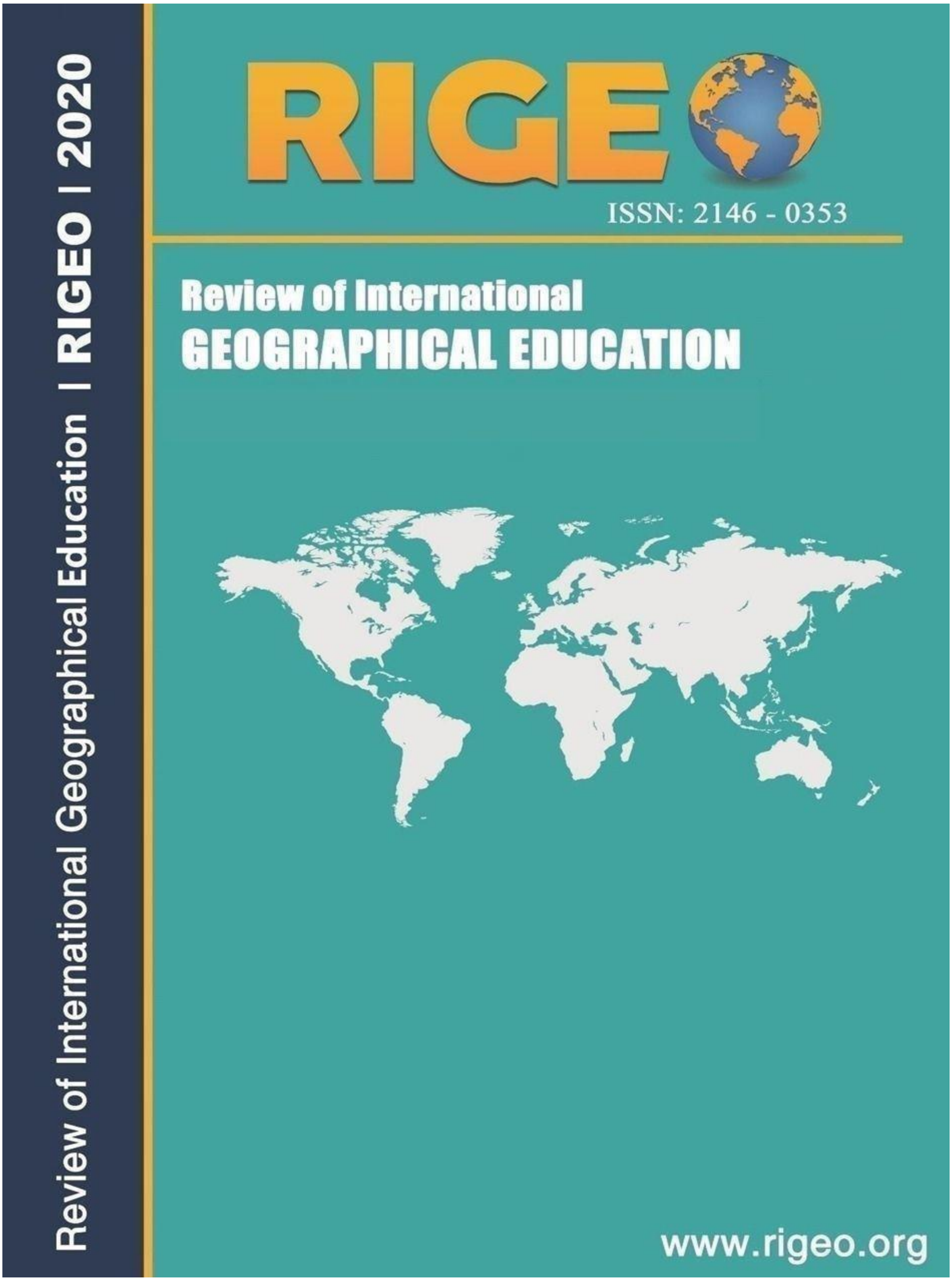

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Structure and Protein Interactions in Carbon Nanotubes: A Comprehensive Review

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Abstract:

