

On the cytotoxicity of carbon nanotubes

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The cytotoxicity of carbon nanotubes (CNTs) is a major concern today well before its unusual physico-chemical, mechanical, and electrical properties are fully exploited for commercial interests and subsequent mass production leading to greater possibilities for its exposure to humans and the environment. Contradictory reports on cytotoxicity of CNTs often appear in the literature and a mechanistic explanation of the reported toxicity remains obscure. We review here the conflicting results to focus categorically on an array of issues in CNT cytotoxicity. They include dispersion, aggregation status, coating or functionalization and immobilization, cellular uptake or internalization, purity in terms of metal catalyst contaminants, size and size distribution, surface area, surface chemistry and surface reactivity, cell types selected for experimentation as well as bioassay of nanotoxicity itself attesting as an issue in cytotoxicity. Recently a general agreement has emerged towards the potential toxicity of CNTs, although various paradigms explaining the mechanisms of CNT cytotoxicity continue to be elusive in the literature. A lack of synergy among various issues while studying cytotoxicity and most developed paradigms for the mechanism of CNT toxicity is highlighted.

Keywords: Carbon nanotubes, cytotoxicity, nanocomposite, tissue engineering.

THE urgent need for toxicological studies on carbon nanotubes (CNTs) has arisen from the rapidly emerging applications of CNTs well beyond materials science and engineering. Especially the potential medical and environmental problems, including the associated toxicity and biocompatibility issues have attracted a great concern among scientists¹⁻⁶. Therefore, determining the cytotoxicity of CNTs has been one of the most pressing questions in nanotechnology. Before reviewing the details on toxicity, we briefly describe CNTs in the next section.

CNTs are well-ordered, high aspect ratio allotropes of carbon. The two main variants, single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) both possess a high tensile strength, are ultralight weight, and have excellent chemical and thermal stability. They also possess semi- and metallic-conductive properties. The SWCNT is a one-atom thick sheet of graphite (called graphene) rolled up into a seamless

cylinder with diameter of the order of a nanometre. This results in a nanostructure where the length-to-diameter ratio exceeds 1,000,000. Such cylindrical carbon 'molecules' have novel properties that make them potentially useful in many applications in nanotechnology, electronics, optics and other fields of materials science. They exhibit extraordinary strength and unique electrical properties, and are efficient conductors of heat as well. All these unique physico-chemical properties of CNTs and the unusual one-dimensional hollow nanostructure have also rendered them useful in biological applications, particularly as a novel drug delivery tools and imaging agents. Scientists also eye upon a great promise of CNTs to impart mechanical strength to relatively weak but biologically important biomaterial scaffolds in the area of regenerative medicine or tissue engineering. However, such biomedical applications will not be realized if there is no proper assessment of the potential hazards of CNTs to humans and other biological systems. Several issues relevant to cytotoxicity have been discussed and results from various scientific tests on cells have so far proven confusing⁷⁻¹⁵, with some results indicating it to be highly toxic¹⁶⁻²¹, and others showing low toxicity or no signs of toxicity at all²²⁻²⁴.

In this review we compile a range of scattered issues in CNT nanotoxicology as published in the literature so far and also critically review major reports concerning CNT biocompatibility in following sections.

Issues in CNT nanotoxicology

As seen in the literature, CNT cytotoxicity can be attributed to a range of issues such as metal impurities, length and size distribution, surface area, dispersion and aggregation status, coating or functionalization, immobilization, cellular uptake or internalization and cytotoxic response of different cell types to CNTs as well, among others. In this section we review and organize the published results from the literature into sub-sections to focus on these different issues in CNT cytotoxicity.

Cell types and CNT cytotoxicity

Relatively more challenged and easily accessible organs in a given CNT-polluted environment are the skin, lungs and blood-borne-cells. Therefore, these organs and organ-

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specific cell lines have been studied substantially^{7,16-18,25-35}. Nevertheless, nanotoxicity data of CNTs have been made available on several other types of cell lines as well, namely kidney cells⁹, stem cells²⁹ and cancer cells^{7,34}.

