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Biomedical applications of carbon-nanotube composites

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TABLE OF CONTENTS

1. ABSTRACT

The unique physical, chemical and mechanical properties of carbon nanotubes make them attractive for a variety of biomedical applications. Carbon nanotubes have been used to modify conventional biomedical materials to enhance mechanical properties, biocompatibility, or to impart other functionalities. New multifunctional composite materials using carbon nanotubes have been developed by combining them with inorganic, polymeric or biological materials. Biomedical applications for which novel carbon nanotube composites have been investigated include antimicrobial coatings, neural implants, tissue engineering scaffolds and electrochemical biosensors. In this paper, research on development and application of carbon nanotube composites for biomedical applications has been reviewed.

2. INTRODUCTION

Since their discovery in 1991 by Iijima (1), the unique electrical, chemical, mechanical, and thermal properties of carbon nanotubes (CNTs) have stimulated tremendous scientific interest in them. CNTs have been extensively investigated for wide variety of applications in structural materials, sensors, field emission displays, hydrogen storage materials, tips for scanning probe microscopy, and nano-electronics (2-8). CNTs are materials in the class of fullerene materials. They exhibit hollow cylindrical shapes and can be conceptualized to be formed by rolling up a graphene sheet into a cylinder (9). In CNTs, carbon atoms are linked by sp^2 bonds. The higher strength of sp^2 bonds, in comparison to sp and sp^3 bonds, imparts the excellent mechanical strength to CNTs (10). In addition, the electrons that form π bonds in the CNTs are delocalized; this results in very high thermal and electrical conductivity along the axis of the tube. Carbon nanotubes may be classified into single-walled carbon nanotubes (SWCNT) or multiwalled carbon nanotubes (MWCNT) (9). SWCNTs are formed with only one layer of graphene sheet, while MWCNTs are formed with multiple graphene sheets, which are rolled up concentrically around the tube axis (Figure 1). SWCNTs have smaller diameter ranging from 0.5 to 1.5 nm and MWCNTs usually have larger diameter up to 100 nm. Since their diameter is in range of nanometers, but the length may extend to several micrometers, the length to diameter ratio of CNTs can be as high as 28 million to 1 (11). Because of the very high aspect ratio of CNTs, they can be bent and twisted to large angles without breaking. This rare combination of high flexural and mechanical strengths is desirable for many structural and biomedical implant applications (12-13). Owing to hollow cylindrical structure, density of SWCNTs can be as low as 0.6 g/cm^3 (9).

The current focus in biomaterials research is to develop next generation materials which can stimulate specific cellular responses at the molecular level (14-17). Prevalence of nano-dimensionality and hierarchically organized structure in nature has aroused significant interest in developing next generation biomaterials using nanotechnology to mimic nature (18-19). Their size and interesting properties make CNTs ideal components for advanced biomimetic materials. This has motivated research exploring use of CNTs in biomedical applications (20-23). The ability to functionalize CNTs provides an exciting opportunity to make CNTs biocompatible and bioactive (12, 24). Carbon nanotubes have been used to modify conventional biomaterials to enhance properties and functionalities. More significantly, novel composites have been developed for biomedical applications by combining CNTs with other inorganic, polymeric or biological materials. The biomedical applications for which CNT composites have been investigated include antimicrobial coatings, neural electrodes, biosensors and tissue engineering prosthetics (25-29). It is believed that CNT composites will be the basis for novel technologies of diagnostics and therapeutics (30). In the present paper, we have reviewed the research studies focusing on biomedical application of carbon nanotube composites.

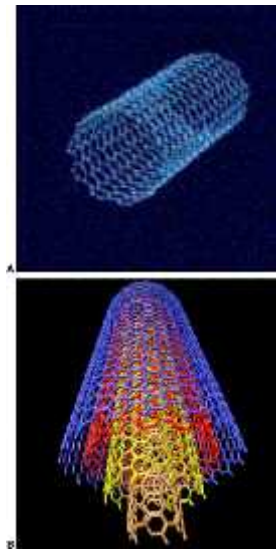

Figure 1. Schematic showing an individual (A) SWCNT and (B) MWCNT. This figure is reproduced with permission from (6).

3. ANTIMICROBIAL APPLICATIONS

Bacterial infections originating from implanted medical devices such as catheters, artificial prosthetics and sensors, are a very serious issue in healthcare (31). These infections start with adhesion of infectious bacteria to the implant surface. The possible sources of the infectious bacteria may be surgical instruments, clothing of medical staff, patient's skin, or ambient atmosphere of hospital (32). Once attached to the implant surface, the infectious bacteria can quickly develop into a biofilm. Bacterial biofilms exhibit very strong resistance to immune response or antibiotic treatment (33). The current methods used to prevent microbial growth, such as treatment of implants in microbicidal solutions and biocidal pharmacologic coatings, are not as effective in their application. For example, while microbicidal solutions do provide a reduction in microbial count, the solution does not always completely eradicate the microbes; and biocidal pharmacologic coatings are only active for short periods of time and are not universally effective against all microbes (34).