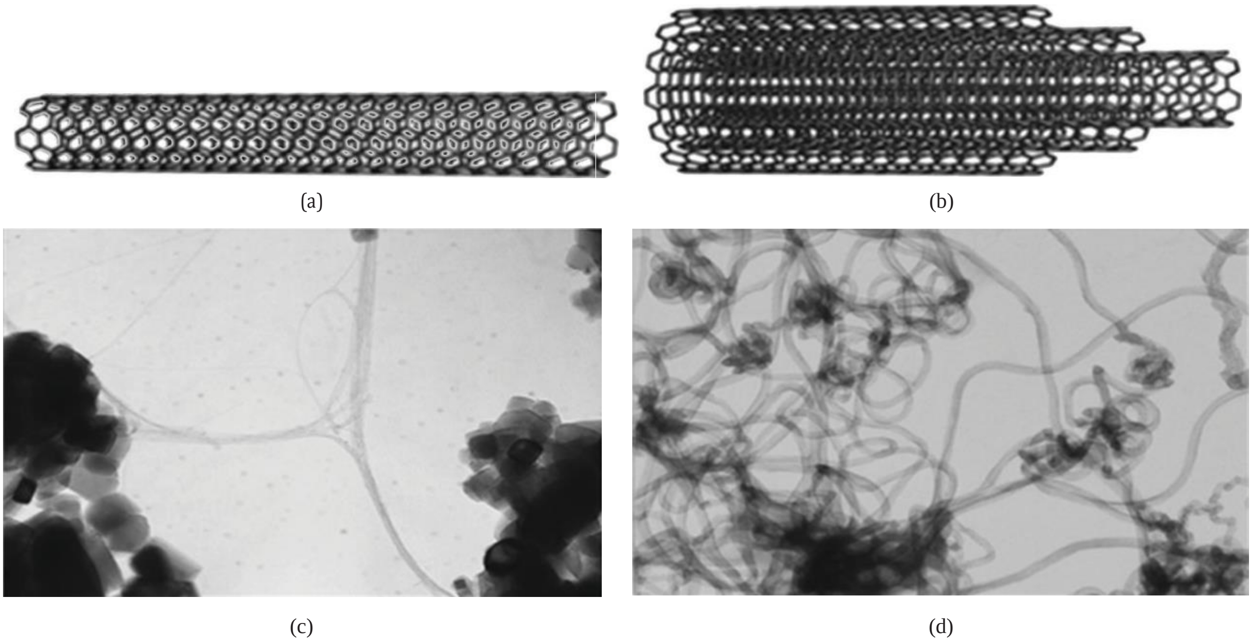
A carbon allotrope with a nanostructure and a length-to-diameter ratio more than 1,000,000 is known as a carbon nanotube (CNT). Methods such as chemical vapour deposition, arc discharge, and laser ablation have been developed to manufacture nanotubes in significant amounts. Recent advancements have shown the revolutionary potential of nanomaterials, particularly in the fields of biomedical imaging, drug delivery, biosensing, and functional nanocomposites design. In order to bring these uses to fruition, methods for efficiently integrating proteins with nanomaterials are constantly developing. The immobilised entity may be concentrated to a much greater extent than with other materials due to the high surface-to-volume ratio provided by nanoparticles. The impact of nanomaterials on protein structure and function is another area of growing interest. The specialised attachment of enzymes to carbon nanotubes has garnered a lot of interest among the several immobilisation techniques that have been devised. As research into cascade enzymatic reactions continues to advance, multienzyme coimmobilization may emerge as a promising next step. Our research here primarily aims at the latest developments in the technique of enzyme immobilisation on carbon nanotubes.

1. Introduction

Diamond and graphite are considered as two natural crys- talline forms of pure carbon. In diamond, carbon atoms exhibit $ssss^3$ hybridization, in which four bonds are directed towards the corners of a regular tetrahedron. The resulting three-dimensional network (diamond) is extremely rigid, which is one reason for its hardness. In graphite, $ssss^2$ hybridization occurs, in which each atom is connected evenly to three carbons (120°) in the xxx plane, and a weak $\pi\pi$ bond is present in the zz axis. The $ssss^2$ set forms the hexagonal (honeycomb) lattice typical of a sheet of graphite[1]. A new form of carbon, Buckminster fullerene (C_{60}), wasdiscovered in 1985 by a team headed by Korto and coworkers[2]. Besides diamond, graphite, and fullerene (C_{60}), quasi- one-dimensional nanotube is another form of carbon first reported by Ijima in 1991 when he discovered multiwalled carbon nanotubes (MWCNTs) in carbon soot made by an arc-discharge method [3]. Carbon nanotubes (CNTs) areallotropes of carbon. CNTs are tubular in shape, made of graphite. The tubes contained at least two layers, often many more, and ranged in outer diameter from about 3 nm to 30 nm. About two years later, he made the observation of single-walled carbon nanotubes (SWCNTs) [4]. At about the same time, Dresselhaus et al. synthesized single-walled carbon nanotubes by the same route of producing MWCNTs but adding some transition metal particles to the carbon electrodes [5]. The single-walled nanotubes are generally nar-rower than the multiwalled tubes, with diameters typically in the range 1-2 nm, and tend to be curved rather than straight (Figure 1). A significant amount of work has been done in the past decade to reveal the unique structural, electrical, mechanical, electromechanical, and chemical properties of CNTs. Recent research has focused on improving the quality of catalytically-produced nanotubes [6, 7].

2. C1assification of Carbon Nanotubes

Carbon nanotubes are classified in following two types: SWCNTs—Single-walled carbon nanotubes and MWCNTs—Multiple-walled carbon nanotubes. Comparison between SWCNT and MWCNT is as presented in Table 1[9–11].



(a) (b)

(c) (d)

Figure 1: Molecular representations of SWCNT (top left) and MWCNT (top right) with typical transmission electron micrographs (below) [8].