The lungs as a whole have been subjected to testing for the potential hazards of inhalation exposure to carbon nanotubes *in vivo* in rat models. The pulmonary toxicity in such experiments was found due to mechanical blockage of the large airways^{16,25}. The physiological relevance of these findings remains to be determined, since the SWCNTs have a strong tendency to agglomerate following intratracheal exposures¹⁶. Lam *et al.*¹⁷ also reported similar work on the pulmonary toxicity of SWCNTs in mice, where SWCNTs were found to induce dose-dependent interstitial granulomas and pulmonary injuries. In addition to mechanical blockage and distinct granulomas, the pharyngeal aspiration of CNTs in animal models resulted in a pronounced cellular response and increase in various cytotoxicity/inflammatory markers in the lungs²⁶. These included a significant increase in total bronchoalveolar lavage (BAL) cells and polymorphonuclear leukocytes and also protein, lactate dehydrogenase (LDH), tumour necrosis factor (TNF)-alpha, interleukin (IL)-1beta, and mucin levels²⁶. Not only have the lungs as a whole at the organ level and *in vivo*, but the cytotoxicity effect of CNTs has also been evaluated *in vitro* on cultured lung cells. Davoren *et al.*²⁷ reported SWCNTs to have low acute toxicity to the A549 cells cultured *in vitro*. However, the presence of increased number of surfactant storing lamellar bodies as seen in TEM ultrastructural studies of SWCNT-exposed lung cells indicated a defensive response of these cells that might be the cause of the low acute cytotoxicity observed⁷. Casey *et al.*²⁸ demonstrated the cause of such apparent cytotoxicity to the A549 cells to be due to media constituent depletion and referred to it as a case of a false positive result. In their experiment Casey *et al.*²⁸ suspended SWCNTs in the culture medium and then removed all the tubes through ultracentrifugation and filtration. Media constituent depletion caused due to nutrient biomolecule deposition on SWCNTs may immediately appear as a convincing explanation given by the authors, since it makes the cells starve for essential nutrients in the medium. Also, any hydrophobic surface is a preferred site for biomolecule deposition and hence being inherently hydrophobic, well-dispersed CNTs will adsorb most of the nutrient biomolecules from the medium proportional to the concentration and surface area of CNTs, thereby depleting nutrient biomolecules in the culture medium. Nevertheless, if media depletion is the cause for the loss in cell viability, this needs further exploration as there are reports at the molecular level showing genotoxicity being induced due to SWCNTs. Kisin *et al.*²⁹ have reported loss of viability in lung fibroblast (V79) cell line in a concentration and time-dependent manner after the exposure of cells to SWCNT, demonstrating the geno-

toxic effect of SWCNTs at the molecular level in terms of DNA damage.

Since alveolar macrophage constitutes the first line of immunological defence against the invading particles in the lung, researchers have conducted a cytotoxicity study of carbon nanotubes with macrophages as well. Jia *et al.*¹⁸ observed profound dose-dependent cytotoxicity of SWCNTs in alveolar macrophage isolated from guinea pigs *in vitro* for 6 h. The macrophages exposed to SWCNTs or MWCNTs showed characteristic features of apoptotic cell death at different dosages, toxic response being more with SWCNTs compared to MWCNT, quartz and fullerene used in this study¹⁸. There are reports showing contradictory results of CNT cytotoxicity to macrophages. Kalbacova *et al.*³⁰ found SWCNTs to be toxic to monocytes/macrophage (THP-1) cells, while Fiorito *et al.*³¹ reported insignificant toxicity of SWCNTs to human macrophage cells.

Apart from the concern for inhalational entry of carbon nanotubes into the biological system, a direct contact with the skin is of equal concern. Carbon fibre dermatitis, hyperkeratosis, and naevi for example, have been linked with exposure to carbon nanomaterials and graphite. Hence several investigations have been carried out to determine cytotoxicity of carbon nanotubes on skin cells. Shvedova *et al.*⁷ reported ultrastructural and morphological changes, formation of free radicals, accumulation of peroxidative products, antioxidant depletion, and loss of cell viability in a culture of immortalized human epidermal keratinocytes (HaCaT) exposed to unrefined SWCNTs, indicating dermal toxicity of SWCNTs. Surprisingly, Tian *et al.*³³ used highly refined SWCNTs and found that they induced more cellular apoptosis/necrosis in human fibroblast cells and proved to be more toxic than their unrefined counterpart. However, a serum supplementation to the cell culture medium probably made it non-toxic, as demonstrated recently by Yehia *et al.*³⁴, when they dispersed the purified or refined and characterized carbon nanotubes termed as DM-SWCNTs in the medium with cultured human epithelial-like HeLa cells. Even MWCNTs and MWCNOs (multiwalled carbon nano onions) have been shown to be toxic to human skin fibroblast. The toxicity was demonstrated by cell-cycle arrest and increased apoptosis/necrosis at cytotoxic doses, with MWCNOs showing ten times less cytotoxicity than MWCNTs³⁵. MWCNT cytotoxicity was also demonstrated by Monteiro-Riviere *et al.*³⁶ on human epidermal keratinocytes, where TEM examination of cells confirmed the presence of chemically unmodified MWCNTs within the cytoplasmic vacuoles. The study further showed that MWCNTs induced the release of the pro-inflammatory cytokine interleukin-8 from human epidermal keratinocytes in a time-dependent manner³⁶. Compared to oral and nasal ingestion, dermal penetration route of nanotubes into the living tissue does not warrant much attention. More so for nanoparticles with size exceeding

100 nm, with no evidence in the literature showing penetration through the skin barrier. Nevertheless, a size less than 100 nm should be thoroughly investigated³⁷.