Research on CNTs has demonstrated their excellent antimicrobial properties (35-36). The antimicrobial activity of CNTs is believed to arise from physical damage to microbial cells by direct contact with CNT. CNTs can act as nanosyringes and penetrate through the bacterial cells walls (35, 37). Diameter of CNTs is a key factor governing their antibacterial effects and it has been found that SWCNTs are much more toxic to bacteria than MWCNTs (38). The antimicrobial property, combined with excellent mechanical and chemical stability, make carbon nanotubes attractive material for antimicrobial applications. However, for such applications, CNTs must be firmly anchored to surfaces in order to overcome the concern that individual CNTs may induce foreign body neoplasia (39). Also, pristine CNTs are too expensive for large scale application. Hence, for practical antimicrobial applications, various composites of CNTs have been investigated.

Narayan *et al.* developed CNT composite on silicon substrates by pulsed laser ablation of graphite (39). *In vitro* testing of these composites showed significant antimicrobial activity of CNT composite films against *Staphylococcus aureus* and *Staphylococcus warneri* colonization. Such antimicrobial CNT composite films may be useful in hemodialysis catheters and other medical devices (39). CNT-polymer composites have been also found promising for antimicrobial applications. Alsan *et al.* developed SWCNT and poly (lactic-co-glycolic acid) (PLGA) composites for antimicrobial applications (40). It was found that viability and metabolic activity of *Escherichia coli* and *Staphylococcus epidermidis*, which are two common biomedical implant pathogens, were significantly reduced on SWCNT– PLGA composites. Up to 98% of the pathogens died within one hour on SWCNT–PLGA composites, compared to only 15–20% on pure PLGA.

In many cases, rather than being used for their antimicrobial properties, CNTs have been used to enhance properties of other antibacterial materials (41-42). For example, Nepal *et al.* fabricated a biomimetic composite using SWCNTs, DNA and lysozyme (LSZ), a natural antibacterial protein (41). The SWCNTs in this composite were used to impart mechanical strength to LSZ. This composite showed significant antimicrobial activity and high Young's Modulus. In another study, SWCNTs were used to develop electroactive antiseptic bandages (42). SWCNTs were coated with polyvinylpyrrolidone-iodine (PVPI) and deposited as a composite film. The antiseptic property of this composite were derived from self contained slow-release of antiseptic iodine, which was non-covalently bound on SWCNT surface. This composite had low enough resistance for electrical stimulation of cells which has been shown to enhance cell growth and accelerate healing process (42).

Protein adsorption onto surfaces is often the first step leading to microbial adhesion and eventually biofilm formation (43). Hence, microbial growth can also be countered by designing surfaces which will resist protein adsorption. Asuri *et al.* developed polymer–nanotube–enzyme composite films which resist protein fouling (44). The enzyme serine protease subtilisin carlsberg (SC) was absorbed onto SWCNTs in an aqueous solution. The resulting SWCNT-enzyme conjugates were dispersed in poly (methyl methacrylate) (PMMA) to prepare PMMA-SWCNT-SC composite films. The use of CNTs in this composite was critical in incorporating the enzyme in the composite (44). This composite exhibited excellent anti bio-fouling properties when exposed to two model proteins, human serum albumin and fibrinogen, which are present in blood plasma.

4. NEURAL APPLICATIONS

Electrical stimulation of the nervous system can be used to treat a wide range of health problems such as hearing loss, paralysis, chronic pain, diabetes, retinal degeneration, and Parkinsons and Alzheimer's disease (45-49). The stimulation of nervous system is achieved through implanted neural electrodes (NE). The neural electrodes work by delivering electrical pulses to the target tissue to modulate neural response (50). The ideal neural electrodes

should chronically provide safe levels of therapeutic stimulation (46). For safe stimulation, the electrode must deliver appropriate charge at tissue-NE interface without inducing any chemical reactions on the electrode or in the tissue (47). Currently, biocompatible metals such as gold, platinum, iridium and titanium and some other nonreactive materials such as stainless steel, iridium oxide and silicon are used for making neural electrodes (47, 50). Most of the practical neural electrodes are made by deposition of conductive and high charge capacity metal layer onto a lower performance material (47). The delamination and degradation of the metal layer on the neural electrodes is a serious issue and reduces the efficacy of electrodes (51).The long-term inflammatory response of neural tissues is another major concern for implanted neural electrodes. Chronic inflammation results in the formation of glia around the neural electrode (52). The glial scarring causes the separation of the neural electrode and the neural tissues, which results in the function loss of neural electrode and device failure in long term. The device failure over long term is partly attributed to the micromotion of the neural electrode to the surrounding tissues (53-54).