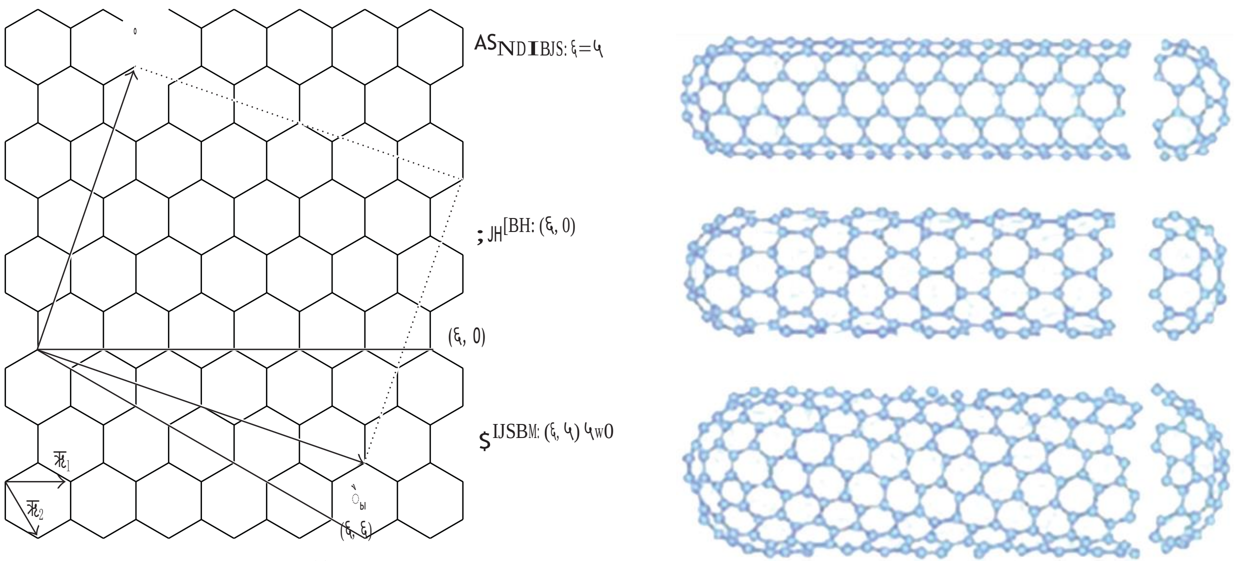
Table 1: Comparison between SWCNT and MWCNT.

SWCNT	MWCNT
Single layer of graphene.	Multiple layer of graphene
Catalyst is required for synthesis.	Can be produced without catalyst.
Bulk synthesis is difficult as it requires proper control over growth and atmospheric condition.	
Bulk synthesis is easy.	
Not fully dispersed, and form bundled structures.	Homogeneously dispersed with no apparent bundled formation.
Resistivity usually in the range of 10^{-4}–10^{-3} Ω·m.	Resistivity usually in the range of 1.8×10^{-5}–6.1×10^{-5} Ω·m Purity is
poor. Typical SWCNT content in as-prepared samples by chemical vapour deposition (CVD) method is about 30–50 wt%. However high purity up to 80% has been reported by using arc discharge synthesis method.	
A chance of defect is more during functionalization. Purity is high. Typical MWCNT content in as-prepared samples by CVD method is about 35–90 wt%.	
A chance of defect is less especially when synthesized by arc-discharged method.	
Characterization and evaluation is easy.	It has very complex structure
It can be easily twisted and are more pliable.	It cannot be easily twisted.

3. Structure and Morphology

Comprised entirely of carbon, the structure of pure SWCNT can be visualized as rolled-up tubular shell of graphene sheet which is made up of benzene type hexagonal rings of carbon atoms (Figure 2(a)). Graphene sheets are seamless cylinders derived from a honeycomb lattice, representing a single atomic layer of crystalline graphite. A MWCNT is a stack of graphene sheets rolled up into concentric cylinders. Each nanotube is a single molecule composed

of millions of atoms and the length of this molecule can be tens of micrometers long with diameters as small as 0.7 nm [11]. The SWCNTs usually contain only 10 atoms around the circumference and the thickness of the tube is only one-atom thick. Nanotubes generally have a large length-to-diameter ratio (aspect ratio) of about 1000, so they can be considered as nearly one-dimensional structures [12]. MWCNTs are larger and consist of many single-walled tubes stacked one inside the other. The name MWCNT is restricted to nanostructures with outer diameter of less than 15 nm, above which the structures are called carbon nanofibers. CNTs are distinct from carbon fibers, which are not single molecules but strands of layered-graphite



(a) (b)

Figure 2: Schematic representation of (a) formation of single-walled carbon nanotubes by rolling of a graphene sheet along lattice vectors which leads to armchair, zigzag, and chiral tubes and (b) the three types of carbon nanotubes [14].

In addition to the two different basic structures, there are three different possible types of carbon nanotubes. These three types of CNTs are armchair carbon nanotubes, zigzag carbon nanotubes, and chiral carbon nanotubes. The difference in these types of carbon nanotubes are created depending on how the graphite is "rolled up" during its creation process. The choice of rolling axis relative to the hexagonal network of the grapheme sheet and the radius of the closing cylinder allows for different types of SWCNTs.

The chiral vector is represented by a pair of indices, nn and mm, where these two integers correspond to the number of unit vectors along the two directions in the honeycomb crystal lattice of grapheme. When $mm\ m\ m$ the nanotube is called "zigzag", when $nn\ m\ mm$ the nanotube is called "armchair", and all other configuration are designated as chiral. Figure 2 shows the three different types of SWCNTs: armchair, zigzag, and chiral. Further details of the structure can be found in reviews by [12, 15, 16].