Among other cell types tested for toxicity include mouse embryonic stem cells²⁹ and human embryo kidney cells⁹, popularly known as HEK293. These cells were evaluated for toxicity at the molecular level and CNTs were found to inhibit the proliferation of these cells by inducing cell apoptosis and decreasing cellular adhesive ability. By and large, all the above-mentioned studies report an assorted degree of CNT cytotoxicity to the cells. These discrepancies may find bases in various other issues mentioned below, rather than the cell types alone.

Bioassay of nanotoxicity

One of the most recent topics that has been added to the list of issues in nanotoxicology of carbon nanomaterials is the commonly used colorimetric and fluorescence-based assay method itself³⁸⁻⁴⁰. Bioassay of cell viability normally involves assessment of metabolic activity (using alamar blue or AB), lysosomal activity (using neutral red dye or NR), mitochondrial activity (using MTT assay), total protein content of the cells (using coomassie brilliant blue or CB assay), loss of cell membrane integrity (using adenylate kinase or AK release assay) and inflammation response (using interleukin-8 or IL-8 assay). Carbon nanotubes have been found to interact with these colorimetric and fluorescent dyes used to determine toxicity and interfere with absorption/fluorescence data. For instance, NR for cell lysosomal activity was found to adsorb onto carbon nanomaterials to yield false reading of absorption spectra³⁹. Similarly, cytokine assay was postulated to be objectionable as being the probable case of IL-8 adsorption to carbon black by the same group³⁹. Wörle-Knirsch *et al.*⁴⁰ demonstrated interference of SWCNTs with the MTT assay. Due to such interactions between organic indicator dyes and SWCNTs, many investigators are not in favour of employing such dyes for cytotoxicity screening of carbon nanoparticles^{5,39-42}. An alternative way to use these dyes ensuring complete absence of SWCNTs in the test solution was attempted²⁸ using the highly sensitive alamar blue assay that is nontoxic, water-soluble and stable in the culture medium. However, complete elimination of nanotubes from the test medium needs great care, including centrifugation, filtration and spectroscopic characterization²⁸. Nevertheless, the removal of nanotubes from the test solution or medium so that they do not interfere with the absorption spectrum of the dye, does not completely solve the problem of cytotoxicity determination; rather it creates another concern, i.e. of media nutrient depletion. A notion of an indirect cytotoxicity to the cells has developed due to media depletion of essential nutrients not being available to the cells simply because the organic

nutrients get eliminated through adsorption on hydrophobic and inherently adsorptive surfaces of carbon nanomaterials²⁸. Indeed, SWCNTs have been observed to bind various organic molecules such as sugars, proteins and culture medium components^{41,43-45} as well as lung surfactant proteins⁴⁶. Therefore, another best alternative to address the problems with colorimetric or fluorescence assay remains the old clonogenic assay that does not involve any absorbance or fluorescence measurements of indicator dyes⁴⁷⁻⁵⁰. The clonogenic assay can further distinguish between the effect, for example, of carbon nanotubes on cell viability and cell proliferation^{28,50}. Media depletion due to carbon nanomaterials and an indirect cytotoxicity to cells influencing cell proliferation^{28,50} also find support from the well-known effect of nutrient-deficient environments on cells that respond by reducing cell proliferation leading to reduced colony size⁵¹. Such indirect effect reflecting on the cytotoxicity of carbon nanomaterials may possibly be a wrong information or a false positive result in many cytotoxicity studies rather than the toxicity being an inherent property of the carbon nanomaterials themselves specially SWCNTs.

Purity vs catalyst metal contaminants in CNTs

Among the issues that complicate the matter are the catalyst metal contaminants in CNTs, which have so far been impossible to remove entirely without destroying the structural entity of CNTs. A recent report on the genetic response to CNTs indicates that risk-assessment studies of CNTs to date may be viewed as a sum of the effects of CNTs and the transition metals³⁵, some of which are known to be toxic by themselves¹. But whether impurity of CNTs is the concern that induces cytotoxicity to cells remains a dilemma when investigators find both positive and negative toxicity while using highly refined preparations. Kalbacova *et al.*³⁰ claimed using pure SWCNT free of metal catalyst contaminant as confirmed by Raman and UV-VIS-NIR spectroscopy to investigate cytotoxicity on macrophage and showed it to be toxic to monocytes/macrophage (THP-1) cells used in this study through metabolic activity assessment. On the other side of the spectrum, Fiorito *et al.*³¹ also claimed using highly purified fullerenes and SWCNT on murine and human macrophages to investigate the cytotoxic effects, but found that these materials did not stimulate the release of the inflammatory marker nitric oxide by murine macrophage cells in culture and that each possessed a low toxicity against human macrophage cells. Another such study claims focusing on the transition-metal impurity-free preparation of well-characterized homogenous solution of SWCNTs of approximately 130 nm size showing uptake into the cytoplasm and causing only low level of cytotoxicity³⁸. SWCNT with varying metal content has been evaluated for toxicity. A 30 wt%, iron-