There is a need for miniaturization of neural electrodes to achieve selective excitation and recording of neuron cells. This is highly desirable since it will promote new therapy and novel neuroprosthetic devices (47). Research results indicate that inflammation response can also be reduced with reducing neural electrode size (55-56). For example, a study showed that 50 µm diameter implants elicited smaller tissue reactions and resulted in the survival of larger numbers of neurons compared to 200 µm diameter implants (55). In addition, small device size also reduces the tissue damages during device implantation. However, the mechanical and electrical properties of the present neural electrode materials limit the size reduction. The unique electrical and mechanical properties, combined with their surface characteristic and good biocompatibility, make CNTs attractive material for neural neuroprosthetic devices. The chemical and mechanical stabilty of CNTs make them ideally suited for long-term implants. The high surface area of CNTs can drastically increase charge injection capacity of neural electrodes and decrease interfacial impedance with neurons (48). This will allow reduction in neural electrode size. Another important attribute of CNTs is their high mechanical compliance with neural tissue that can help to alleviate micromotions. The reduction of micromotions is extremely beneficial since micromotions can cause immune cell response or foster inflammatory reactions (47, 48, 51).

A large number of studies have been done to investigate the biocompatibility and feasibility of CNTs and CNT based composites for neural implant applications (57-60). These studies have established the feasibility of applications of CNTs in neuroprosthetics. In one of the first work in this direction, Mattson *et al.* (57) developed a method for growing embryonic rat-brain neurons on MWCNTs. They found that the neurons were able to survive, attach and grow on unmodified CNT surfaces. However, no neurite branching was observed on unmodified CNTs. In contrast, extensive neurite branching and outgrowth was seen on the CNTs modified with bioactive molecule 4-hydroxynonenal, which is known to induce increased intracellular Ca^{2+} levels and promote neurite outgrowth (57). A detailed neuron growth study, in which effect of functionalization of MWCNTs with carboxylic group, poly-m-aminobenzene sulfonic acid and ethylenediamine was investigated, has shown that neurite outgrowth could be controlled by surface charge of MWCNTs (58). In this study, hippocampal neuronal cultures were used, and it was found that neurons grown on positively charged MWCNTs showed more growth cones with longer average neurite length and more neurite branching. Conductivity of CNT-composites can also have significant effect on the neuronal growth. For SWCNT - poly (ethylene glycol) (PEG) composite films it was observed that neuronal growth and neurite outgrowth were promoted in narrow range of conductivity. At conductivity higher than this optimal range for SWCNT-PEG composites, these effects on (out)growth of neuritis were diminished (59). Gheith *et al.* investigated the properties of free-standing CNT- poly (diallyldimethylammonium chloride)- poly (acrylic acid) composite membranes (60). The biocompatibility of CNT-polymer membranes, prepared by layer-by-layer (LBL) assembly method, was tested by incubation with NG108-15 neuronal cells (60). It was found that SWCNT-polymer composite membranes supported neuronal attachment and differentiation. In another interesting work, neural network activity and neural signal transmission of neuronal circuits on a CNT grid were investigated (20). In this study, the spontaneous postsynaptic currents (PSCs) from a single neuron using single-cell patch-clamp recordings were measured. PSCs clearly indicated the existences of synapses and functional network. These results showed that neuronal circuits could be grown on a CNT grid with considerable increase in network activity (20).

Wang *et al.* demonstrated the first prototype of CNT based neural electrodes (48). In this study, a microelectrode array consisting of vertically aligned MWCNTs was integrated onto a quartz substrate with pre-patterned microcircuitry (48). Each microelectrode consisted of a bundle of MWCNTs with individual addressing ability. The embryonic rat hippocampal neurons were grown and differentiated on these microelectrodes. More importantly, successful and repeated electrical stimulation of neurons was demonstrated using the CNT electrode array. It was also found that CNT microelectrodes operated mainly with capacitive current, which is ideal for neural stimulation. The charge injection limit of CNT array was found to be $1-1.6$ mC/cm^2, which is higher than that for bare platinum. Electrical stimulation of neurons has been also demonstrated using LBL-assembled CNT-polymer films (30). It was found that LBL-assembled CNT-polymer films had sufficiently high electrical conductivity to stimulate neurons. The electrophysiological measurements of NG108 cells indicated the excitation of the neuronal cells when current was passed through CNT-polymer film substrate. In another important study, indium tin oxide multi-electrode array (MEA) and conventional tungsten and steel wire electrodes were coated with carbon nanotubes using electrodeposition (49). Electrical

stimulation of neurons in culture and *in vivo*, in rats and monkeys, was demonstrated using these CNT coated electrodes (49). It was found that CNT coating enhanced both electrical stimulation and recording by increasing the charge transfer and decreasing the electrode impedance. In addition, CNT coated electrodes were mechanically robust (49).