4. Properties

The strength of the $ssss^2$ carbon-carbon bonds gives car- bon nanotubes amazing mechanical properties. No previ- ous material has displayed the combination of superlative mechanical, thermal, and electronic properties attributed to them. Their densities can be as low as 1.3 g/cm^3(one-sixth of that of stainless steel). CNTs Young's moduli (measure of material stiffness) are superior to all carbon fibres with values greater than 1 TPa which is approximately 5x higher than steel [17]. However, their strength is what really sets them apart. Carbon nanotubes are the strongest materials ever discovered by mankind. The highest measured tensile strength or breaking strain for a carbon nanotube was up to 63 GPa which is around 50 times higher than steel [17]. Even the weakest types of carbon nanotubes have strengths of several GPa [18]. Besides that, CNTs have good chemical and environmental stability and high ther- mal conductivity (~3000 W/m/K, comparable to diamond). These properties, coupled with the lightness of carbon nan-otubes, give them great potential in applications such as aerospace.

The electronic properties of carbon nanotubes are also extraordinary. It has high electrical conductivity (comparable to copper). Especially notable is the fact that nanotubes can be metallic or semiconducting. The rolling action breaks the symmetry of the planar system and imposes a distinct direction with respect to the hexagonal lattice and the axial direction. Depending on the relationship between this axial direction and the unit vectors describing the hexagonal lattice, the nanotubes may behave electrically as either a metal or a semiconductor. Semiconducting nanotubes have bandgaps that scale inversely with diameter, ranging from approximately 1.8 eV for very small diameter tubes to 0.18 eV for the widest possible stable SWCNT [19]. Thus, some nanotubes have conductivities higher than that of copper, while others behave more like silicon. There is great interest in the possibility of constructing nanoscale electronic devices from nanotubes. There are several areas of technology where carbon nanotubes are already being used. These include flat- panel displays, scanning probe microscopes, sensing devices, and fuel cell.

5. Carbon Nanotubes Synthesis Techniques

High-quality nanotube materials are desired for both fun- damental and technological applications. High quality refers to the absence of structural and chemical defects over a significant length scale (e.g., 1–10 microns) along the tube axes. The number of patents and publication on the synthesis of carbon nanotube is increasing rapidly. However there are many challenges remaining that must be resolved regarding synthesis of CNT. Currently, there are four main challenges in the field of nanotube synthesis. (a) Mass production, that is, the development of low-cost, large-scale processes for the synthesis of high-quality nanotubes, including SWCNTs.

(b) Selective production, that is, control over the structure and electronic properties of the produced nanotubes. (c) Organization, that is, control over the location and orien- tation of the produced nanotubes on a flat substrate. (d) Mechanism, that is, the development of a thorough under- standing of the processes of nanotube growth. The growth mechanism is still a subject of controversy, and more than one mechanism might be operative during the formation of CNTs.

A variety of techniques have been developed to produce CNTs and MWNTs with different structure and morphology in laboratory quantities. There are three methods commonly used to synthesize CNT: arc discharge [20, 21], laser ablation [22], and chemical vapor deposition (CVD) [23–26]. The basic elements for the formation of nanotubes are catalyst, a source of carbon, and sufficient energy. The common feature of these methods is addition of energy to a carbon source to produce fragments (groups or single C atoms) that can recombine to generate CNT. The energy source may be electricity from an arc discharge, heat from a furnace (∼900°C) for CVD, or the high-intensity light from a laser (laser ablation).

6. Arc Discharge and Laser Vaporization

Arc discharge and laser ablation were the first methods that allowed synthesis of SWCNTs in relatively large (gram) amounts. Both methods involve the condensation of hot gaseous carbon atoms generated from the evaporation of solid carbon [27]. For the growth of single-wall tubes, a metal catalyst is needed in the arc-discharge system [28]. The growth of high-quality SWCNTs at the 1–10 g scale was also produced using a laser-ablation (laser oven) method [22]. Besides the laser-oven method, there are reports regarding usage of a typical industrial continuous wave CO_2-laser system for production of SWCNTs [29]. Nevertheless, the equipment requirements and the large amount of energy consumed by these methods make them less favorable for nanotube production. With the arc and laser methods, only powdered samples with nanotubes tangled into bundles can be produced. The common feature of arc discharge and laser ablation methods is the need for high amount of energy to induce the reorganization of carbon atoms into CNTs. The temperature used is even higher than 3000°C, which is beneficial for good crystallization of the CNTs, thus, the products are always produced with good graphite alignment. However, the basic requirements of these systems, including vacuum conditions and continuous graphite target replacement, pose difficulties to the large-scale production of CNTs.

7. Chemical Vapor Deposition (CVD)

The CVD method involves the decomposition of a gaseous or volatile compound of carbon, catalyzed by metallic nanopar-ticles, which also serve as nucleation sites for the initiation of carbon-nanotube growth. In contrast the previous two methods, CVD has been proven to be a preferred route for large-scale production of carbon nanotubes [27, 30]. Here the carbon is deposited from a hydrocarbon (or other carbon bearing source) in the presence of a catalyst at temperatures lower than 1200°C. The CNT structure, such as its wall number, diameter, length, and alignment, can be well controlled during the CVD process. Thus, the CVD method has the advantages of mild operation, low cost, and controllable process. Over the last twelve years, several methods have been developed that have the potential for industrial-scale preparation of nanotubes. All of them are based on CVD methods. Among these methods, five different approaches have been shown to be the most promising:

(i) Methane CVD- It was first reported in 1998, where bulk amount of SWCNTs were synthesized by CVD from methane at 900°C [23, 31]. Su et al. [26] signifi- cantly improved the yield of this method using Al_2O_3 aerogels impregnated with Fe/Mo nanoparticles as a catalyst.

