12. Applications of Carbon Nanotubes in Bio-Nanotechnology

12.1 Introduction

Patients today are seeking for better health care, while healthcare providers and insurance companies are calling for cost-effective diagnosis and treatments. The biomedical industry thus faces the challenge of developing devices and materials that offer benefits to both patients and healthcare industry. The combination of biology and nanotechnology, is expected to revolutionize biomedical research by exploiting novel phenomena and properties (physical chemical and biological) of material present at nanometer length (10^{-9}m) scale [1–5]. This will lead to the creation of functional materials, devices and systems through control of matter on the nanometer meter scale and the direct application of nano-materials to biological targets.

The nano-materials were existed in nature, long before mankind was able to identify forms at the nanoscale level. Today, nano-materials have been designed for a variety of biomedical and biotechnological applications, including biosensors, enzyme encapsulation, neuronal growth, drug delivery and bone growth [6–10]. The advances in bio-nanotechnology is based on the introduction of novel nano-materials which can result in revolutionary new structures and devices using extremely biological sophisticated tools to precisely position molecules and assemble hierarchal structures and devices. The application of the principles of biology to nanotechnology provides a valuable route for further miniaturization and performance improvement of artificial devices. The feasibility of the bottom-up approach that is based on molecular recognition and self-assembly properties of bio-molecules has already been proved in many inorganic-organic hybrid systems and devices [11]. Nanodevices with bio-recognition properties provide tools at a scale, which offers a tremendous opportunity to study bio-chemical processes and to manipulate living cells at the single molecule level. The synergetic future of nano-and bio-technologies holds

great promise for further advancement in tissue engineering, prostheses, pharmacogenomics, surgery and general medicine.

In this chapter, we discuss about various aspects of carbon nanotubes that have been successfully applied to bio-nanotechnology. We focus particularly on biological applications of carbon nanotubes, and take a comprehensive look at the advances in this fast-moving and exciting research field. We review the results on modifications of carbon nanotubes, and highlight some of the recent achievements in the fabrication and evaluation of carbon nanotube-based biological devices and implants.

12.2 Bio-Nanomaterials

Many nanomaterials have novel chemical and biological properties and most of them are not naturally occurring [12]. Carbon nanotubes (CNTs) are in the top list of artificial bio-nanomaterials [16–20], which has won enormous popularity in nanotechnology for its unique properties and applications. CNTs have highly desirable physicochemical properties for use in commercial, environmental and medical sectors. The inclusion of CNTs to improve the quality and performance of many widely used products, as well as potentially in medicine, will dramatically affect occupational and public exposure to CNT-based bio-nanomaterials in the near future.

Even since the discovery of carbon nanotubes, researchers have been exploring their potential in bio applications [21]. One focal point has been the development of nanoscale biosensor [22] and drug delivery systems [23] based on carbon nanotubes, which has been driven by the experimental evidence that biological species such as proteins and enzymes can be immobilized either in the hollow cavity or on the surface of carbon nanotubes [24]. Recently, hopes have been raised for the use of carbon nanotubes as superior biosensor materials in light of the successful fabrication of various electroanalytical nanotube devices, especially those modified by biological molecules [25]. These prototype devices, sometimes prepared as ordered arrays or single-nanotube transistors, have shown efficient electrical communications and promising sensitivities required for such applications as antigen recognition, [26] enzyme-catalyzed reactions [27] and

DNA hybridizations [28]. The CNT/hydroxyapatite composite coated [29] bio-implants has also received much attention recently, for the surface modification of implant materials to promote interaction with living bone tissues owing to its similar chemical composition and crystal structure as natural apatite in the human skeleton.

12.3 Carbon Nanotubes

12.3.1 Introduction

The discovery of carbon nanotubes [30] in 1991 has stimulated significant scientific interest and research leading to rapid progress in the field. Since their discovery, enormous research have been focused on the problems of synthesizing nanotubes, on their physical properties and on possible applications in nanoelectronics [31–35], catalysis [36–38] and other fields including bio-applications [39–45]. The highly impressive structural, mechanical, and electronic properties such as small size and mass, high strength, higher electrical and thermal conductivity, etc. are some of the fascinating properties of this remarkable material that are ideal for various potential applications.

12.3.2 Synthesis

Carbon nanotubes can be manufactured using a variety of methods that includes (Figure 12.1): (i) Laser ablation [46] uses a high-power laser to vaporise a graphite source loaded with a metal catalyst (Figure 12.1a). The carbon in the graphite reforms as predominantly single-wall nanotubes on the metal catalyst particles. (ii) Arc discharge [47] involves an electrical discharge from a carbon-based electrode in a suitable atmosphere to produce both single and multi-wall tubes of high quality but in low quantities (Figure 12.1b). (iii) Chemical vapour deposition (CVD) [48], where a hydrocarbon feedstock is reacted with a suitable metal-based catalyst in a reaction chamber to grow CNTs (Figure 12.1c) which are subsequently removed from the substrate and catalyst by a simple acid wash.

The laser-vaporization method is widely used for the production of single walled (SW) CNTs. The laser is suitable for materials with

a high boiling temperature, such as carbon, as the energy density of lasers is much higher than that of other vaporization devices. The basic principle of this method is as follows: a CO_2 laser beam is introduced onto the target (carbon composite doped with catalytic

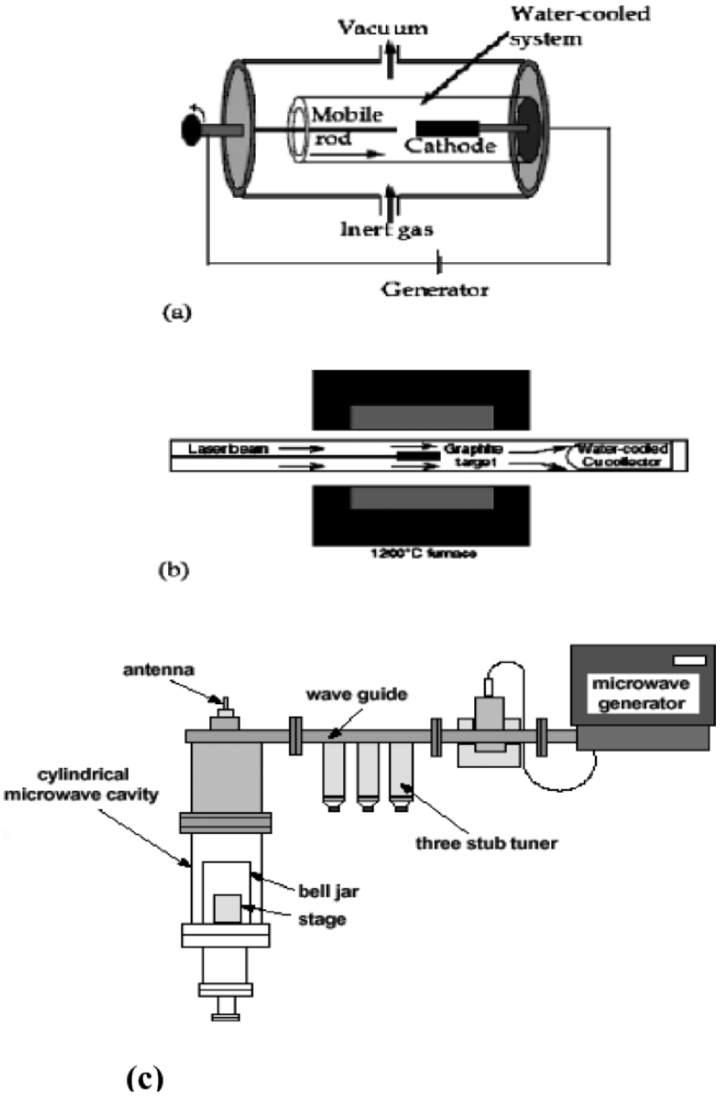

Fig. 12.1. Methods of manufacture for carbon nanotubes: (a) laser ablation; (b) arc discharge; and (c) microwave chemical vapor deposition

metals) located in the center of a quartz tube furnace; the target is vaporized in a high-temperature argon atmosphere and SWCNTs are formed; and, the SWCNTs produced are conveyed by the gas to a special collector. The method has several advantages, such as the high quality of the diameter and controlled growth of the SWCNTs. The change of the furnace temperature, catalytic metals and flow rate directly affect the SWCNT diameter [49].