Jan *et al*. have done a comparative evaluation of the CNT composite films with two other state of the art neural electrode coating materials; iridium oxide (IrOx) and poly (3,4-ethylenedioxythiophene) (PEDOT) (50). The results of this study indicated that LBL-assembled CNT-polymer coatings were much superior to IrOx and PEDOT coatings in their ability to reduce impedance, increase cathodic charge capacity, and facilitate charge transfer on conventional platinum-iridium wire electrodes. In another study, which explored usage of CNT-composites for improving electrode neural interface, Lu *et al.* co-deposited polypyrrole (PPy)/ SWCNT films on Pt microelectrodes (61). The PPy/SWCNT coated microelectrodes exhibited a particularly high safe charge injection (Qinj) limit of ~7.5 mC/cm^2 and low electrode impedance. In addition to *in vitro* studies, *in vivo* tests were done on these electrodes by implanting in the cortex of rats. These tests showed excellent biocompatibility of PPy/SWCNT film coated electrodes. These characteristics are highly desirable for chronic implantable neural electrodes. Kam *et al.* fabricated "humanized" composites of CNT with laminin, which is an essential part of the extracellular matrix (51). This study established the ability of CNT-laminin composites to mediate the differentiation and electrical stimulation of neural stem cells. The structural and chemical similarity of such "humanized" composites allows better integration with tissue (51).

5. BIOSENSORS

Biosensors are analytical devices that convert a biological response into a measurable signal (62-63). Amongst different types, electrochemical biosensors, which convert biological event to an electronic signal, are most popular for biomedical applications because of their sensitivity and selectivity (64). Electrochemical biosensors consist of a biological recognition element that selectively reacts with the target analyte. The transducer or electrode is another important component in the electrochemical sensor which converts analyte specific chemical reaction into an analyte concentration dependent electrical signal. Based on the biological element used, electrochemical biosensors can be classified as enzymatic or affinity sensors (65). Enzymatic sensors are based on a biocatalytic event between sensor element and target analyte which produces electroactive species. Affinity sensors rely on a selective binding interaction between the analyte and a biological component such as an antibody, nucleic acid, or a receptor (65).

In enzymatic sensors, enzyme plays a role of catalyst and electrons need to be transferred from the active sites of the enzyme to the electrode. The processes of coupling an enzyme reaction to the electrode is realized either using low molecular weight redox mediators or using mediatorless (direct) electron transfer (66). In the first approach, enzyme catalyzes the oxidation or reduction of the mediators, after which mediators exchange electrons with the electrode. In such mediator-assisted processes, mediators act as second substrate for the enzymes and the electrochemical transformation of mediators on the surface of the electrode has to be reversible to maintain the function of the device. In mediatorless processes, electron is transferred directly between the electrode and the substrate molecule. Direct electron transfer between enzymes and electrodes offers the opportunity to create reagentless biosensors which are highly desirable for biomedical applications. However, the redox center of most enzymes is usually insulated by a protein cell, making direct electron transfer unfeasible (67-68). Conventional electrodes have to be surface-modified in order to support efficient, direct electron transfer. CNTs have been demonstrated to be one of the materials well-suited for effective electrode surface modification. Modified electrodes not only allow realization of mediatorless enzyme sensors, but also provide the possibility to investigate redox processes of proteins. In general, lower overvoltages and higher peak currents are observed for a variety of analyte molecules at electrodes modified with CNTs (69).

In one of the first reports on use of CNTs for biosensors, Britto *et al.* constructed MWCNT paste electrodes using bromoform as a binder and used them for detection of dopamine (an important neurotransmitter) (70). Detection of dopamine, which is based on its characteristic cyclic voltammetric oxidation on electrode surface, was investigated on the CNT- paste electrodes and was compared with conventional carbon paste electrode (CPE) (70). Compared to CPE, the sensitivity of CNT paste electrodes exhibited higher sensitivity towards dopamine. This study paved the way for use of CNTs in biosensors. Wang *et al.* prepared an enzyme-dispersed MWCNT electrode by mixing glucose oxidase (GOx) with MWCNT (71). This biocomposite electrode was packed in a needle and was used for biosensing of glucose. Compared to conventional graphite electrodes which did not respond to glucose below +0.6V, a highly selective low potential (-0.1 V) biosensing of glucose was reported on CNT composite electrodes. In a more recent study, a DNA biosensor was developed by modifying the surface of a carbon paste electrode with a nanocomposite of MWCNT, polyaniline (PANI) nanofibers and chitosan (CHIT) (72). The synergistic effect between PANI and MWCNT resulted in highly conductive and biocompatible MWCNT-PANI-CHIT composite, which lead to a big improvement in immobilization of probe DNA on the surface of the electrode. The dynamic detection range for this DNA biosensor was 1.0×10^{-13} to 1.0×10^{-7} mol/L, with a minimum detection limit of 1.0×10^{-14} mol/L (72). These examples of CNT composite electrodes combine the advantages of paste electrode materials and the fast electron kinetics offered by carbon nanotubes (68).