(ii) HiPCO, which stands for high-pressure catalytic decomposition of carbon monoxide, uses high- pressure CO as the carbon source for the preparation of SWCNTs [32]. The catalysts used in a HiPCO process are in the gas phase produced from a volatile organometallic catalyst precursor.

(iii) CO CVD uses CO as a feed gas. Compared with samples made using the same catalyst and methane, the amount of amorphous carbon can be reduced. An important advance in the CO CVD method is the development of the Co-Mo catalyst [33]. In that process Co-Mo bimetallic catalysts and a fluidized- bed CVD reactor was used to produce a large quan- tity of SWCNTs. The most important advantage of fluidized-bed reactors is that they permit continuous addition and removal of solid particles from the reactor, without stopping the operation.

(iv) Alcohol CVD, was reported in 2002 by Maruyama et al. [34], which produce high-purity SWCNTs without any amorphous carbon coating using alcohols such as methanol and ethanol as a carbon source. It was proposed that the OH radical formed at high tem- perature from alcohols can remove the amorphous carbon efficiently during nanotube growth, leaving only pure SWCNTs as a product.

(v) Plasma-enhanced CVD (PECVD) methods have also been widely used for making carbon materials includ- ing MWCNTs and SWCNTs recently [35–39]. The reactive species in the plasma system could affect the growth of very small diameter tubes, with implica- tions to both diameter control and selective etching of metallic SWCNTs.

8. Catalyst

Catalysts play a crucial role in the CVD synthesis of CNTs and therefore improving the desired characteristics of catalyst will enhance the obtained CNTs quality as well as the process yield. Carbon nanostructures are commonly synthesized using transition metal nanoparticles as catalysts [22]. The structure of CNTs has been found to be determined by the size and chemical composition of the metal catalysts. However, at present, the diameter, length, and chirality of CNTs have not been controlled sufficiently in a single process due to incomplete understanding of the role of the catalyst in nanotube nucleation and growth [41]. It is accepted that CNT growth by CVD involves surface and/or bulk diffusion of carbon at a metal catalyst particle. CNT-CNT or CNT- substrate interactions in addition to the arrangement and activity of the catalytic sites determine if CNTs grow in an isolated, tangled, or aligned configuration. At all stages of CNT growth, chemical and mechanical interactions are highly coupled, and these interactions must be further under- stood for efficient synthesis of CNTs [42].

Transition metals in the form of nanoparticles are con- sidered as the most effective catalysts. The peculiar ability of transition metals to promote CNT growth is strongly related to these factors: (a) catalytic activity for decomposition of volatile carbon compounds, (b) ability of metastable carbides formation, and (c) diffusion of carbon through and over the metallic particles [43–45]. The catalytic CVD process for CNTs growth utilizes heterogeneous catalysts, which are the catalytically active metal particles, typically with a diameter of 1–10 nm, anchored on a high surface inert area. The Transition metals used to date as catalysts include Fe, Ni, Co, and Mo. More recent studies however, have shown that other metals such as In, Cu, Ag, Pd, Mn, Mo, Cr, Al, and Au can also be used for SWCNT formation [46, 47]. Since SWCNT nucleation requires a catalyst particle, a great deal of attention has been paid to the catalysts role in forming the embryonic stage of SWCNT. However, their full role has yet to be determined and this is in part due to conflicting results, which may indicate that several mechanisms exist.

In order to obtain CNTs, the catalyst must be prepared as a nanoparticle catalyst [48]. The absorption of carbon in catalyst particles and the precipitation rates of CNTs from the catalyst particles both show great dependence on the size of the catalyst particles. Under a given CVD there is an optimal particle size diameter to nucleate SWCNTs with a fixed feeding rate of carbon. Smaller catalyst nanoparticles are easily poisoned by excess feeding, and larger catalyst particles are inactive due to under feeding [49].

So far, the published results concerning catalysts have mainly focused on the synthesis of monodispersed CNTs, but there have been very few detailed experimental studies of the reaction pathways. Furthermore, the interaction between catalysts and the surface of substrates need to be further investigated and characterized. Efforts in future will be geared towards finding optimal CVD conditions to discover the detailed mechanism of catalysis, and facilitating control over the growth of CNTs for future fabrication of nanotube-based devices.

8.1. Carbon Sources. One of the major barrier to the indus-trial application of CNTs, lies on the cost of their carbona- ceous precursors. Various carbon sources have been used to produce CNTs since its first discovery by Iijima in 1991. Different methods in producing the CNTs show different usage of carbon source. The arc discharge was the first technique used for the production of carbon nanotubes. The CNTs produced by this method were grown on the negative end of graphite electrode under inert atmosphere of helium or argon with a very high temperature needed in order to evaporate the pure graphite or coevaporate the graphite and metal [50–53]. Similar to arc discharge method, graphite is used in the laser ablation method. Graphite is vaporized by laser irradiation under an inert atmosphere with the presence of metal catalyst to produce the carbon nanotubes [22, 51, 54, 55].