Large-scale synthesis of Multiwalled (MW) CNTs by arc-discharge was reported [50] in a helium, argon, and methane atmosphere. It was found that methane is the best gas for the synthesis of MWCNTs. This is due to the thermal decomposition of methane producing hydrogen that achieves higher temperature and activity compared to inert gases, such as Helium or Argon. The hydrogen is also found to be an effective factor in the synthesis of MWCNTs [51–53]. The drawback of arc-discharge method is purification of CNTs. Removal of non-nanotube carbon and metal catalyst material in as-grown CNTs is much more expensive than production itself.

The first two methods, arc-discharge and laser furnace, also have the drawback that they do not allow control of the location and the alignment of the synthesized CNTs. CVD is suggested as an alternate method which uses hydrocarbon vapor (e.g., methane) that is thermally decomposed in the presence of a metal catalyst and CNTs are deposited directly on desired substrate.

12.3.3 Structure and Properties

A CNT can be regarded as one gigantic carbon molecule obtained by folding graphite planes into a cylinder (Figure 12.2) whose diameter is measured in nanometers and whose length can reach macroscopic dimensions [54].

This linear structure determines the extremely high mechanical strength of CNT [55] whereas their electrical conductivity depends strongly on the diameter and the helicity that is the angle between the most highly packed chains of atoms and the axis of the cylinder [56].

Two types of CNTs exist: (i) whose walls contain a single layer of carbon atoms, SWCNTs [57] and (ii) nanotubes with walls consisting of several concentric cylindrical graphite layers, MWCNTs [58] as illustrated in Figure 12.3. The hexagonal lattice structure of

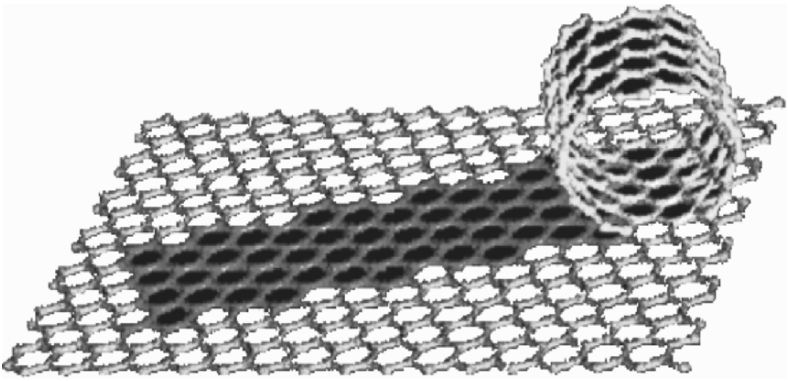

Fig. 12.2. Folding of graphite sheets to form a carbon nanotube

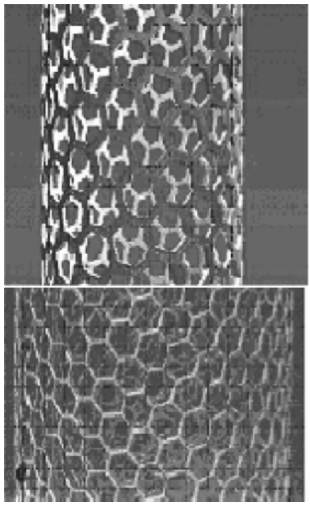

Fig. 12.3. Nanotubes with walls consisting of several concentric cylindrical graphite layers known as multi-walled carbon nanotubes (MWCNTs)

CNTs, gives rise to three types of SWCNTs and their diameter varies between 0.4 and 2 nm. Based on the unit cell of a CNT (Figure 12.4), it is possible to identify armchair nanotubes, formed when n=m and the chiral angle is 30; zig-zag nanotubes, formed when either n or m are zero and the chiral angle is 0; and chiral tubes, with chiral angles intermediate between 0 and 30. SWNTs are either metallic or semi-conducting depending on their diameter and helicity. All armchair nanotubes are metallic, while zig-zag and chiral nanotubes can be metallic or semiconducting.

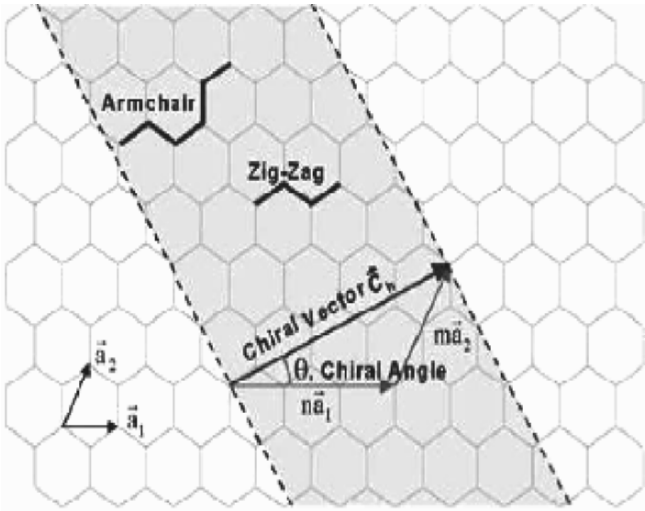

Fig. 12.4. Unit cell of a carbon nanotube

12.3.4 Applications

There is a wealth of potential applications for CNTs [59–62] due to their extraordinary properties [63–66]. They are probably the best electron field-emitter possible. They are polymers of pure carbon and can be reacted and manipulated using the tremendously rich chemistry of carbon. This provides opportunity to modify the structure and to optimize solubility and dispersion. Very significantly, CNTs are molecularly perfect, which means that they are free of property-degrading flaws in the nanotube structure. These extraordinary characteristics give CNTs potential in numerous applications including electronic, mechanical, chemical, thermal and biological applications. The electrical properties of single wall carbon nanotubes are highly sensitive to surface charge transfer and changes in the surrounding environment as the walls of nanotubes constitute a monolayer of atomic arrangement. Due to their surface sensitivity, surface charge mechanisms can cause covalent/non-covalent interactions and van der Waals forces to induce sufficient change in their electronic properties and local density of states. The diversity of available chemistries and easiness of modification makes CNTs viable candidates for

biological applications. The biological assembly of CNTs can be attained by their manipulation, dispersion and separation.

12.3.4.1 CNTs as Biosensors

The first application of CNTs for biosensors was proposed in 1996 by Britto et al. [67]. Later, the study by A.Star et alia, on SWNTs have shown to exhibit a significant change in response to the presence of small biomolecules and proteins [68]. The adsorption of cytochrome c, a redox catalyst in the respiratory chain of mitochondria, has been detected in situ using a SWCNT device [69]. Biotin-modified SWCNTs have been used to electronically detect biotin-streptavidin binding [68]. It has been demonstrated that the binding of streptavidin to biotin-functionalized SWCNTs results in a reduced conductance of the carbon nanotubes. Although the mechanism of chemical sensing exhibited by SWNTs has not been unequivocally identified, it seems probable that the resistance changes experienced by these devices originate from the doping of the carbon nanotubes as a result of charge transfer processes that are associated with interactions between the SWCNTs and the analyte. Nevertheless, the interpretation of the electrical responses in thin film devices is complicated by the nature of carbon nanotube networks that are a mixture of bundled semiconducting and metallic SWCNTs.

In some cases the conductance change originates from electronic effects occurring at the metal-nanotube contacts during adsorption. Despite the absence of a definitive understanding of the sensing mechanism, remarkable achievements in electrical biosensing have been reported [70,71]. Covalent coupling of the alkaline phosphatase (ALP) enzyme to CNTs has lead to the highest sensitivity (detection limit of 1 pg L^{-1}) reported thus far for electrical detection of DNA. This CNT-ALP-linked assay can be modified for antigen detection by using specific antibody-antigen recognition. Thus, it could provide a fast and simple solution for molecular diagnosis in pathologies where molecular markers exist, such as DNA or protein [72].

Demand for the reliable monitoring of blood glucose has stimulated further research on the development of biosensors based on CNTs. The voltametric behavior of oxidized SWCNTs with physically adsorbed glucose oxidase has been investigated [73]. The magnitude of the

catalytic response to the addition of D-glucose was 10-fold greater than that observed with a glassy carbon electrode. Further improvements in sensitivity and temporal resolution were made by using glucose oxidase-functionalized individual SWCNTs in a (Field emission transistor) FET configuration which allowed for the measurement of enzymatic activity at the level of a single molecule.