rich SWCNT was reported to cause oxidative stress and loss of cell viability, including ultrastructural and morphological changes in human epidermal keratinocytes (HaCaT)⁷ and a 26 wt%, iron-rich SWCNT resulted in a significant loss of intracellular low molecular weight thiols (GSH) and accumulation of lipid hydroperoxides in murine macrophages³². Tian *et al.*³³ compared the refined nanomaterial that introduced the strongest toxic effect to its unrefined version and found that refined SWCNTs are more toxic than their unrefined counterparts. Yehia *et al.*³⁴ characterized and confirmed the purity of the SWCNT sample using scanning electron microscopy, thermal gravimetric analysis, atomic force microscopy, inductively coupled plasma–mass spectrometry, and absorption and Raman spectroscopies. They have concluded that dispersions of purified SWCNTs are not inherently cytotoxic to HeLa cells used. However, unlike others, serum was used in this study³⁴.

Size, size distribution and surface area of CNTs

A structure–activity relationship for asbestos-like pathogenicity linked with carbon nanotubes based on length-conforming asbestos and other pathogenic fibres has been demonstrated by Poland *et al.*⁵². The long exposure of MWCNTs to the mesothelial lining of the body cavity of mice resulted in asbestos-like, length-dependent, pathogenic behaviour, including inflammation and granulomas formation⁵². Sato *et al.*⁵³ conducted a detailed study on the effect of length on CNT cytotoxicity using the human acute monocytic leukaemia cell line THP-1 *in vitro* and could not see any significant effect. However, the degree of inflammatory response in subcutaneous tissue in rats showed length-dependent inflammation. Carbon nanofibres being lengthier compared to SWCNTs, have been shown to be comparatively more cytotoxic in a study³⁰ involving mesenchymal stem cells and monocyte/macrophage cell line THP-1. Magrez *et al.*⁵⁴ studied the cellular toxicity of MWCNTs and other carbon-based nanomaterials as a function of their aspect ratio and surface chemistry using lung tumour cells *in vitro* and found the hazardous effect to be size-dependent. Kang *et al.*⁵⁵ conducted experiments to show the antibacterial effect of the size of SWCNTs and MWCNTs. They found that SWCNTs were much more toxic to bacteria than MWCNTs, attributing size to the degree of cytotoxicity. Similarly, ultrafine/nanoparticles were found to produce enhanced toxicity responses compared with larger-sized particles of similar chemical composition^{56,57}. These investigators have also indicated transmigration of ultrafine/nanoparticles to the pulmonary interstitium escaping alveolar macrophage watch^{56,57}. Tian *et al.*³³ found surface area as an important variable that best predicts the potential toxicity of these refined carbon nanomaterials. SWCNTs produced by high pressure carbon monoxide

(HiPCO) chemical vapour deposition process^{58,59} having greater surface area showed higher toxicity compared to SWCNTs produced by arc discharge method, in media depletion experiments²⁸. However, while considering various carbonaceous materials, Raja *et al.*⁶⁰ reported an inversely proportional relationship between carbon nanomaterial size regimes and cell growth inhibition.

Surface chemistry and surface reactivity at the CNT interface

Particle surface and interfaces have significance as important nanoscale material components. With a reduction in particle size, surface atoms are proportionately enhanced compared to the proportion inside its volume, resulting in more reactive nanoscale particles. Reactive surfaces may act as an effective catalyst, but in terms of biological use reactive groups present on surfaces may find health implications, thereby making surface chemistry at the interface or of the shell, an important parameter to look at from the toxicity point of view. Pertaining to lung cytotoxicity, Warheit *et al.*^{61,62} have indicated the importance of particle surface reactivity in playing a definite role in terms of eliciting inflammatory response rather than the core particle or simply the particle size and surface area. Saxena *et al.*⁶³ demonstrated the role of surface charge present on acid-functionalized SWCNT preparation unlike pristine SWCNTs in eliciting strong cytotoxicity *in vitro* and *in vivo*, that could be reversed by neutralizing their surface charge with poly (L)-lysine.

Dispersion/suspension, aggregation status and sedimentation of CNTs

Another issue is the lack of methods to prepare water-soluble CNT particles that are homogeneous enough to ensure validity of the studies on the alleged nanosize effects. The uncontrollable aggregation behaviour of CNTs, for instance, bundle formation, poses a primary problem that hampers risk assessment studies^{4,5}. Therefore, an effort to achieve a stable suspension of CNTs in water has been a familiar concern^{64–76}. While most specific ways to solubilize CNTs are mentioned in the next section dedicated to functionalization of nanotubes, we summarize the commonly used surfactant-based methods here. Even though the use of surfactant is straightforward and simple, it may be noted that the surfactants used widely for solubilization of CNTs^{77,78} are toxic by themselves and must therefore be avoided. Monteiro-Riviere *et al.*⁷⁹ used nontoxic surfactants, namely pluronic F127 and Tween, to see the effect on reducing aggregation of MWCNTs and achieving dispersion to confirm whether large aggregates of MWCNTs had any contribution to cytotoxicity. This study⁷⁹ found surfactants to disperse and