Conventional electrochemical biosensors are based on either glassy carbon electrodes or metal electrodes

such as Au, Pt or Cu (73). There have been numerous studies to modify the conventional electrodes with CNTs to design improved biosensors. One of the easiest methods to modify the electrodes is to cast a dispersion of CNTs on the electrodes followed by evaporation of the solvent. This method has been used very frequently to modify glassy carbon electrodes as well as metal electrodes (74- 77). For example, Musameh *et al.* modified a glassy carbon electrode by casting with CNTs dispersed in concentrated sulphuric acid (75). The coating was dried at 200 ^{o}C for 3 h. The CNT modified electrodes developed in this study offered a stable low potential detection of β-nicotinamide adenine dinucleotide (NADH). The CNT coating resulted in a decrease in the overvoltage for NADH oxidation and eliminated surface fouling effects. Wang *et al.* investigated label free detection of DNA on a glassy carbon electrode modified by solution casting of CNTs (77). The electrode surface modified with CNTs facilitated adsorptive accumulation of the guanine nucleobases and resulted in greatly enhanced detection of DNA and DNA hybridization in comparison to unmodified glassy carbon, carbon paste or graphite pencil electrodes (77).

The CNT modification of biosensor electrodes has been also done using CNT-polymer composites. Nafion is one of the polymers which have been often used for these applications (26, 74). Nafion has good ion exchange and biocompatibility properties and promotes formation of well connect CNT networks on electrodes (73). For example, Lyons *et al.* also used SWCNT/GOx/Nafion composite to modify gold and glassy carbon electrodes (74). This procedure resulted in fabrication of a highly porous and randomly dispersed SWCNT/GOx mesh on the electrode surface. Using these SWCNT/GOx/Nafion composite modified electrodes, amperometric glucose detection was achieved at very low applied potential. Wang *et al.* used Teflon as a binder to prepare 30/70 wt % CNT/Teflon composite electrode for biosensors (78). It was found that Teflon did not affect the catalytic properties of CNTs. The excellent catalytic activity of the CNT-Teflon composite electrode facilitated low-potential amperometric sensing of glucose and ethanol. Polypyrrole (PPy), a highly conducting polymer, has been also used for immobilizing CNTs on glassy carbon electrode. Li *et al.* deposited a PPy/SWCNT composite film on a glassy carbon electrode by casting a solution containg PPY and SWCNT. Subsequently this composite film was oxidized at a potential of +1.8 V (79). The resulting overoxidzed PPY/SWCNT modified glassy carbon electrodes exhibited excellent electrocatalytic properties for species such as ascorbic acid, dopamine, and uric acid. These favorable properties make such electrodes attractive for practical biosensor applications (79).

There have been many studies to understand electron-transfer kinetics on CNT modified electrodes (80-81). Gooding *et al.* have investigated electron-transfer of redox enzyme (microperoxidase MP-11), covalently attached on the ends of aligned SWCNTs (80). Shortened nanotubes were aligned vertically on the surface of a gold electrode by self-assembly. Redox enzyme was then attached on the free end of the CNT. The high rate of electron-transfer between the electrode and redox enzyme observed in this study was attributed to the nanotubes. It was proposed that CNTs act as molecular wires to allow electron communication between electrode and enzyme. In a similar study on glucose oxidase attached to SWCNTs on a gold electrode surface, Patolsky *et al.* concluded that SWCNTs act as a nanoconnector, which enabled the electrical contact between the active site of the enzyme and the electrode (81). In this study, the rate of electron-transport was found to depend on the length of the SWCNTs.