Enzyme immobilization is central to bioreactor and biosensor technologies. The current immobilization methods include covalent binding and physical adsorption of enzymes on high surface area materials (carbon silica and polymers). The first step in enzyme immobilization on CNTs is to create active sites on their stable walls. The immobilization of antibodies on the sensor platform to convert a non-electrical, physical or chemical, quantity into an electrical signal is the key for the control and the improvement of the performance of such a biosensor. Several immobilization methods have been reported for the improvement of the anti- body –antigen binding to increase detection sensitivity or for covalent binding of antibody or protein on solid surface [74]. T.S. Huang et alia studied the antibody immobilization on the surfaces of various oxidation processed nanodiamond and carbon nanotubes [75].

Electron transfer in biological systems is one of the leading areas of biochemical and biophysical sciences, and has received more and more attention [76–83]. The direct electron transfer of enzymes with electrodes can be applied to the study of enzyme-catalyzed reactions in biological systems and the development of an electro- chemical basis for the investigation of the structure of enzymes, mechanisms of redox transformations of enzyme molecules and metabolic processes involving redox transformations. Enzyme-modified electrodes provide a basis for constructing biosensors, biomedical devices, and enzymatic bioreactors. If an enzyme immobilized on an electrode surface is capable of direct electron transfer and keeping its bioactivity, it can be used in biosensors without the addition of mediators or promoters onto the electrode surface or into the solution. Unfortunately, it is difficult for an enzyme to carry out a direct electrochemical reaction due to several factors. For example, enzymes would be adsorbed on the electrode surface, resulting in the de-naturation, and loss of their electrochemical activities and bioactivities. In addition, usually, the larger three-dimensional structure of enzymes and the resulting

inaccessibility of the redox centers have made it generally difficult to obtain direct electron transfer between enzymes and electrode surfaces, so that promoters and mediators are needed to obtain their electrochemical responses. Therefore, suitable electrode materials and immobilization methods of enzymes onto the electrode surface are important for obtaining their direct electrochemical reaction and keeping their bioactivities.

For the immobilization and/or modification of cells, there are mainly two types of interface interactions being used between a substrate and cells; one is chemical modification of substrate surface to have high affinity to cells, and the other is attaching biomolecules on substrate to recognize the cells [84]. Since the pioneering work of Decher [85], there has been great interest in using the layer-by-layer immobilization of polyelectrolytes for the development of biosensors [86].

Since carbon nanotubes show good electric conductivity, they have been used to modify electrodes and catalyze various biomolecules electrochemically [87]. Direct electrochemistry of redox proteins may provide a model for the study of electron transport of enzymes in biological system [88] and establish a foundation for fabricating a new generation of electrochemical biosensors without using mediators [89]. The CNT has been demonstrated as biochemically compatible, electrically conductive nano-scale interface between redox enzymes and macro-scale electrodes [90]. CNTs are capable of maintaining the functional properties of redox enzymes while linking biomolecules into nanoelectronic platforms. Guiseppi-Elie et al. [91] have developed biosensors of exceptional sensitivity by exploiting the efficiency and specificity inherent in redox proteins. The direct electron transfer of redox enzymes to an electrode surface of CNT has been reported by Cai and Chen also [92]. A relatively new approach to realize direct electrochemistry of proteins is to incorporate proteins into films modified on surface of solid electrodes [93]. Li and co-workers [94] reported direct electrochemistry of cytochrome c (Cyt c) in SWCNT films cast on glassy carbon (GC) electrodes.

Several other important investigations have also been directed to the attachment of natural proteins and DNA immobilization onto MWCNT to construct biosensors. Davis et al. [95] have reported the high surface area possessing multiply acidic sites that may make an

offer of special opportunities for the immobilization of enzymes. MWNTs can be activated in acid oxidation conditions due to the residues such as –COOH, –COH, and –OH introduced on the surface of MWNTs [96,97]. The schematic of self-assembly of DNA probes onto MWCNTs described by them are illustrated in Figure 12.5. Shim et al. [98] have described that BSA can be covalently attached to SWNTs and MWNTs by way of diimide-activated amidation under ambient conditions, while the majority of the protein in the nanotube–BSA conjugates remain bioactive. CNTs have been used as modified electrodes to catalyze the electrochemical reaction of some biomolecules, such as dopamine, β-nicotinamide adenine dinucleotide (NADH), cytochrome *c*, etc., [99].

GC electrodes, in which they dipped the MWCNT electrodes into Mb solution and Mb was absorbed into MWCNT films. A reversible CV peak pair of catalase in SWCNT films cast on gold (Au) electrodes and used the films to electrochemically catalyze reduction of hydrogen peroxide was also observed by them. Cai et al. [104] coated a mixture of hemoglobin (Hb), glucose oxidase (GO_X), or horseradish

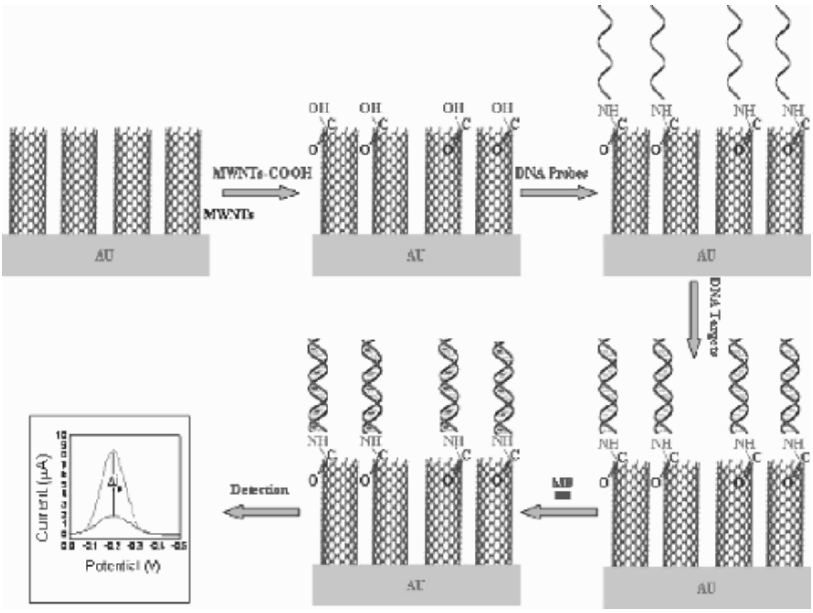

Fig. 12.5. Schematic diagram of self-assembly of DNA probes to MWCNTs

peroxidase (HRP) solution with carbon nanotube dispersions onto GC electrodes. After drying, the films demonstrated good direct electrochemistry of Hb, GO_X, or HRP in blank buffers. Rusling and coworkers [105] covalently attached Mb or HRP onto the ends of vertically oriented SWCNT forest arrays assembled on pyrolytic graphite (PG) electrodes. Quasi-reversible heme Fe(III)/Fe(II) CV response of Mb or HRP at this electrode was observed. There have also been suggestions of using nano-diamond [106] as the signal transducer for glucose sensing enzyme due to the biocompatibility and chemical robustness of these systems.

12.3.4.2 Processibility

A major drawback of CNTs particularly relevant to biological applications is their complete insolubility in all types of solvents. However, CNTs have been found to exhibit a certain degree of chemical reactivity towards many reagents, thus leading to increased solubility and processability. An aqueous medium is highly essential in order to study CNTs in the presence of live cells and therefore the solubilization of CNTs in aqueous solutions is the focus of biological research. Several strategies have been developed to introduce carbon nanotubes into solvent systems, including dispersion and suspension under special experimental conditions and the chemical modification and functionalization. The well-dispersed and solubilized carbon nanotubes make it possible to characterize and study the carbon nanotubes by using solution-based techniques, to realize some of the unique properties of the nanotubes, and to carry out further chemical transformations. The recent bloom of chemical modification and functionalization methods 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. Equally important is the recent experimental demonstration that biological and bioactive species such as proteins, carbohydrates, and nucleic acids can be conjugated with carbon nanotubes. These nanotube bioconjugates will play a significant role in the research effort toward bioapplications of carbon nanotubes.