reduce MWCNT aggregation in the medium. However, MWCNTs were found to be cytotoxic to cells independent of surfactant exposure. Polyoxyethylene sorbitan monooleate, yet another surfactant, was found to well-disperse CNTs and is also considered to be nontoxic⁸⁰. While comparing the cytotoxic effects of well-dispersed CNTs using polyoxyethylene sorbitan monooleate with that of conventionally purified, rope-like agglomerated CNTs and asbestos as a reference, suspended CNT bundles were found less cytotoxic than asbestos⁸⁰. Further, the rope-like agglomerates induced more pronounced cytotoxic effects than asbestos fibres at the same concentrations⁸⁰. Murr *et al.*¹⁹ used well-characterized, SWCNTs (ropes) and two different MWCNT aggregates with 1–2 µm mean diameter as shown by TEM and found strong concentration and toxicity relationship for all the carbon nanotube materials relative to the chrysotile asbestos nanotubes and black carbon nanoaggregates being used as toxicity standards. Raja *et al.*⁶⁰ tested the isolated effect of SWCNT aggregates by subjecting rat aortic smooth muscle cells (SMC) to grow in two types of media, both exposed to SWCNTs but later, one type was made free from SWCNT through filtering and the other type was used as such with suspended SWCNT aggregates in it. At low dosage of SWCNTs (below 0.1 mg/ml), removal of nanotube aggregates ensured better growth of SMC in the filtered medium compared to the unfiltered medium. But at 0.1 mg/ml cell growth was affected equally in both filtered and unfiltered media relative to the control. Soto *et al.*⁸¹ demonstrated varying degrees of the cytotoxic effect of a series of nanomaterials, including MWCNT aggregates on murine alveolar macrophage cell line and human macrophage and epithelial lung cell lines.

Coating or functionalization and immobilization of CNTs

Functionalization of the CNTs seems important as solubilization and stable suspension has been reported to be influenced by functionalization⁷⁶. Most importantly, solubilization through functionalization rules out a toxic effect by avoiding the use of surfactants. Several investigators have attempted specific physico-chemical ways to functionalize the nanotubes to achieve solubilization^{82–100}. Recent cytotoxicity studies on carbon nanotubes have shown that the biocompatibility of nanomaterials might be determined mainly by surface functionalization, rather than by size, shape and material^{101,102}. Hu *et al.*¹² reported the use of chemically modified carbon nanotubes as a substrate for cultured neurons, where they systematically varied the chemical properties of carbon nanotubes by attaching different functional groups that conferred known characteristics to the substrate. By manipulating the charge carried by functionalized carbon nanotubes, they could control the outgrowth and branching pattern of

neuronal processes. Magrez *et al.*⁵⁴ studied the cellular toxicity of MWCNTs and other carbon-based nanomaterials as a function of their aspect ratio and surface chemistry using lung tumour cells *in vitro*. They found that the carbon nanomaterials were toxic and cytotoxicity was enhanced when the surface of the particles was functionalized after an acid treatment. Contradictory to this, Sayes *et al.*¹⁰² showed that the cytotoxic response of cells in culture was dependent on the degree of functionalization of the SWCNT, but with an inverse relation; as the degree of sidewall functionalization increased; the SWCNT sample became less cytotoxic. However, functional groups attached to the carbon nanotubes were different in these two studies^{54,102}. Zhang *et al.*¹⁰³ showed that the lower concentration of 5 ng/ml of 6-amino-hexanoic acid-derivatized SWCNTs (AHA-SWCNTs) maintains cell viability and induces a mild cytotoxicity, but 50,000 ng/ml of AHA-SWCNTs demonstrated an irritation response by an increase in IL-8. While proper functionalization of CNTs facilitates their solubilization and makes them suitable for a given application, there is an apprehension that this may also accelerate their uptake in the systemic circulation, thereby sourcing their translocation and distribution to different organs of the body^{4,104}.