6. TISSUE ENGINEERING

Tissue engineering involves enhancing or replacing the damaged tissue to restore normal biological function. Development of biomaterials, which can substitute tissue or facilitate tissue growth, is critical for advancements in tissue engineering (82). Biocompatibility is the basic requirement for any tissue engineering material. In addition, hard-tissue applications, such as bone or dental tissue, require materials with good mechanical properties (84). Currently titanium and some other bio-inert metal alloys and ceramics are the most common materials used for hard tissue implants. Even though these materials are biocompatible, their physicochemical and mechanical properties are very different than the bone material. The excellent mechanical properties of CNTs have lead to many research studies exploring use of CNTs for reinforcing matrix materials used for hard tissue implants (12, 82, 84). Hydroxyapatite (HA) is widely used for biomedical applications for bone tissue engineering because of its excellent biological compatibility and osteoconductivity (14). However, the inherent brittle nature of HA makes it unsuitable for load bearing applications (84). This has lead to efforts to develop HA coating for conventional biomaterials such as Ti-alloys. This approach combines excellent mechanical properties of metals with biocompatibility and bioactivity of HA. There have many studies which focused on reinforcement of HA coatings with CNTs to improve the mechanical properties (28, 85-88). In one of the earliest study in this direction, Chen *et al.* used laser surface alloying to develop CNT-HA composite coatings on Ti-6Al-4V alloys (87-88). This work showed that reinforcing of HA with CNTs resulted in significant improvement in mechanical properties of HA. However, it observed that a fraction of CNTs reacted with Ti during laser irradiation. In another study, plasma spraying was used to coat Ti-6Al-4V substrate with 4 wt % CNT reinforced HA coatings (85). It was reported that CNTs can be uniformly dispersed in HA using plasma spraying technique. The addition of 4 wt% CNT to HA coatings improved fracture toughness by 56% and crystallinity of HA by 27%. Furthermore, biocompatibility studies onto these coatings, with human osteoblast hFOB cell culture, showed good spreading of cells and unrestricted growth of cells near CNTs (85). Hahn *et al.* developed CNT reinforced-HA coatings on conventional Ti alloy plates and studied their biocompatibility (86). In this study, aerosol deposition technique was used to deposit CNT reinforced-HA coatings on Ti substrates. These HA coatings, reinforced with 1 and 3 wt% CNTs, exhibited excellent

adhesion strength (27.3-29 MPA). Nanoindentation studies showed that compared to pure HA coatings, reinforcement with 3 wt% CNTs lead to an increase in hardness and elastic modulus of HA coatings by 27% and 11%, respectively. *In vitro* cell tests showed that proliferation and alkaline phosphatase (ALP) activity of MC3T3-E1 pre-osteoblast cells grown on the HA–CNT composite coatings was higher than those on the bare Ti and pure HA coating. The ALP activity in MC3T3-E1 grown on the composite coatings was considerably improved as the CNT content increased. This study also showed that HA-3 wt% CNT coatings had lower cytotoxicity compared to bare Ti and pure HA coatings. These results suggest that CNTs would be an effective reinforcing agent to enhance both the mechanical and biological performances of HA coatings (86). Singh *et al.* prepared a nanocomposite of poly (methyl methacrylate) (PMMA)-modified HA with MWCNTs (28). They used freeze-granulation technique to prepare this composite to disperse the MWCNTs uniformly. The uniform distribution is important for effective load transfer to MWCNTs. Their study showed that the optimum mechanical properties of the composite were achieved with 0.1% MWCNTs (28). This material has potential application in bone cement and implant coatings (28). Also, there has been efforts to develop bulk CNT-HA composites (89-90).

Bone tissue consists of hierarchically organized collagen fibrils and HA crystals. This hierarchical structure of bone develops in the early stages of bone formation, when collagen acts as nucleation site for HA crystals (91). The diameter of CNTs is around 1 nm and is close to size of triple helix collagen fibrils. A size comparable to collagen fibrils and possibility of functionalization makes CNTs suitable for mineralization of HA. This approach was used to develop MWCNT-HA composites by mineralization of functionalized MWCNTs (92). In this study the MWCNTs were first dispersed in a dilute sodium dodecyl sulfate aqueous solution. The mineralization was achieved by adding dispersed MWCNTs to Ca $(NO_3)_2$ solution followed by sonication. Aqueous $(NH_4)_2HPO_4$ solution was then added into this mixture at 0 ^oC with vigorous agitation. The resulting mixture was then placed in a Teflon-lined autoclave and maintained at 118 ^oC to facilitate mineralization of MWCNTs. MWCNTs–HA composite nanopowders were finally obtained by rinsing, grinding and drying of precipitates resulting from mineralization in autoclave. Zhao *et al.* functionalized SWCNTs with phosphates and poly (aminobenzene sulfonic acid) (PABS) (93). They carried out these studies with CNTs in solution phase as well as films on substrates. They showed that negatively charged functional groups on SWCNTs attracted calcium cations leading to self-assembly of HA crystals (93). These studies demonstrate potential application of CNTs for designing biomimic scaffolds which can induce mineralization of HA (92-93). Porous three dimensional scaffolds are being increasingly used for tissue engineering. The objective of scaffolds is to provide appropriate environment for cell growth and facilitate regeneration of tissues and organs (94). Unlike traditional permanent bio-implants, scaffold materials should be biodegradable. Various synthetic and natural polymers have been investigated for scaffolds applications. There is growing interest in using polymer-CNT composites for developing scaffolds with enhanced bioactivity, mechanical properties and functionality. Jell *et al.* used a thermally induce phase separation technique (TIPS) to form porous CNT-polyurethane (PU) foam nanocomposite to mimic the porous structure of bone (95). Using this method they were able to disperse CNTs uniformly in the PU matrix. Their study showed compressive strength of PU increased by 200% with 5 wt% CNTs. This nanocomposite was not only biocompatible but also showed increased osteoblast production of the potent angiogenic factor vascular endothelial growth factor (VEGF), with increasing CNT wt% in the composite. This indicates possibility of manipulating cellular behavior by varying the CNT content (95). Bhattacharya *et al.* studied the effects of CNT-polymer composites on osteoblasts *in vitro* and bone tissue *in vivo* in rats (97). This study showed the CNT-polymer composites improved the mineral formation. The *in vivo* studies showed that CNT-polymer composite was biocompatible and promoted bone formation. Sitharaman *et al.* studied the *in vivo* biocompatibility of ultra short CNT reinforced -poly (propylene fumarate) (PPF) porous scaffolds in a rabbit model (98). The CNT-PPF composite and control PPF scaffolds were implanted in rabbit and tissue response was analyzed with micro-computed tomography, histology, and histomorphometry at 4 and 12 weeks after implantation. It was concluded that CNT-PPF scaffolds exhibit favorable tissue response and can be potentially used as a prototype bone tissue engineering scaffold (98).