Chemical functionalization of CNTs has been shown to impart solubility in a variety of solvents, to modify their electronic properties

and to cause significant de-bundling. The chemical reactivity of CNTs arises from the curvature-nduced strain due to misalignment of the π-orbitals of adjacent conjugated carbon atoms. The induced strain is higher at the carbon atoms that comprise the CNT caps because they are curved in two-dimensions, and therefore the caps are more reactive than the sidewalls. Hence, treatment in strong oxidizing agents such as HNO_3 or H_2SO_4 preferentially disrupts the aromatic ring structure at the caps of CNTs and introduces carboxylic acid groups that undergo further chemical reactions. Thus, SWNT-COOH are produced by refluxing arc discharge produced SWCNTs in HNO_3 or H_2SO_4. Numerous amidation and esterification reactions of acid functionalised SWCNTs have been reported [107]. In addition to the chemistry that occurs at the oxidized open ends of SWCNTs, it is also possible to react the side- wall carbon atoms with highly reactive reagents, such as carbenes, fluorine, aryl radicals and azomethineylides. Furthermore, the surface chemistry developed for SWCNTs has been applied to MWCNTs in specific cases. End [108] and/or sidewall [109] functionalization, use of surfactants with sonication [110], polymer wrapping of nanotubes [111], and protonation by superacids [112] have been reported. Although acid treatment methods for CNT functionalisation are quite successful, they often indicate cutting the CNTs into smaller pieces (sonication and/or functionalization), thus partly losing the high aspect ratio of SWCNTs. J.E. Riggs et al., have shown, it is possible to solubilize carbon nanotubes in aqueous solutions by covalently attaching water soluble linear polymer [114]. By applying the preceding functionalization scheme, poly-m-aminobenzene sulphonic acid (PABS), has been covalently linked to SWCNTs to form a water soluble nanotube-graft copolymer [115], which could be used for future biological applications.

Titus et al., reported the effective functionalisation of CNT using a novel surfactant and its performance of dispersion of CNT in Poly Vinyl Alcohol (PVA) medium [116]. The as-grown CNTs synthesized using microwave plasma (MP) CVD method was in bundle form (Figure 12.6) and bundles were dispersed effectively in nanodisperse surfactant by ultrasonication (Figure 12.7). The surfactant functionalisation promoted further unbundling of CNT in PVA medium. Titus et al. have also reported the attachment of COOH group onto CNT in non-aqueous medium by metal CVD process [117]. The advantage

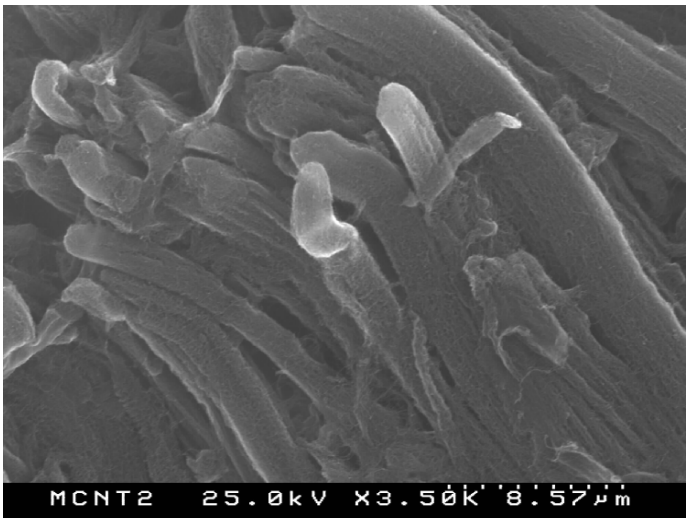

Fig. 12.6. As-grown CNTs in bundle form synthesized using the microwave plasma (MP) CVD method

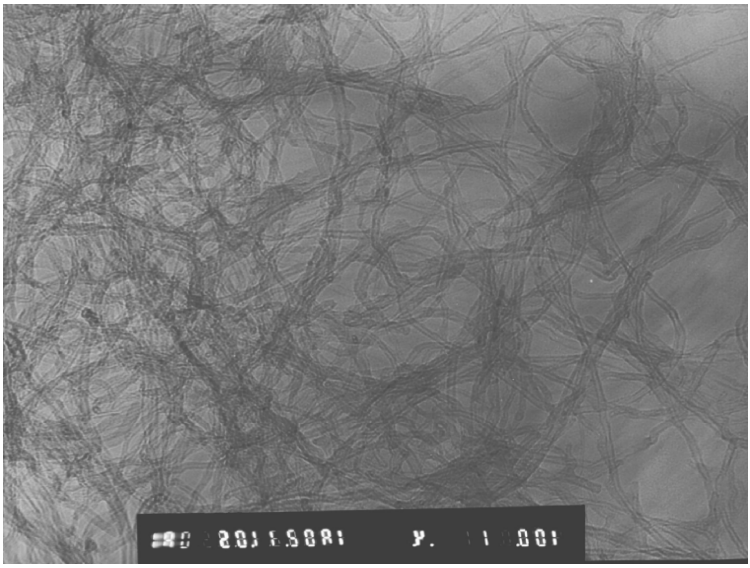

Fig. 12.7. Bundles of CNTs dispersed effectively using a nanodisperse surfactant by ultrasonication

of this method is the formation of high population density of carboxyl groups along the walls of CNT compared to the aqueous method.

A carboxylic acid groups forms an overall electrophylic surface that can minimizes hydrophobic adsorption of biological molecules like protein. In addition, the wall thickness (20–30Å) of high-curvature MWCNT, is dimensionally compatible with typical dimensions of proteins (e.g., GO_x , 60 Å × 52 Å × 77Å). This presumably reduces potential non-specific binding regions, minimizing surface denaturation effects [119].

Biological functionalization of CNTs has come to be of significant interest also due to the possibility of developing sensitive and ultra-fast detection systems that can be addressed using electronic or optical techniques [120]. Most often, biological sensing techniques depend on optical signals derived from the analytes in use, thus involving a series of steps for preparation, varying reagents to differentiate components, and a relatively large sample size. Although these techniques are relatively sensitive, they result in complex data analysis involving unnecessary time consumption and expensive examination techniques. Miniaturizing processes in biological sensing could result in lowering sample size, time and expenses related to detection and sensing. CNTs functionalized with biological assays could be the key to novel nano-biosensing techniques. Several issues are important regarding functionalization of biomaterials on solid-state nanomaterials such as biocompatibility, specificity to the target biomolecule, extent of functionalization, interface effects and the corresponding sensor performance.

12.3.4.3 Fabrication

One of the key focuses of biosensor research is the need to fabricate bio-electrodes which exhibit high selectivity, high-sensitivity and long-term stable response to bioanalytes. Researchers have demonstrated that CNTs have a high electrocatalytic effect, a fast electron-transfer rate, and a large working surface area [121]. CNTs have been used for preparing biosensors employing different strategies: by dispersing them in acidic solutions [122], *N*,*N*_-dimethylformamide [123], Nafion [124] and chitosan [125] among others; by incorporating in composites matrices using different binders like Teflon [126], bromoform [127], mineral oil [128] and inks [129]; by immobilizing on pyrolitic graphite electrodes [130]. Wang et al. [131] have

demonstrated the ability of the perfluorosulfonated polymer Nafion to disperse single wall (SWCNTs) and multi-wall (MWCNTs) carbon nanotubes. They reported a dramatic decrease in the overvoltage for hydrogen peroxide oxidation and reduction as well as highly selective glucose quantification after immobilization of glucose oxidase (GO_x) by cross-linking with glutaraldehyde.

Various chemical sensors and biosensors based on CNTs have been developed to detect some important species that are related to human health, such as glucose, NADH, ascorbic acid, and cytochrome C [132]. Significant research and development efforts have been devoted to producing CNT-based glucose chemical-and biosensors for in vitro or in vivo applications because of the importance of monitoring blood glucose for the treatment and control of diabetes [133–136]. The measurement principle of CNT-based electrochemical glucose sensors relies on the direct measurement of the oxidation current of glucose on the CNT surface [137] or the immobilization of glucose oxidase on the CNT surface to detect the redox current produced by the enzymatic product H_2O_2 [138]. Ye et al. [139] reported non-enzymatic glucose detection using a well-aligned multi-wall CNT electrode in an alkaline medium. The oxidation over-voltage of the glucose on CNT electrodes was reduced 400 mV compared to that on a glassy carbon electrode. The direct oxidation of glucose on the CNT surface avoids the use of glucose oxidase and over comes the problem of the sensors' life and stability; however, the interference from other electroactive species, such as uric acid and ascorbic acid, still exists. Most of the CNT based electrochemical glucose biosensors are based on glucose oxidase (GO_x), which catalyzes the oxidation of glucose to gluconolactone:

$$\text{Glucose} + O_2 \xrightarrow{GO_x} \text{Gluconolactone} + H_2O_2$$

The quantification of glucose can be achieved via electro- catalytic redox detection of the enzymatic product H_2O_2 on the CNT transducer at reduced oxidation or reduction over voltage. Here, CNTs play multiple roles: (1) a substrate to immobilize GO_x, (2) electrocatalytic oxidation or reduction of H_2O_2 at the CNT surface to reduce over-voltage and avoid interference from other co-existing electroactive species, and (3) an enhanced signal because of its fast electron transfer and large working surface area.