Cellular uptake or internalization of CNTs

There have been difficulties in spotting the CNTs entering the cells and differentiating them from other organic carbon-based cell structures, such as membranes. This has warranted the need for alternative ways to study their uptake and cytotoxic effects in cells. A recent study by Alexandra *et al.*¹⁰⁵ shows that once the CNTs are inside the cell, they accumulate in the cytoplasm and cause cell death in a dose-dependent manner. Chemically unmodified MWCNT uptake was demonstrated by Monteiro-Riviere *et al.*³⁶ with human epidermal keratinocytes under TEM examination. They confirmed the presence of MWCNTs within the cytoplasmic vacuoles. Yehia *et al.*³⁴ used confocal micro Raman spectroscopy to demonstrate that SWCNTs were taken up by HeLa cells in a time- and temperature-dependent fashion. They also used TEM that spotted SWCNT-like materials in intracellular vacuoles. Earlier, Kam *et al.*²³ also observed internalization of functionalized carbon nanotubes by adherent as well as nonadherent human cancer cells showing no toxicity to the cells. However, when a fluorescinated protein, streptavidin that cannot enter the cells by itself was allowed to bind biotin-functionalized nanotubes, it could be internalized through adsorption-mediated endocytosis of the so-called CNT-cargo complex and caused dose-dependent cytotoxicity. Similarly, internalization of MWCNTs by phagocytic cells and brain cells was demonstrated and was not found toxic

to the cells tested, suggesting the potential use of MWCNTs as a novel, non-toxic, and biodegradable nano-vehicle for targeted immunotherapy in brain cancers¹⁰⁶. Recently, Saxena *et al.*¹⁰⁷ have patented a new technique to isolate deposited carbon particles from lung epithelial cells and alveolar macrophages.

CNT nanocomposites and biocompatibility

CNTs can form several categories of nanobiocomposites, including biometals, bioceramics such as hydroxyapatite, tricalcium phosphate, wollastonite, bioactive glasses and polymer (natural and synthetic) e.g. collagen, polyhydroxyalkanoates, polylactides and polyglycolides. Other general-purpose, hydrogel-forming polymers are polyethylene oxide, polyvinyl alcohol, polyvinyl pyrrolidone and polyhydroxyethyl methacrylate.

Several investigators who worked with nanocomposites of CNT with biomaterials have reported non-cytotoxicity of CNTs^{108–110}. The application of 'fixed' or embedded CNTs in nondegradable nanocomposite scaffolds has been felt advantageous over 'loose' or unattached CNTs from a toxicological point of view¹⁰⁸. Purified SWCNTs, SWCNTs functionalized with 4-*tert*-butylphenylene, and ultra-short SWCNTs at 1–100 µg/ml concentrations were mixed with biodegradable poly(propylene fumarate) and the crosslinking agent propylene fumarate-diacrylate to prepare injectable nanocomposites, which when tested for cytotoxicity to fibroblast cell line *in vitro* were not found to be toxic. Nearly 100% cell viability was observed on all crosslinked nanocomposites, except the degradation products of the nanocomposites that displayed a dose-dependent adverse effect on the cells¹⁰⁹. Kawaguchi *et al.*¹¹⁰ reported non-cytotoxicity and usefulness of CNT-alginate hydrogel nanocomposite as a scaffold material in tissue engineering. Studies of bone cell interactions with the 3D polyurethane and CNT nanocomposite foams were not found cytotoxic and revealed no detrimental effects on osteoblast differentiation or mineralization¹⁰⁸.

Paradigms for the mechanism of CNT cytotoxicity

Among the current hypothesized toxicity mechanisms – disruption of intracellular metabolic pathways, oxidative stress, and physical membrane damage causing ruptures – the generation of reactive oxygen species (ROS) and oxidative stress is the most developed paradigm for the mechanism of CNT toxicity⁵⁵. All stress-related reports on toxicity are mostly investigated at the molecular level exploring the genotoxicity of CNTs in mammalian cells. Cytotoxic doses of MWCNTs induce cell-cycle arrest, increase apoptosis/necrosis, perturb multiple cellular pathways, activate genes involved in cellular transport,

metabolism, cell-cycle regulation, stress response and show that interferon and p38/ERK-MAPK cascades are critical pathway components in the induced signal transduction³⁵. Cui *et al.*⁹ carried out extensive molecular characterization of HEK293 responses to SWCNTs showing secretion of some 20–30 kDa proteins, aggregation of cells attached by SWCNTs, G1 arrest and cell apoptosis, up-regulation expression of cell cycle-associated genes such as *p16*, *bax*, *p57*, *hrk*, *cdc42* and *cdc37*, down-regulation expression of cell-cycle genes such as *cdk2*, *cdk4*, *cdk6* and *cyclin D3*, and down-regulation expression of signal transduction-associated genes such as *mad2*, *jak1*, *ttk*, *pcdha9* and *erk* and of adhesion-associated proteins such as laminin, fibronectin, cadherin, FAK and collagen IV, suggesting that down-regulation of G1-associated *cdks* and *cyclins* and upregulation of apoptosis-associated genes may contribute to SWCNT-induced G1 phase arrest and cell apoptosis. Zhu *et al.*¹¹¹ assessed the DNA damage response to MWCNTs in mouse embryonic stem (ES) cells and found that MWCNTs can accumulate and induce apoptosis in mouse ES cells and activate the tumour suppressor protein p53 within 2 h of exposure. They have warned for a careful scrutiny of the genotoxicity of nanomaterials like MWCNTs reportedly to be of limited or no toxicity. Garza *et al.*¹¹² have investigated the cytotoxicity and ROS generation for various carbonaceous materials, including MWCNT aggregates. The data demonstrate that cytotoxicity is related to ROS generation.