Some tissue applications, such as cardiac muscle and neural tissues, where bio-functioning of the tissue involves electrical signals, the materials should have suitable electrical properties (83). Edwards *et al.* have demonstrated three-dimensional CNT yarn based composite scaffolds which can support cell growth (96). They first prepared a tubular knitted scaffold from a 9 ply MWCNT yarn followed by electrospinning of PLGA. The advantage of the knitted CNT structure is that the continuity of CNT network is maintained which leads to effective load transfer and retention of highly desirable mechanical and electrical properties. The MWCNT knit structure had a breaking load of 0.7 N (compared to 0.35 for the rat acellular sciatic nerve) and electrical resistance of 1.01 kΩ/cm (compared to 0.95 kΩ/cm for 5 ply MWCNT). Such composites may be particularly useful in applications, such as nerve regeneration, where electrical stimulation of nerve cells is required. Studies on NR26 mouse fibroblast cells indicated that MWCNT yarn- PLGA composite supported cell growth with a uniform distribution of distribution cells on the scaffold surface (96). Type 1 collagen is one of the most important biomolecule and plays an important role in physical and biochemical functions of many tissues. MacDonald *et al.* (83) prepared CNT-collagen composites, with 0-2 wt% CNTs. In this study, Type 1 collagen and carboxylated SWCNT were used. The composite was seeded with living rat aortic smooth muscle cells. It was also found the CNTs in the matrix did not affect the cell viability or cell proliferation. This makes such materials potential candidates for soft and hard tissue engineering applications. The self-assembly properties of collagen also offer the possibility of producing ordered composites in which CNTs are aligned.

Table 1. A brief summary of selected studies on toxicity of CNTs

Objective of the study	Result	Reference
Investigated and compared cell response of MWCNTs and graphite compacts using a myoblastic mouse cell (C2C12) culture	It was found that CNTs might induce cellular functions by adsorbing more proteins	99
Investigated CNTs at various degrees of agglomeration using an *in vitro* cytotoxicity study with human MSTO-211H cells	Suspended CNT-bundles exhibited lower cytotoxicity compared to rope-like CNT agglomerates	100
Investigated effect of SWCNTs on human HEK293 cells to explore biocompatibility of SWCNTs	SWCNTs were found to inhibit HEK293 cell growth by inducing cell apoptosis and decreasing cellular adhesion ability	101
Assessed toxicology of carbon nanomaterials (SWCNT, MWCNT, active carbon, carbon black and carbon graphite) on human fibroblast cells *in vitro*	Toxicity was found to be inversely dependent on surface area (SWCNTs with lowest surface area were most toxic) and refined SWCNT were more toxic compared to unrefined SWCNTs	102
Studied cytotoxicity of SWCNT, MWCNT and fullerene (C60) with alveolar macrophage	The cytotoxicity was found to follow a sequence order on a mass basis: SWCNT > MWCNT >C60	103
Evaluated the acute lung toxicity of intratracheally instilled SWCNTs in rats	High-dose of SWCNTs produced mortality in ~15% of the rats because of mechanical blockage of the upper airways; pulmonary exposures to SWCNT produced a non-dose-dependent series of multifocal granulomas in rats	104
Assessed various markers of inflammatory and fibrogenic pulmonary responses as well as oxidative stress response to SWCNTs in mice	Pharyngeal aspiration of SWCNTs induced inflammatory reaction and pulmonary exposure to SWCNTs caused persistent changes in pulmonary functions	105
Studied effect of MWCNT exposure on mesothelial lining of the body cavity of mice	Length-dependent pathogenic behavior, including inflammation and the formation of lesions, was observed	106