The approaches, such as covalent binding [140], direct adsorption [141], and entrapment [142] has also been widely used to construct GO_x/CNT biosensors. A drawback to physical adsorption and entrapment is that the distribution of enzyme molecules is not uniform, is sometimes unstable, and tends to leach with time. Covalent binding of GO_x on the functionalized CNT needs a relatively longer reaction time. The ideal immobilization method should employ mild chemical conditions and a short immobilization time to allow for large quantities of enzyme to be immobilized, provide a large surface area for enzyme –substrate contact within a small total volume, minimize barriers to mass transport of substrate and product, and provide a chemically and mechanically robust system.

12.3.4.2 Carbon Nanotubes for Neuronal Growth

Neurons are electrically excitable cells that on network formation serve as conduits for information transfer. A vast amount of information is transferred through the cells in the spinal cord via synaptic and gap junctions in an electroionic fashion mediated by neurotransmitters. The growth of neurites and formation of synapses during development and regeneration is controlled by a highly specialized motile structural specialization at the tip of the neurite called the growth cone. Carbon nanotubes (CNT) are strong, flexible and conduct electrical current. They are biocompatible and non-biodegradable. They can be functionalized with different biomolecules like neuron growth factors and adhesion agents. These properties are useful in the formation of neuron hybrids [143]. These capabilities of carbon nanotubes make them potentially successful candidates to form scaffolds to guide neurite outgrowth.

Xuan Zhang et al., established the ability of the growth cone to grasp onto carbon nanotube matrices functionalized with neuron growth factors [144]. The need, however, is the ability to guide the formation of neuronal networks and establishment of synaptic connections essential for signal transmission leading to re-generation. Their latest research shows the ability of forming highly directed neural networks in vitro over patterned nanotube substrates (Figure 12.8). The nanotubes not only function as scaffolds for the neurons, but the patterned

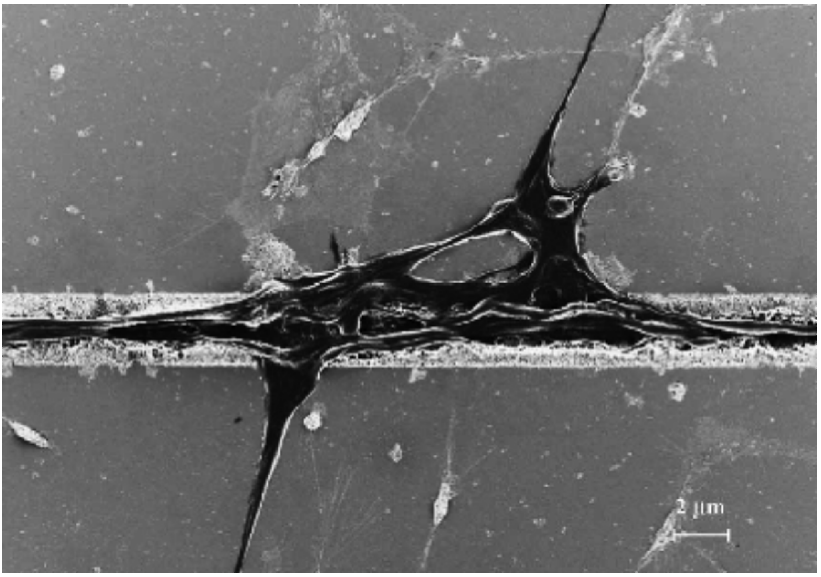

Fig. 12.8. Highly directed neural networks in-vitro over a patterned nanotube substrate

boundary serves as markers for directing the growth and network formation. A number of patterns of vertically aligned multiwalled carbon nanotube (MWNT) substrates are used to determine the geometry and NT length most suitable for scaffolding purposes. Surface characterization is performed using scanning electron microscopy. The interaction between the neuron membrane and the CNT scaffold is also visually analyzed to obtain an insight into network formation in in vitro conditions. This is an essential pre-requisite in forming three-dimensional scaffolds.

The growth of cells and neurons on carbon nanotube films also have been reported [145]. The two-dimensional network of aligned nanotube arrays are proposed to be a good substrate for the cell growth. Elena Bekyarova et al. [146], demonstrated the growth of cultured hippocampal neurons on MWCNTs deposited on poly- ethyleneimine-coated coverslips. SEM was used to identify the morphological changes of neuron growth brought about by the presence of the MWCNTs. The neuronal bodies were found to adhere to the surface of the MWCNTs with their neurites extending through the bed of CNTs and elaborating into many small branches (Figure 12.9). The

neurons remained alive on the nanotubes for at least 11 days, and it was shown that the physisorption of 4-hydroxynonenal on the MWNTs enhanced both neurite outgrowth and branching.

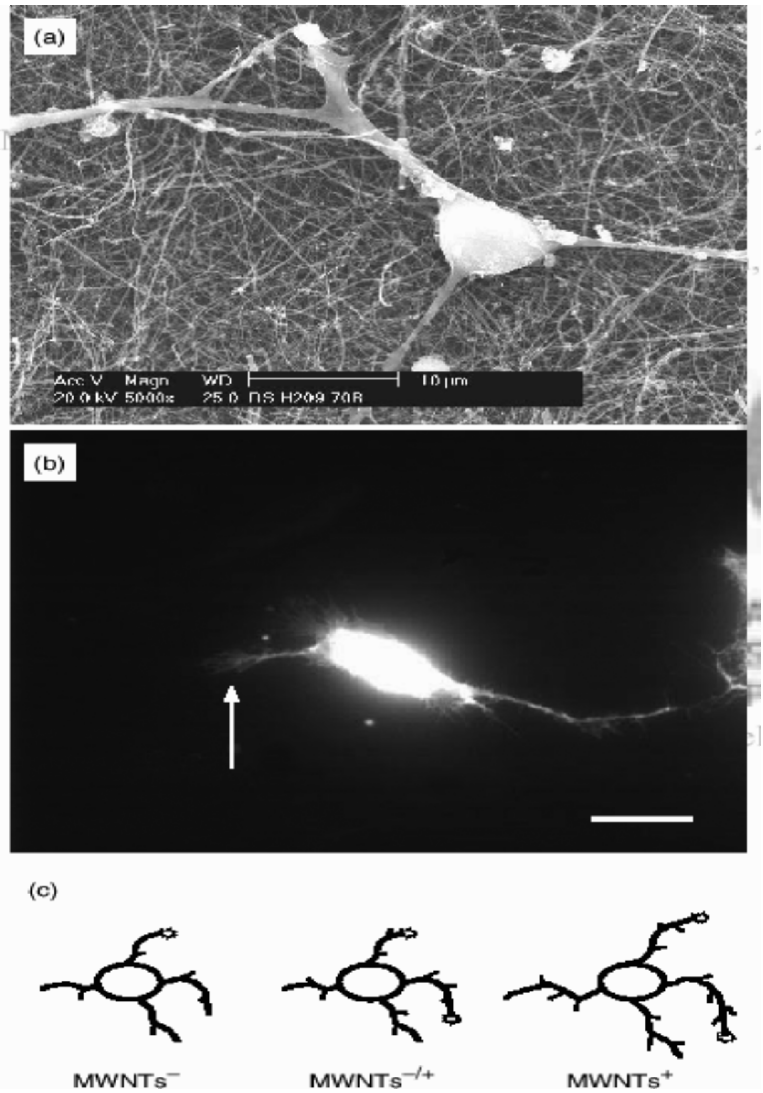

Fig. 12.9. (a) Neuronal bodies found to adhere to the surface of the MWCNTs with their neurites extending through the bed of CNTs (b); and (c) elaborating into many small branches

12.3.4.3 Drug Delivery by CNTs

The diversity of available chemistries and cell-penetrating structures makes CNTs viable candidates as carriers for the delivery of drugs, DNA, proteins and other molecular probes into mammalian cells [147]. An important issue in intracellular drug delivery is the poor permeability of the plasma membrane to many drugs. Thus, various carriers, including polyethylene glycol, peptides and lipids, have been developed to facilitate the cellular entry of drugs. One of the prerequisites for such a task is therefore the ability of the carrier to bind to biologically relevant molecules [148].