Conclusion and future considerations

As mentioned in the preceding sections, there exist discrepancies among the reports on cytotoxicity of CNTs. A close look at the published literature on this issue reveals that the resulting positive or negative reports on cytotoxicity may be due to the way CNTs have been used in the experiments by different researchers. By and large, two ways could be identified. First, CNTs are used as suspension in the media and secondly, they are immobilized as a layer, on a culture dish using a polymer or through functionalization to the scaffold in tissue-engineering pursuits. Invariably CNTs are shown to be toxic to cells when used as a suspension in cell culture media in any given experiment, while they appear as non-toxic if immobilized to a matrix or to a culture dish. A summary of such trends reported in various studies is given in Table 1.

Further, nanocombinatorial library approach may be a good futuristic consideration in nanomedicine and nanotoxicity research, as recently demonstrated by Zhou *et al.*¹¹³. Finally, adequate material characterization remains a challenging and exhaustive exercise to be practised by all prudent investigators in order to make the cytotoxicity results meaningful in future¹¹⁴.

REVIEW ARTICLES

Table 1. Cytotoxicity trend as dictated by different modes of carbon nanotube (CNTs) usage irrespective of various other issues mentioned in this review

Experimentation	Cell type	Toxicity result
CNTs in suspension Suspended in cell culture media ^{9,27,33,35,54,60,80,111,115}	Human HEK293 cells ⁹ , human fibroblast ³³ , 3T3 fibroblast cell ¹¹⁵ , mesothelioma cell line (MSTO-211H) ⁸⁰ , lung tumour cell lines (H596, H446, Calu-1) ⁵⁴ , human skin fibroblast (HSF42) and human embryonic lung fibroblast (IMR-90) ³⁵ , human A549 lung cell ²⁷ , smooth muscle cells ⁶⁰ , stem cells ¹¹¹	+
CNTs on substratum, adhered/static Nonwoven SWCNTs with nanotopographic structure and macroscopic volume ¹¹⁶ ; chemically modified CNT to have neutral, negative and zwitterionic charge ¹¹⁷ ; SWCNT films on poly styrene ³⁰ ; CNTs as substrate for neuronal growth ¹²	3T3-L1 mouse fibroblasts ¹¹⁶ , osteoblast ¹¹⁷ , mesenchymal cells, monocytes/macrophage ³⁰ , neurons ¹²	-
CNTs composite CNF and PCU ¹¹⁸ ; MWCNT and hydroxyapatite ¹¹⁹ ; MWCNT and hydroxyapatite ¹²⁰ ; CNT and collagen composite ¹⁴ ; porous ultra-short SWCNT nanocomposite ¹²¹ ; PLA-CNT conducting polymer nanocomposite ¹²² ; polystyrene-SWCNT nanocomposite ¹²³	Osteoblast ^{118,119,122} , rat aortic smooth muscle cells ¹⁴ , MSC ¹²¹ , mouse fibroblast (L-929) ¹²³	-

+, Toxic; -, Non-toxic.