There are two main carbon sources for the synthesis of CNTs-using CVD method: fossil-based hydrocarbon and plant based hydrocarbon. Hydrocarbon was long and widely used as the conventional carbon source in the field of CNTs research. Natural gas becomes the most preferable carbon source to many researchers. Because its stability at high temperature against self-decomposition, methane catalytic decomposition by transition metal catalyst particles is the dominant process in carbon nanotubes growth. Besides methane several other carbon species such as acetylene, benzene, xylene, toluene, and so forth, have been used as a carbon feedstock to synthesize CNTs [56–60]. These carbon precursors are related to fossil fuels and in view of the insufficient available in near future and its environmental effects, it is necessary to consider developing carbonaceous materials from the natural resource. Efforts are now directed to the use of nonpetroleum products. Syntheses of CNTs from natural precursors are rare, however, over the past several years natural renewable resources have become more attractive because of their environmental benefits and lower cost [61]. One such appreciable effort is to use nondegradable polymers for synthesis of CNTs [62]. There have been reports on the use of natural precursor such as: camphor ($C_{10}H_{16}O$), turpentine oil ($C_{10}H_{16}$), eucalyptus oil ($C_{10}H_{18}O$), castor oil ($C_{54}H_{100}O_7$), coconut oil ($C_{39}H_{74}O_6$), and palm oil ($C_{67}H_{127}O_8$) for synthesis of CNTs [63–70]. Published data show that, some researchers have used waste cooking palm oil as the biocarbon precursor in their studies [71]. Waste cooking oil, which is much less expensive than virgin veg- etable oil, is a promising alternative to vegetable oil for CNT production. Suriani and coworkers [71] reported the use of waste cooking palm oil for the synthesis of vertically aligned carbon nanotubes (VACNT). The result showed that the complex composition of the waste oil (leaching of fats and other hydrocarbons from the fried objects) did not affect the synthesis process.

9� Purification and Dispersion of Carbon Nanotubes

As-synthesized CNTs prepared by the above methods inevitably contain carbonaceous impurities and metal catalyst particles, and the amount of the impurities commonly increases with the decrease of CNT diameter. The fundamental problems that still exist are how to (1) remove impurities, such as amorphous carbons and metallic catalysts, and (2) obtain uniform dispersions of the carbon nanotubes in dispersing media or polymer solutions. The impurities in unpurified carbon nanotubes severely reduce the mechanical or electrical properties. The as-produced CNTs soot contains a lot of impurities. Up to now, all currently known production methods generate CNTs with impurities. Purification has been an important synthetic effort since the discovery of carbon nanotubes. In general, the main impurities in the soot are graphite (wrapped up) sheets, amorphous carbon, metal catalyst, and the smaller fullerenes. Also, structural defects, such as dangling bonds, are often found in most types of CNTs. These impurities will interfere with most of the desired properties of the CNTs. Purification difficulties are considerable because CNTs are insoluble and, hence, liquid chromatography is limited. Thus, extensive research has been dedicated to the purification of carbon nanotubes in order to remove foreign nanoparticles that modify the physicochemical properties of carbon nanotubes. Here, we just intend to give a brief overview of the principles with a few examples. Good review articles on the purification of nanotubes are available in the recent literature [72, 73]. Much effort has therefore been expended in the development of purification techniques; the resulting approaches are summarized in Table 3. These methods utilize either one or a combination of several elemental techniques.

Obtaining pure monodisperse SWCNTs of specific structures in large quantities is a problem. SWCNTs have attractive electronic properties, since they become metallic or semiconductive depending on chiral indices ($nnn\ nn$). However, their strong tendency to form bundles (or ropes) due to strong van der Waals interactions prevents their utilization as the ultimate nanomaterial. Successful dispersion of SWNTs could lead to the diameter and/or chirality-selective separation of individual SWNTs. Disaggregation and uniform dispersion are critical challenges that must be met, since carbon nanotubes tend to self-associate into microscale aggregates. Hence, the thermodynamic drive toward aggregation must be overcome. There are two distinct approaches for dispersing carbon nanotubes: the mechanical method and methods that are designed to alter the surface energy of the solids, either physically (noncovalent treatment) or chemically (covalent treatment) [74, 75]. Chemical methods use surface functionalization of CNT to improve their chemical compatibility with the target medium (solvent or polymer solution/melt), that is, to enhance wetting or adhesion characteristics and impeded the full realization of their potential. Chemical functionalization of CNTs has been shown to impart solubility in a variety of solvents, to modify their electronic properties, and to cause significant debundling. Problems of solubility of CNTs due to the formation of bundles, make them hardly soluble in common solvents. The solubility of carbon nanotubes in water is limited and proper amounts of stabilizers are required to avoid flocculation and phase separation. One disadvantage of the CNTs concerning their use in biochemistry and biomedical applications is that they are highly hydrophobic and generally form insoluble aggregates. Due to the less solubility of CNTs in any of the solvents, it is also very difficult to isolate one carbon nanotube from the other. Like graphite, CNTs are relatively nonreactive, except at the nanotube caps which are more reactive due to the presence of the dangling bonds. The reactivity of the sidewalls of the carbon nanotube $\pi\pi$-system can be influenced by the tube curvature or chirality. The hydrophobic surfaces of carbon nanotubes adsorb a wide class of substances by $\pi\pi$-$\pi\pi$ and/or van der Waals interactions [81–83]. Therefore, proper stabilization of CNTs dispersions is a prerequisite for technological applications.