The feasibility of using SWCNTs for intracellular drug delivery has been demonstrated [149]. Water soluble SWCNTs were functionalized with a fluorescent probe, FITC, to allow tracking of SWCNTs. When murine and human fibroblast cell lines were exposed to SWCNT-FITC, the nanotubes could be shown to accumulate within the cells. Similarly, SWCNTs, covalently functionalized with biotin and reacted with streptavidin, were internalized within human promyelocytic leukemia (H60) cells, human T cells, Chinese hamster ovary (CHO) and 3T3 fibroblast cell lines.

While the mechanism of the CNT cell entry remains undelined, these experiments suggest the viability of CNTs as carriers for delivering relatively large molecules to mammalian cells.

MWCNTs are also demonstrated in drug delivery. F. Balavoine et al., reported the interactions between MWCNTs and proteins and revealed the self-organization of streptavidin molecules and the growth of its helical crystals on the CNT surface [150]. Similarly, DNA molecules may be adsorbed on MWCNTs, and small protein molecules, such as cytochrome c and -Lactase I, can be inserted within the interior cavity of open CNT. CNTs have also been used to deliver proteins and peptides inside a cell [151] by the direct covalent bonds between a CNT and biomolecules formed by attaching functional groups via acidic treatments [152]. As an alternative to the binding of molecules to the outside of the CNTs, it would also be convenient to fill the interior cavity of tubes, whose open ends might be capped to generate a nanopill containing a drug for delivery to the cell. Towards that end, a template method has been used to synthesize nano test tubes, which are CNTs with one end closed and the other open. Such an approach might be construed as a first step toward the development of a nanopill,

in which the substance to be delivered is introduced into the interior of the nanotube and then bottled by resealing the open end.

12.3.4.4 Biomedical Implant Applications of CNT

The primary issues in materials science of new bone biomaterials are mechanical properties and biocompatibility. Although mechanical properties of biomaterials have been well characterized, the term biocompatibility is only a qualitative description of how the body tissues interact with the biomaterial within some expectations of certain implantation purpose and site [153]. Materials scientists have investigated metals, ceramics, polymers and composites as biomaterials. The general criteria for materials selection for bone implant materials are:

- It is highly biocompatible and does not cause an inflammatory or toxic response beyond an acceptable tolerable level.
- It has appropriate mechanical properties, closest to bone.
- Manufacturing and processing methods are economically viable.

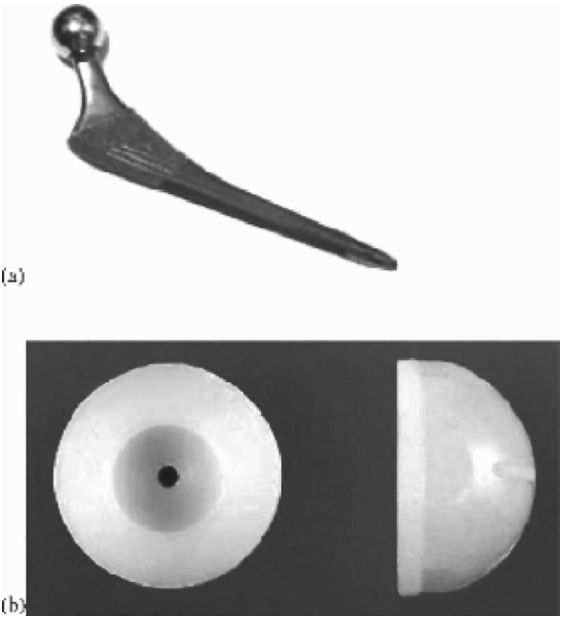

Fig. 12.10. Artificial hip implant fabricated from titanium alloy: (a) artificial hip implant; and (b) femoral head manufactured from UHMWPE

The hip joint consists of two complementary articular surfaces separated by articular cartilage and the synovial fluid that has a pH between 7.29 and 7.45. Excessive wear of the interfaces due to degenerative disease (such as osteoarthritis) or injury requires a replacement of the entire hip joint. Historically, a total hip replacement the articulation of a human hip is simulated with the use of two components, a cup type and a longfemoral type element [154 K.S. Katti, Colloids and surfaces]. A typical hip implant fabricated from titanium is shown in Figure 12.10.

The head of the femoral element fits inside the cup to enable the articulation of the human joint. These two parts of the hip implant have been made using a variety of materials such as metals, ceramics, polymers and composites. Typically polymeric materials alone tend to be too weak to be suitable for meeting the requirement of stress deformation responses in the THR components. Metals typically have good mechanical properties but show poor biocompatibility, cause stress shielding and release of dangerous metal ions causing eventual failure and removal of implant. Ceramics generally have good biocompatibility but poor fracture toughness and tend to be brittle. A hip implant therefore should be such that it exhibits an identical response to loading as real bone and is also biocompatible with existing tissue. The average load on a hip joint is estimated to be up to three times body weight and the peak load during other strenuous activities such as jumping can be as high as 10 times body weight. In addition hipbones are subjected to cyclic loading as high as 10^6 cycles in 1 year [154]. The compatibility issue involves surface compatibility, mechanical compatibility and also osteocompatibility. These materials are also classified as bioactive (illicit a favorable response from tissue and bond well), bioinert and biodegradable. The commercial metallic total hip replacement (THR) implants are five to sixtimes stiffer than bone and result in significant problems associated with stress shielding. Titanium (Ti) alloys in the femoral elements of the THR have shown improvement in wear properties [155]. The regenerative and remodeling processes in bone are directly triggered by loading, i.e., bone subjected to loading or stress regenerates and bone not subjected to loading results in atrophy. Thus, the effect of a much stiffer bone implant is to reduce the loading on bone resulting in the phenomenon called as stress shielding. The key problems associated with the use

of these metallic femoral stems are thus release of dangerous particles from wear debris, detrimental effect on the bone remodeling process due to stress shielding and also loosening of the implant tissue interface. It has been shown that the degree of stress shielding is directly related to the difference in stiffness of bone and implant material [156]. Ti alloys are favorable materials for orthopedic implants due to their good mechanical properties. However, Ti does not bond directly to bone resulting in loosening of the implant. Undesirable movements at the implant-tissue interface results in failure cracks of the implant.

One approach to improving implant lifetime is to coat the metal surface with a bioactive material that can promote the formation and adhesion of hydroxyapatite, the inorganic component of natural bone [157]. The application of bioactive coatings to Ti-based alloys enhance the adhesion of Ti-based implants to the existing bone, resulting in significantly better implant lifetimes than can be achieved with materials in use today. Typically, several silicate glasses are used as bioactive coatings [158]. Some ceramic coatings are known to be bioactive and have also been tested on Ti implants. As compared to metals, ceramics often cause reduced osteolysis and are regarded as favorable materials for joints or joint surface materials. Several ceramics due to their ease of processing and forming and superior mechanical properties were investigated as bone substitute materials [159].

Conventional ceramics such as alumina were evaluated due to their excellent properties of high strength, good biocompatibility and stability in physiological environments [160]. Alumina, because of the ability to be polished to a high surface finish and its excellent wear resistance, is often used for wear surfaces in joint replacement prostheses. Femoral heads for hip replacements and wear plates in knee replacements have been fabricated using alumina. In year 2003, the United States Food and Drug Administration (FDA) has approved alumina ceramic-on-ceramic articulated hips for marketing in the United States of America. Other ceramic materials have also been investigated for potential applications in orthopedics. Considerable research has focused on zirconia and yttria ceramics that are characterized by fine-grained microstructures. These ceramics are known as tetragonal zirconia polycrystals (TZP). TZP in the body have been limited by the low strength and low fracture toughness of the synthetic

phosphates. Alumina and titanium dioxide have been used as nanoceramics separately or in nanocomposites with polymers such as polylactic acid or polymethlyl methacrylate. The nanoceramic formulations promote selectively enhanced functions of osteoblasts (bone-forming cells). These functions include cell adhesion, proliferation, and deposition of calcium-containing minerals, an indication of new bone formation in a laboratory setting. Despite of many advantages, the lack of chemical bonding between sintered alumina and tissue, however limited its applications as a potential bone substitute to a certain extent. The other problems arise when attempting to coat metals with ceramics are: the thermal expansion coefficients of the ceramic and metal are usually different, and as a result, large thermal stresses are generated during processing. These stresses lead to cracks at the interface and compromise coating adhesion. In addition, chemical reactions between the ceramic and metal can weaken the metal in the vicinity of the interface, reducing the strength of the coated system.

