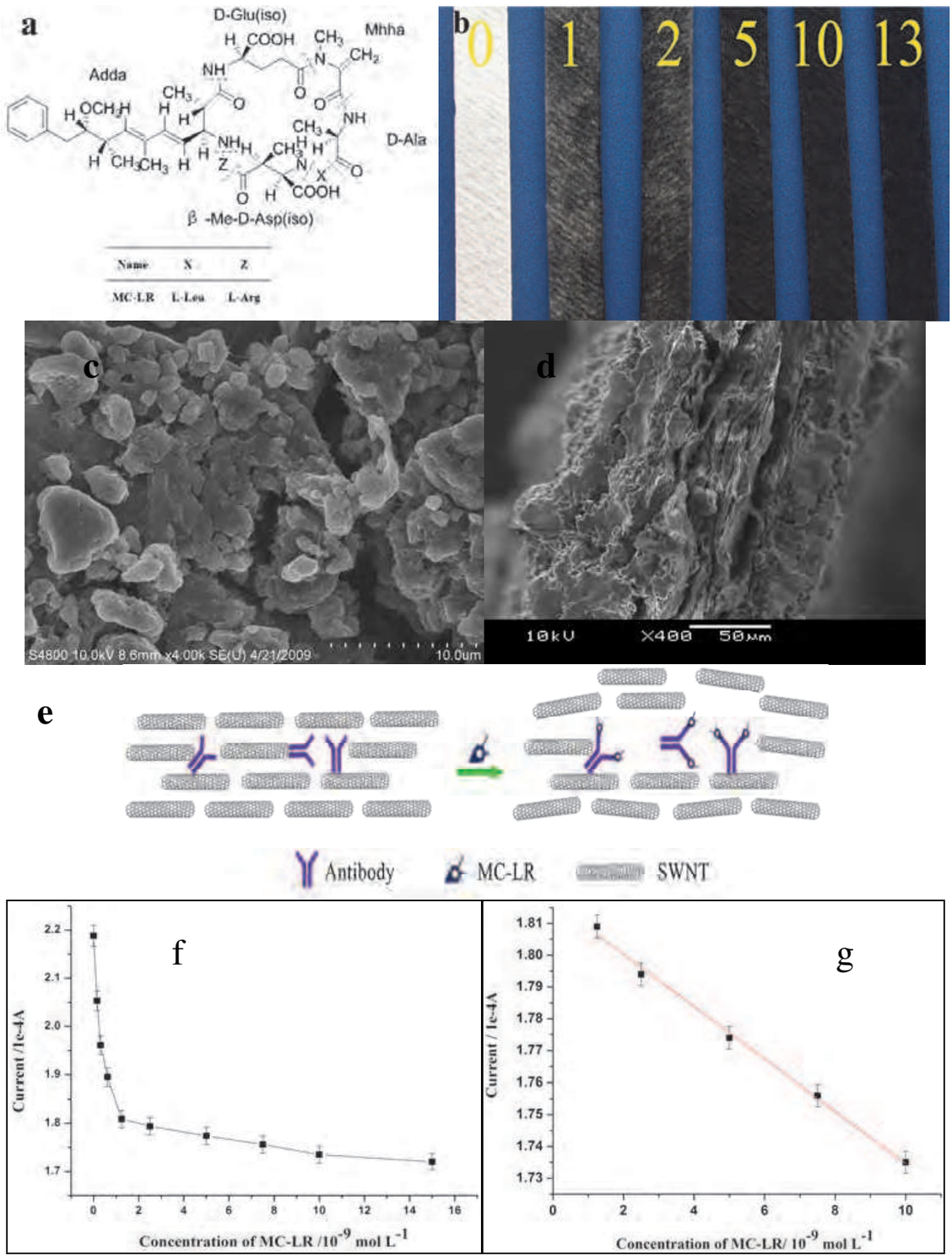


Fig. 9. (a) Chemical Structure of MC-LR. (b) Optical photographs of the SWNT-impregnated filter paper with a different number of the deposition cycles. The SEM images of (c) the surface morphology and (d) the edge of the paper electrode. (e) The sense mechanism of the developed method. The calibration curve of the determination for MC-LR (f) and (g).