Loung et al. [84] reported that when CNTs were sonicated in organic solvents, they produce dangling bonds that will undergo further chemical reactions. Many efforts in recent years have led the development of versatile chemical modification methodologies in order to solve the insolubility problem. The recent expansion in methods to chemically modify and functionalize carbon nanotubes has made it possible to solubilize and disperse carbon nanotubes in water, thus opening the path for their facile manipulation and processing in physiological environments. The surface functionalization of CNTs by chemically attaching an organic functional groups will aid the carbon nanotube materials in becoming biocompatible, improving their solubility in physiological solutions and selective binding to biotargets. Two main paths are usually followed for the functionalization of CNTs: attachment of organic moieties either to carboxylic groups that are formed by oxidation of CNTs with strong acids or direct bonding to the surface double bonds [85]. Important early reports by Georgakilas et al. (2002) have shown method to functionalize CNTs using organic compounds. Approximately one organic group per 100 carbon atoms of the nanotube is introduced to yield remarkably soluble bundles of nanotubes [86]. The solubilization of the nanotubes generates a novel, interesting class of materials, which combines the properties of the nanotubes and the organic moiety, thus offering new opportunities for applications in materials science, including the preparation of nanocomposites. Fluorination, addition of carbenes and nitrenes, electrophiles, or peroxy radicals were found to be successful reactions for sidewall covalent functionalization of CNTs [85, 86].

In recent years, efforts have been devoted to explore the potential biological applications of CNTs, motivated by their interesting size, shape, and structure, as well as attractive optical and electrical properties. First, with all atoms exposed on the surface, SWNTs have ultrahigh surface area (theoretically 1300 m^2/g) that permits efficient loading of multiple molecules along the length of the nanotube sidewall. Second, supramolecular binding of aromatic molecules can be easily achieved by $\pi\pi$-$\pi\pi$ stacking of those molecules onto the polyaromatic surface of nanotubes [87, 88]. It has been demonstrated that biological and bioactive species such as proteins, carbohydrates, and nucleic acids can be conjugated with carbon nanotubes [89–91]. Both noncovalent and covalent strategies have been explored to engineer the interface between biological molecules and CNTs with the goal of preserving the functional properties of the biomolecules. The biomolecule immobilization on the sidewall of the CNTs, and more interestingly inside the CNTs has been reported in both computational and experimental fields [92–97]. Based on these exciting observations and potential applications, the conformational changes of biomolecules in these confined environments tend to be of great significance mainly because these conformational changes affected by the biomolecules- CNT interactions could directly impact their biological functions. However, the atomic details of the interactions at the molecular level, and the dynamic mechanisms of the biomolecules-CNT systems are still challenging due to the complexity of the biomacromolecules. The interaction between nanostructured materials and living systems is of fundamental and practical interest and will determine the biocompatibility, potential utilities, and applications of novel nanomaterials in biotechnological processes. However, the studies on the CNT-organic nanoparticle hybrid architec- tures are poorly developed comparatively. For example, there are not enough studies on the influence that the nanomaterial properties (such as composition, morphology, and surface chemistry) have on the structure and function of conjugated proteins. The most important parameter in all such studies is the type of carbon nanotubes used, which is determined by (i) the preparation and manufacturing process followed; (ii) the structural characteristics of the CNTs; (iii) the surface charac- teristics of the CNTs and the characteristics of the functional groups at the surface of CNTs. Interactions with cells have to be performed using biocompatible CNTs, achieved by either covalent or noncovalent surface functionalization to produce water-soluble CNTs [98].

10.2. Immobilization of Proteins and Enzymes. Practical use of enzymes has been realized in various industrial processes, and is being expanded in new fields, such as fine-chemical synthesis, pharmaceuticals, biosensors, and biofuel cells [99]. To improve enzyme stability, enzymes have generally been studied with the enzymes immobilized on a solid support [100]. Nanomaterials can serve as excellent supporting mate- rials for enzyme immobilization, because they offer the ideal characteristics for balancing the key factors that determine the efficiency of biocatalysts, including surface area, mini- mized mass transfer resistance, and effective enzyme loading [100–102]. Carbon nanotubes are receiving a great deal of attention as alternative matrices for enzyme immobilization. CNTs are better support material for enzyme immobilization compared to common support like zirconia, silica, and epoxy. They are more stable under harsh condition, provide higher loading of enzyme, and enhanced catalytic activity of by allowing the reaction of the free amine groups (on the protein surface) with carboxylic acid groups that are gener-ated by sidewall oxidation of CNTs, which is facilitated by 1-ethyl-3-(3dimethylaminipropyl) carbodiimide [112, 113, 118, 119]. The method has been widely applied to the covalent immobilization of proteins on carboxylated CNTs [108]. For some enzymes, the enzyme-loadings are higher than 1000 $\mu\mu$g enzyme per mg of CNTs [121, 122]. The conjugates are stable at high temperatures, providing a combination of useful attributes such as low mass transfer resistance, high activity and stability, and reusability. It has been reported that the uncovered surface of CNTs may promote the accessibility of substrate to the enzyme and the CNTs can facilitate heat transfer [122]. Ji et al. [121] have showed that covalently attached lipase on CNTs has advantages over free lipase in catalysis in organic solvent. The immobilized lipase greatly improves the conversion of the substrate compared to the native lipase. It has been demonstrated that enzymes on SWCNTs have much higher activity than those conjugated to MWCNTs [109].