7. CNT COMPOSITES: TOXICITY

As discussed in previous sections, CNT composites are very promising for a wide range of biomedical applications. However, a thorough investigation of toxic effects of CNTs needs to be done before they can be used in practical biomedical devices. Considerable research has been done to investigate *in vitro* (99-103) and *in vivo* (104-106) toxicity of CNTs. A brief account of selected research studies on toxicity of CNTs is given in Table 1. Though, there are many reports which rule out any toxic effects of CNTs, the results on CNT toxicity studies are often contradictory (11, 107). Toxicity has not been generally observed for CNT coatings and composites (20, 47, 57). However, if CNTs are used even in the form of composites for human implants, it is likely that a fraction of CNTs from the composite will find their way to human body because of composite degradation. Hence toxicity effects of CNTs in native or in the form of dispersions need to be taken into consideration for any biomedical application of CNT composites (47, 107).

Research on known toxic fibres, such as asbestos, has shown that non-degradable fibres thinner than 3 µm and longer than ~ 20 µm present potential health hazards (108). These results, and morphological resemblance of CNTs with asbestos, point to the potential toxicity of CNTs. Indeed, various *in vitro* and *in vivo* studies have associated toxicity with CNTs. Many *in vitro* studies suggest that CNTs can reduce cell viability, damage DNA and negatively affect neurite branching (47, 101, 103). In a recent *in vivo* study, Poland *et al.* have shown that exposing the mesothelial lining of the body cavity of mice to long MWCNTs resulted in asbestos-like, length dependent, pathogenic behavior (106). Inflammation and formation of lesions were reported in the host animal model (106).

CNTs, like other nanoparticles, can stimulate generation of reactive oxygen species (ROS), resulting in oxidative stress (11). The oxidative stress can lead to decreased cell viability, and even damage to DNA (47, 11). CNTs can cross the cell membrane and accumulate in the cytoplasm or reach the nucleus of human fibroblast cells (109). The resulting changes in cytoskeleton and cell morphology can also explain the cytotoxicity associated with CNTs. It has been reported that both surface area and length can affect the toxicity of CNTs (102, 110). The toxicity of CNTs has been reported to increase with decreasing surface area, and SWCNTs with lower surface area were found to be more toxic than MWCNTs (102). Also, it has been reported that longer CNTs are more likely to lead to an inflammatory response (110). The effect of state of aggregation on toxicity has been also studied but the results are not very consistent (100,102, 111). While some reports suggest that aggregates of CNT's are more toxic than the well-dispersed CNTs (100, 111), there are also reports that suggest reduced toxicity in the aggregated state (102).The difference in the toxicity of aggregated CNTs, compared to well-dispersed CNTs, has been related to their ability to traverse the cell membrane and the reduced surface area (102). The surface functionalization of CNTs can alter cell-CNT interactions and play an important role in toxicity of CNTs (112). For example, very strong functional group dependence has been reported for *in vitro* neuron growth on MWCNTs functionalized with carboxylic group, poly-m-aminobenzene sulfonic acid, or ethylenediamine (58). In addition, surface chemistry can modulate toxicity of CNTs by affecting the state of aggregation (102). In CNT preparation, transition metals such as Fe or Ni are used as catalyst. Trace amount of these transition metals may also contribute to the toxicity of CNTs (47).

To summarize, toxicity in CNTs appears to be a function of preparation methods, size and surface functionalization. Incorporating them in polymers, careful preparation and appropriate surface functionalization can help mitigating the toxic effects of CNTs. Well functionalized CNTs with biocompatible surface coatings have been shown to be non toxic *in vitro* to cell and *in vivo* in animal models (113). However, further studies are required to understand toxicity in animal models and

humans. Needless to say, such studies will also need to clarify long-term *in vivo* effects of CNTs.

8. CONCLUSIONS

A wide range of CNT composites have been developed for various biomedical applications. As a component of antimicrobial coatings, CNTs have shown their ability to inhibit microbial growth which can be utilized to reduce risk of implant related infection. Due their high charge injection capability and excellent mechanical properties, CNT composite hold promise to revolutionize the field of neural prosthetics. Use of CNT composites will allow development of miniaturized neural implants which will be more efficient. This will help to treat a variety of health issues caused by neural damage or disease. CNTs have also found applications in the field of biosensors where they have been used to detect a wide range of biomolecules. Compared to conventional electrodes, CNT composite electrodes allow detection of biomolecules at lower overvoltage potential with enhanced sensitivity and selectivity. In the field of tissue engineering, CNTs have been used to enhance the properties of conventional biomaterials. In addition, CNT-composites have been used to develop tissue engineering scaffolds. Such scaffolds can interact with cells and stimulate accelerated growth of damaged tissues. Despite extensive research, biomedical applications of CNT composites are still limited to laboratory. Toxicity of CNTs is a very concerning issue in realization of biomedical devices based on CNT-composites. *In vivo* studies in animal models and humans are required to clarify long term toxic effects of CNTs.

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