the reaction cell one by one at specific time points to obtain the trend from low to high concentrations. After each addition at least *300 s* was allowed to pass to make sure that immunoreaction has enough time to proceed before the corresponding *i-t* curve was recorded. The reaction time 300 s adopted here was based on the optimization result with the best signal intensity. The current values after the start of detection where the *i-t* is transient plateaus (i.e. in the "flat" portion of the curve in the Supporting Information) were used as the analytical signal to be correlated to the concentration of MC-LR. (Figure 9e) As indicated in Figure 9e, the presence of the target analyte, i.e. MC-LR in this case reduces the current through the electrode. This corresponds to the reduction of the conductivity of SWNT-paper composite, which is quite different than the observations made for SWNT and anti-albumin Ab on cotton, where the resistivity decreased when antigen was present in solution. It was explained by the removal of Ab from the SWNT layers, resulting in shrinking of nanotubes-nanotube gaps and improvement of charge transport. In the case of electrodes described here, a different mechanism is apparently at play. Antigen penetrates through the SWNT polymer layer on the surface of paper fibers and forms the immunocomplex with Ab. This spreads apart the nanotubes, increases the nanotubes-nanotube contact resistance and hence, reduces the current passing through the material.

The SWNT-paper electrode can sense even the minor change of the MC-LR in the solution with limit of detection (LOD) of 0.6 nmol/L, which is correspond to 0.6 ng/mL and the sensitivity to the detection of MC-LR is 0.6 ng/mL (Figure 9g). It is also highly specific. The control sample of ochratoxin, which belongs to the family of micotoxints and is also a carcinogen, produced only slight variations in the current probably due to manipulations with the solution but no systematic correlation with the concentration of the control sample was observed. The calibration curve for MC-LR on SWNT-paper electrodes in the range of 0.125 to 40 nmol/L has a prevalent L-shape (Figure 9f). In the most important range of 1.25 to 10 μmol/L the calibration curve displayed excellent linearity with R^2 of 0.99426. Such behavior is indicative of the saturation phenomena when most of the antibodies in the SWNT-paper electrode formed complexes with MC-LR. According the requirements of the WHO, the content of the MC-LR in the daily water should be less than 1ng/mL, which corresponds to 1 nmol/L. The SWNT-paper based sensor could be used to monitor the quality of the drinking water for safety control. Comparing with the traditional ELISA method, the newly developed method has the similar detection range, LOD, and sensitivity with the ELISA, but in much shorter detection time. It is also much easier to operate. The time necessary for the analysis by ELISA usually exceeds 2 hours. In cases of our method, the entire analysis takes no longer than 30 min. This is much more suitable for the task of everyday monitoring of water supply. The water from Tai lake was spiked with MC-LR, the technique affords excellent recoveries of the spiked samples and acceptable relative standard deviation (n=3). Overall, excellent correlation between the MC-LR concentration values obtained by ELISA and SWNT-paper method was observed.

8. Conclusion

Different types of CNT delivery has been explored in various biomedical applications. The mechanisms of the cellular uptake of CNTs are primarily dependent on the cell type and the chemical nature and characteristics of the molecules used to functionalize the nanotube surface. Consideration of all possible mechanisms leading to CNT uptake by cells is

essential to transform one of the most promising types of novel nonmaterial into a useful and clinically relevant biotechnological and biomedical tool.

The introduction of a probe biomolecule on the surface of the CNTs as recognition element results in highly specific recognition and detection of the biomolecules from the biological samples. Meanwhile, CNT is in direct contact with the environment, which permits them to act as chemical and biological sensors in single-molecular detection of biomolecules.

To meet the urgent demand of monitoring different analytes, the carbon nanotubes based sensor may provide a very simple, rapid, sensitive, and inexpensive electrical sensor. The detection limit, sensitivity, specificity and the repeatability of the developed sesnor can be compared to that of other analytic methods while the sensor is much easier to use. It is believed that the carbon nantube based sensor could be a potential and powerful method for the monitoring of targets. Importantly, future researches on CNTs-based biosensing have attractive interest in vivo detection with less cytotoxicity, high sensitivity, and long-term stability for reliable point-of-care diagnostics under physiological conditions.

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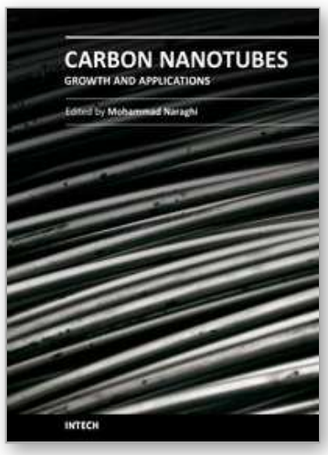
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