Calcium hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is the principal calcium phosphate commonly used for biomedical implant applications. However, the high brittleness and poor strength of sintered hydroxyapatite (HA) restricts its clinical applications under load-bearing conditions and therefore coating of HA on metal implants is an alternate option. The excellent biocompatibility and osteointegration are the key characteristics of existing bioceramic hydroxyapatite coatings. Synthetic HA elicits a direct chemical response at the interface and forms a very tight bond to tissue [161]. Attempts have been made to form high strength consolidated HA bodies [162]. However, its poor mechanical properties such as low strength and limited fatigue resistance restrict its applications. Bending strength as high as 90 MPa has been achieved by colloidal processing of HA [163].

The first priority for the development of a better coating is therefore improvement of the interfaces (metal-coating and coating-bone interfaces) so that the coating binds well with both metal and bone. Thermal stresses, chemical reactions between coating and metal, and biocompatibility are the key issues to be considered. Although HA coverings are able to enhance bone ingrowth and reduce early loosening of hip and knee prostheses, the optimum coating quality and surface texture are still a matter open to debate. Moreover the significance of coating resorption is controversial. It has been suggested that resorption

disintegrates the coating and reduces the bonding strength between the implant and bone, and the strength of coating implant interface, which might lead to implant loosening, coating delamination and acceleration of third body wear process.

The extremely light-weight, extra strong and nano sized carbon nanotubes (CNTs) are highly recommended as an additional ingredient for the synthesis of HA coating. Carbon materials are known to be inert to cells and tissues because of their pure carbon composition. Some recent investigations indicated that carbon nanotubes may have promising potentials in biomedical applications both at molecular and cellular levels [164]. Functionalisation of CNTs are important in HA/CNT coating since chemical reactions can take place at functionalised sites on CNT in a colloidal state and it play a potential role

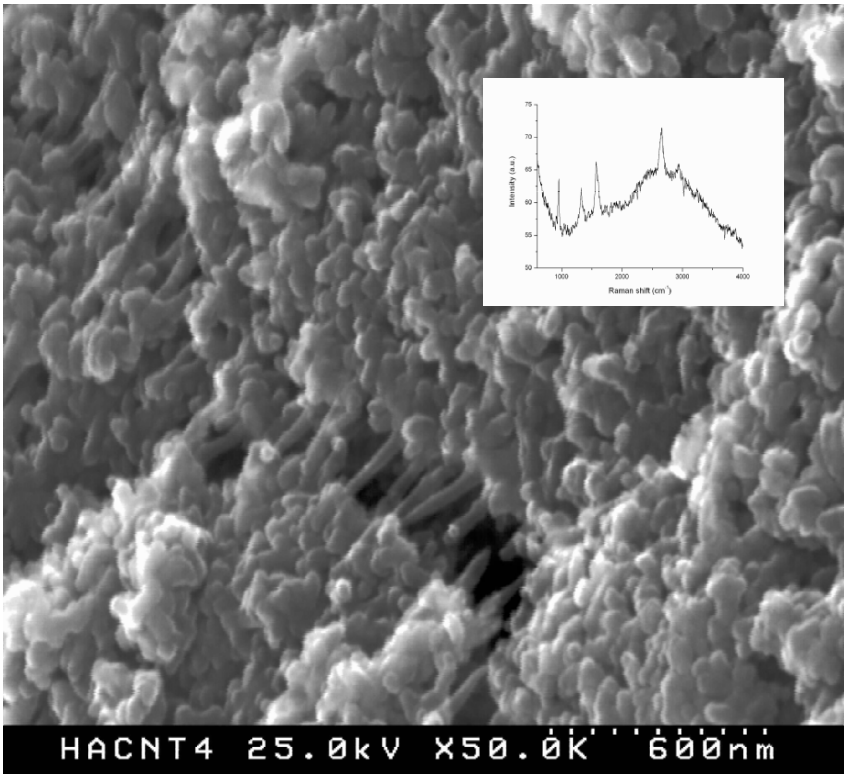

Fig. 12.11. SEM image of uniform distribution of CNT in HA matrix. The uniform distribution of CNT in HA matrix was confirmed using Raman analysis (inset)

in its uniform dispersion in composite media. E. Titus et al., achieved, uniform dispersion of CNT into HA matrix by ultrasonication and other novel methods. Figure 12.11 shows the SEM image of uniform distribution of CNT in HA matrix. The uniform distribution of CNT in HA matrix also was confirmed using Raman analysis (inset of Figure 12.11).

12.4 Analysis

The determination of structure function relationships in biological macromolecules is central to elucidating biochemical pathways, and thereby designing new drugs and understanding their mode of action. Structural biology has played and will continue to play a key role in these endeavors because bio-molecule function is closely tied to three-dimensional structures. The workhorse tools of structural biology, X-ray diffraction, electron diffraction and NMR, can almost routinely be used to determine atomic resolution structure of single bio-molecule. Continued advances in these methods are pushing the limits of the size and complexity of systems that can be characterized [165], although in the future, expanded needs for biomolecular structure analysis are expected in several areas, including: (i) increased throughput to characterize new gene products discovered by genomic DNA sequencing [166], (ii) routine analysis of multimeric protein, protein and protein-nucleic acid structures involved in, for example, signaling and gene regulation; and (iii) elucidation of dynamic processes in these multimeric systems. It is unlikely that the conventional structural tools will meet all of these needs, both because of the increased difficulty of crystallizing large signaling and regulatory protein complexes, which will limit diffraction methods, and the challenges of using solution NMR for large biomolecular systems [167]. Future progress in understanding complex processes in biological systems will therefore clearly require additional, perhaps revolutionary techniques for structural analysis.

AFM [168] is one technique with the potential to probe both structure and dynamics of large macromolecular systems, since it permits direct visualization of individual biological structures in vitro [169]. The potential for AFM to impact structural biology has been suggested

by beautiful images of, for example, two-dimensional arrays of proteins with 'sub-molecular resolution' [170], although such captivating data are not without limitations. These shortcomings, which if overcome could dramatically extend the applicability of AFM to structural biology, can be understood by reviewing the key features of an AFM (Figure 12.12).

Common to all AFMs are an integrated cantilever-tip assembly, a detector to measure cantilever displacement as the sample is scanned, and electronics to acquire and display images. The basic features of AFM and methods of imaging have been reviewed recently [171]. Central to reproducible high-resolution characterization of biological

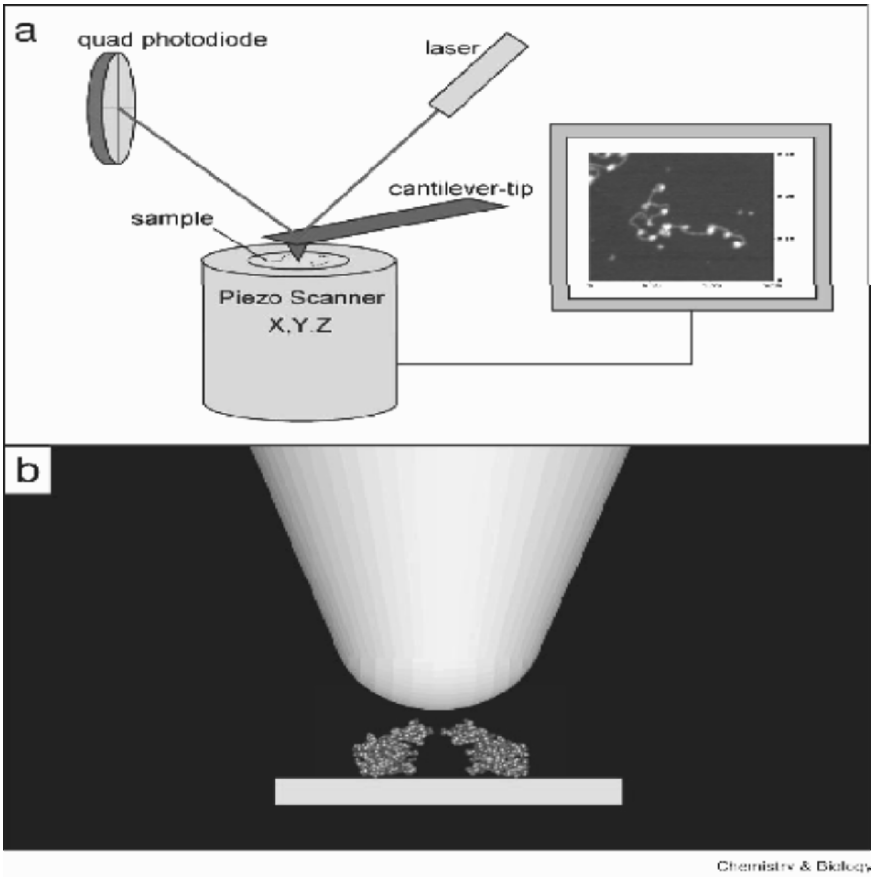

Fig. 12.12. Schematic diagram of the atomic force microscope

macromolecules with AFM especially new systems not crystallographically characterized is the size and shape of the probe tips used for imaging.