10.1.1. Covalent Attachment of Proteins onto CNTs with Linking Molecules. Linking molecules which act as “bridge” between the material and protein are frequently used for covalent immobilization of protein and enzymes onto CNTs. They bind to CNTs through hydrophobic and $\pi\pi$-$\pi\pi$ inter- actions [123, 124] and also covalently bind the enzyme through, for example, an amide bond [125, 126]. These linking molecules present advantages in the immobilization of enzymes. In the immobilization of horseradish peroxidase, the highly reactive succinimidyl ester groups were covered on CNTs, using 1-pyrenebutanoic acid succinimidyl ester as the linking molecule [123]. Pang et al. [124] have reported that with aminopyrene, the amino functional groups were intro- duced uniformly on the CNT surface and the immobilized- laccase enzyme showed higher electrocatalytic activity and better stability than the laccase immobilized on the pristine CNTs. Linking molecules can provide specific sites for CNTs to immobilize enzymes [102, 127]. Figure 3 summaries the three main method of biomolecule immobilization on CNTs.

11. Structure and Catalytic Behavior of Immobilized Enzymes

The premise of using nanoscale structures for immobiliza- tion is to reduce diffusion limitations and maximize the functional surface area to increase enzyme loading [128]. In addition, the physical characteristics of nanoparticles such as enhanced diffusion and particle mobility can impact inherent catalytic activity of attached enzymes [101]. Immobilization of enzymes is advantageous for commercial application due to convenience in handling, ease of separation of enzymes from the reaction mixture and reuse, and a possible increase in thermal and pH stability [129, 130]. Poor biocatalytic efficiency of immobilized enzymes, however, is a main draw- back that hinders the large-scale application. Noncovalent techniques, which employs simple physical adsorption and usually do not require the harsh processing conditions are

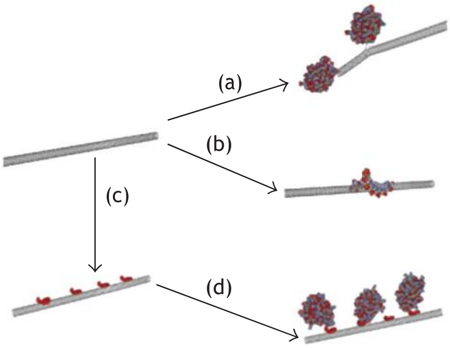


Figure 3: The three main approaches for modifying carbon nan- otubes with biomolecules: the covalent approach (step a), noncova- lent approach (step b), and hybrid approach where a small molecule "anchor" is first noncovalently absorbed to the carbon nanotube (step c), followed by a chemical reaction between the anchor and the biomolecules of interest (step d). (Adapted from Yang et al. [133])

simpler in practice, however leads to lower amounts of loading than more involved methods as reported by a study by Zhang and Henthorn [131]. The authors found that the amount of enzyme loading was significantly less for the direct adsorption method (66 micrograms of enzyme per milligram of dry carbon nanotubes) than for the linker molecule method (140 $\mu\mu$g/mg). Over the past 6 years or so some research groups have investigated the activity-structure relationship of the immobilized enzymes. A study of retained enzyme activity was then conducted and they found that only 27% of the enzyme activity remained when the conjugation was produced using direct adsorption, while 57% of the enzyme activity was retained using the linker molecule [131]. Karajanagi et al. [96] had reported that direct physical adsorption cause a significant change in the secondary structure of the protein [132], used circular dichroism spectroscopy and fluorescence to analyze the structural changes that occurred upon protein adsorption. In many cases, the higher stability is attributed in a more rigid structure that the enzyme adopts.

Several spectroscopic techniques have been used in order to monitor possible structural changes upon immobilization. Table 7 shows the results of some recent works in this area. The type of the enzyme and the nanotubes, the functional groups on the surface of the CNTs, and immobilization techniques are crucial factors which affect the structure of the enzyme upon immobilization [116].

12. Conclusion

Various modified synthesis techniques have been developed in order to produce CNTs in large scale for commercial appli- cation. At the moment, CVD method is the most promising method to produce large quantity of CNTs since the cost is relatively low compared to other methods. Commercial applications of CNTs have been rather slow to develop, however, primarily because of the high production costs of the best quality nanotubes. The chemistry of CNTs has made enormous strides, and it is clear that this subject will drive the applications of carbon nanotubes. Functionalization of CNTs, and particularly CNTs of defined length, diameter, and chirality, will lead to the better control of CNT-based materials and devices at the molecular level. The present paper shows that their immense potential for biotechnology and biomedicine are only just starting to be realized. Various biomolecules (proteins, enzymes, or DNA/RNA) can interact and be immobilized on the CNTs, leading to a wide field of application. However, there is not a universal enzyme support and the best method of immobilization might differ from enzyme to enzyme, from application to application, and from carrier to carrier. In the future, information derived from protein sequences, 3D-structures, and reaction mechanism should be further combined with the fascinating properties of CNTs and physical/chemical methods in order to produce the immobilized enzyme with even more stability and higher catalytic activity. Using noncovalent approaches, enzymes can be less denatured upon immobilization and the intrinsic electronic structure and properties of CNTs are preserved.

It is also necessary to study how the linking molecules interact with enzymes and affect the enzyme structure and the arrangement of enzymes on CNTs. The mobility, con- fining effects, solution behaviors, and interfacial properties of nanoscale materials can introduce unique properties to biocatalyst systems, making it possible to develop a revolu- tionary class of biocatalyst that differs from traditional immo- bilized enzymes in terms of preparation, catalytic efficiency, and application potential. In the future, new mechanisms and phenomena may continue to appear. Interest in this field is rapidly growing and is likely to fuel more exciting developments in the near future.

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