An ideal AFM probe tip should have (i) a sub-nanometer radius, (ii) a zero degree cone angle, (iii) mechanical and chemical robustness and, (iv) the potential for molecularly precise modification of the tip end. Moreover, it should be possible to prepare such tips reproducibly with the same features, such that the resolution and other imaging characteristics are predictable, as is the case in diffraction experiments.

The first demonstration of nanotube probes used mechanical mounting of bundles of MWCNTs onto standard AFM tips [172]. AFM studies with mechanically mounted MWCNT probes yielded only modest improvements in resolution on amyloid fibrils, protofibrils, and gold nanocluster standards with respect to standard Si tips [173]. In contrast, probes fabricated from etched SWCNT bundles, which occasionally have just a few SWCNTs protruding from the end, demonstrated up to five fold better resolution than conventional probes on inorganic nanostructures and DNA [174]. While these results indicated the potential for SWCNTs to enhance AFM resolution, tip radii were still 10 times larger than what would be obtained with a single 0.25 nm radius nanotube [175]. Moreover, the conceptual simplicity of mechanical nanotube tip fabrication is hampered by its difficulty in scale-up and by its intrinsic selectivity towards thicker nanotube bundles. Mechanical nanotube tip assembly in a scanning electron microscope (SEM) [176] allows assembly of somewhat smaller 10 nm diameter tips, but this method is even slower than mounting in an optical microscope. Thus, a different approach is needed for reproducible and scaleable fabrication of ultrahigh resolution nanotube tips. All of the problems associated with manual assembly can be solved by directly growing nanotubes on AFM tips using metal-catalyzed chemical vapor deposition (CVD). By carefully manipulating CVD reaction conditions and the catalyst, one can selectively produce SWCNTs [177] with radii as small as 0.35 nm [178]. Since carbon nanotube probes can provide enhanced imaging in diverse areas, they are particularly suited to the field of biology. The use of SWCNT probes will therefore improve the imaging resolution of small proteins, such as antibodies in DNA analysis. The widespread availability of nanosurgery involves the manipulation of single cells

or cell structures. The advantage of CNT AFM probes is that they can be easily functionalized, and the range of chemical groups that can be added specifically to the tip of a CNT make them an ideal high resolution probe for mapping chemical domains by using chemical force microscopy. Thus probes can be developed to sense polarity, pH and many other chemical characteristics of the sample by adding different residues to the CNT, as shown by studies using CNT AFM tips patterned with terminal carboxylic groups in which it was possible to chemically map the substrate by using tapping mode AFM (Figure 12.13). Figure 12.14 shows the oriented isolated CNTs grown directly on copper substrate by CVD method [Gil Cabral et al.]. The orientation and size of the CNTs were controlled by maintaining growth conditions.

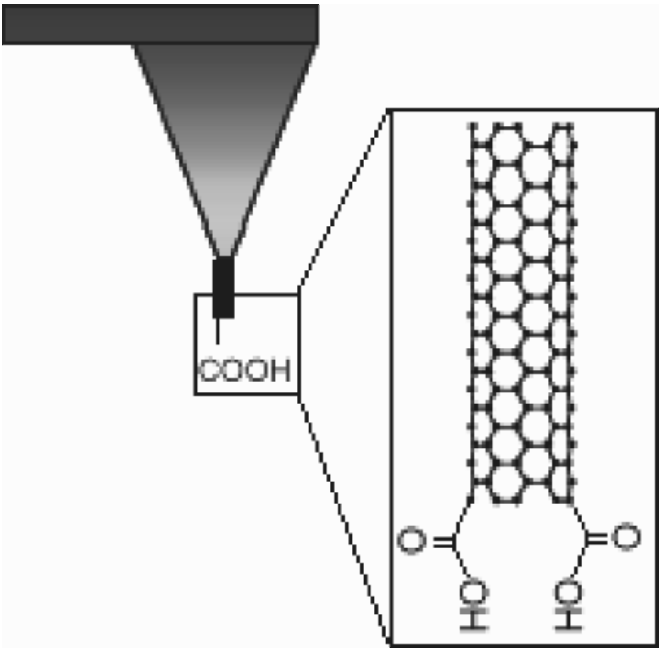

Fig. 12.13. AFM probes are developed to sense polarity, pH and many other chemical characteristics of the sample by adding different residues to the CNT. It is possible to chemically map the substrate by using tapping-mode AFM

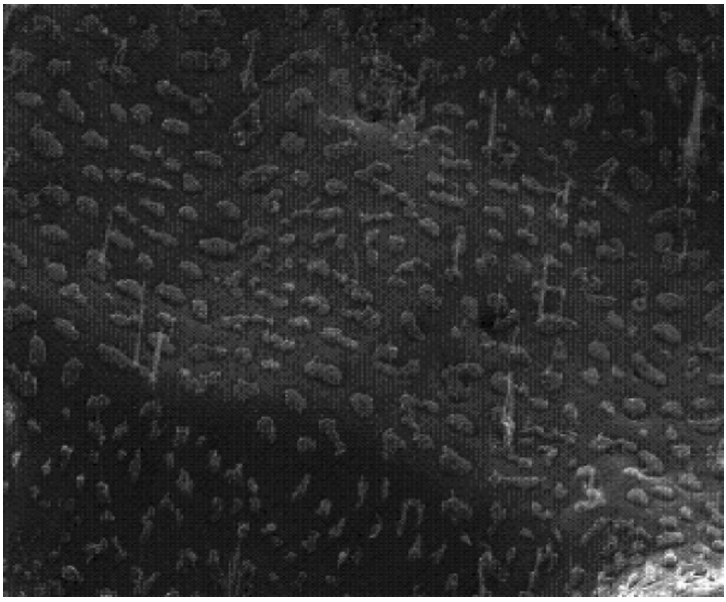

Fig. 12.14. Oriented isolated CNTs grown directly on a copper substrate by the CVD method. Maintaining strict growth conditions controls the orientation and size of the CNTs

12.5 Toxicity of Carbon Nanotubes

Toxicity is one of the important issues regarding the use of CNTs in biology and medicine [179]. Currently, CNTs are under investigation in various laboratories, and therefore, the widespread commercialization and exposure of the general populace to this material must occur only after adequate testing. Detailed toxicological studies are required in this regard, and few of the reported studies have shown negative effects on human health. For example, the exposure of cultured human skin cells to SWCNTs caused oxidative stress and loss of cell viability, indicating that dermal exposure may lead to skin conditions [180]. This is perhaps to be expected, since graphite and carbon materials have been associated with increased dermatitis and kerato sis. Additional studies have investigated the pulmonary toxicity of SWCNTs, and it was shown that exposure to SWCNTs lead to the development of granulomas in rodents. Since these studies used very high concentrations of SWCNTs, which were directly exposed to skin

and instilled into the lungs of the animals, further testing is required to establish their toxicity. Also the toxicity is expected to be less in functionalized CNTs and CNT composites.

12.6 Conclusions

The multidisciplinary field of Bio-nanotechnology holds the promise of delivering the technological breakthrough and is moving very fast from concept to reality. The flexibility to modify or adapt bio-nanotechnology to meet the needs of pathologic conditions either for therapeutic applications or as a diagnostic tool is the important characteristic of the technology.

The CNTs represent one of the most promising materials for application in bio-nanotechnology due to their amazing electronic and mechanical properties. CNTs provide a new generation of biocompatible nanomaterials for sensors and probes, implants, electrochemical devices, reinforcements in composites and nanometer-sized electronics that could revolutionize the world. It provides a wide range of new technologies for developing customized solutions that optimize the delivery of pharmaceutical products. It involves the creation and use of materials and devices at the atomic and molecular level. The scope of nano-material is vast and the potential for breakthroughs is enormous and is being pursued on multiple fronts.

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