- Colvin, V. L., The potential environmental impact of engineered nanomaterials. *Nature Biotechnol.*, 2003, **21**, 1166–1170.
- Service, R. F., Nanotechnology grows up. *Science*, 2004, **304**, 1732–1734.
- Service, R. F., Calls rise for more research on toxicology of nanomaterials. *Science*, 2005, **310**, 1609.
- Oberdorster, G. *et al.*, Principles for characterizing the potential human health effects from exposure to nanomaterials: elements of a screening strategy. *Part. Fibre Toxicol.*, 2005, **2**, 8–43.
- Hurt, R. H., Monthieux, M. and Kane, A., Toxicology of carbon nanomaterials: Status, trends, and perspectives on the special issue. *Carbon*, 2006, **44**, 1028–1033.
- Smart, S. K., Cassady, A. I., Lu, G. Q. and Martin, D. J., The biocompatibility of carbon nanotubes. *Carbon*, 2006, **44**, 1034–1047.
- Shvedova, A. A. *et al.*, Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells. *J. Toxicol. Environ. Health A*, 2003, **66**, 1909–1926.
- McKenzie, J. L., Waid, M. C., Shi, R. and Webster, T. J., Decreased functions of astrocytes on carbon nanofiber materials. *Biomaterials*, 2004, **25**, 1309–1317.
- Cui, D., Tian, F., Ozkan, C. S., Wang, M. and Gao, H., Effect of single wall carbon nanotubes on human HEK293 cells. *Toxicol. Lett.*, 2005, **155**, 73–85.
- Park, K. H., Chhowalla, M., Iqbal, Z. and Sesti, F., Single-walled carbon nanotubes are a new class of ion channel blockers. *J. Biol. Chem.*, 2003, **278**, 50212–50216.
- Mattson, M. P., Haddon, R. C. and Rao, A., Molecular functionalization of carbon nanotubes and use as substrates for neuronal growth. *J. Mol. Neurosci.*, 2000, **14**, 175–182.
- Hu, H., Ni, Y., Montana, V., Haddon, R. C. and Parpura, V., Chemically functionalized carbon nanotubes as substrates for neuronal growth. *Nano Lett.*, 2004, **4**, 507–511.
- Hu, H., Ni, Y., Mandal, S. K., Montana, V., Zhao, B., Haddon, R. C. and Parpura, V., Polyethyleneimine functionalized single-walled carbon nanotubes as a substrate for neuronal growth. *J. Phys. Chem. B*, 2005, **109**, 4285–4289.
- Macdonal, R. A., Laurenzi, B. F., Viswanathan, G., Ajayan, P. M. and Stegemann, J. P., Collagen-carbon nanotube composite materials as scaffolds in tissue engineering. *J. Biomed. Mater. Res. A*, 2005, **74**, 489–496.
- Zhao, B., Hu, H., Mandal, S. K. and Haddon, R. C., A bone mimic based on the self-assembly of hydroxyapatite on chemically functionalized single-walled carbon nanotubes. *Chem. Mater.*, 2005, **17**, 3235–3241.
- Warheit, D. B., Laurence, B. R., Reed, K. L., Roach, D. H., Reynolds, G. A. M. and Webb, T. R., Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. *Toxicol. Sci.*, 2004, **77**, 117–125.
- Lam, C. W., James, J. T., McCluskey, R. and Hunter, R. L., Pulmonary toxicity of single-wall carbon nanotubes in mice 7 and 90 days after intratracheal instillation. *Toxicol. Sci.*, 2004, **77**, 126–134.
- Jia, G. *et al.*, Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene. *Environ. Sci. Technol.*, 2005, **39**, 1378–1383.
- Murr, L. E. *et al.*, Cytotoxicity assessment of some carbon nanotubes and related carbon nanoparticle aggregates and the implications for anthropogenic carbon nanotube aggregates in the environment. *Int. J. Environ. Res. Public Health*, 2005, **2**, 31–42.
- Soto, K. F., Carrasco, A., Powell, T. G., Garza, K. M. and Murr, L. E., Comparative *in vitro* cytotoxicity assessment of some manufactured nanoparticulate materials characterized by transmission electron microscopy. *J. Nanopart. Res.*, 2005, **7**, 145–169.
- Manna, S. K. *et al.*, Single walled carbon nanotube induces oxidative stress and activates nuclear transcription factor-kB in human keratinocytes. *Nano Lett.*, 2005, **5**, 1676–1684.
- Pantarotto, D., Briand, J. P., Prato, M. and Bianco, A., Translocation of bioactive peptides across cell membranes by carbon nanotubes. *Chem. Commun.*, 2004, 16–17.
- Kam, N. W. S., Jessop, T. C., Wender, P. A. and Dai, H., Nanotube molecular transporters: internalization of carbon nanotube-protein conjugates into mammalian cells. *J. Am. Chem. Soc.*, 2004, **126**, 6850–6851.

24. Murakami, T., Ajima, K., Miyawaki, J., Yudasaka, M., Iijima, S. and Shiba, K., Drug-loaded carbon nanohorns: adsorption and release of dexamethasone *in vitro*. *Mol. Pharm.*, 2004, **1**, 399–405.
25. Warheit, D. B., What is currently known about the health risks related to carbon nanotube exposures? *Carbon*, 2006, **44**, 1064–1069.
26. Han, S. G., Andrews, R., Gairola, C. G. and Bhalla, D. K., Acute pulmonary effects of combined exposure to carbon nanotubes and ozone in mice. *Inhal. Toxicol.*, 2008, **20**, 391–398.
27. Davoren, M., Herzog, E., Casey, A., Cottineau, B., Chambers, G., Byrne, H. J. and Lyng, F. M., *In vitro* toxicity evaluation of single walled carbon nanotubes on human A549 lung cells. *Toxicol. in Vitro*, 2007, **21**, 438–448.
28. Casey, A., Herzog, E., Lyng, F. M., Byrne, H. J., Chambers, G. and Davoren, M., Single walled carbon nanotubes induce indirect cytotoxicity by medium depletion in A549 lung cells. *Toxicol. Lett.*, 2008, **179**, 78–84.
29. Kisin, E. R. *et al.*, Single-walled carbon nanotubes: geno- and cytotoxic effects in lung fibroblast V79 cells. *J. Toxicol. Environ. Health A*, 2007, **70**, 2071–2079.
30. Kalbacova, M., Kalbac, M., Dunsch, L., Kataura, H. and Hempel, U., The study of the interaction of human mesenchymal stem cells and monocytes/macrophages with single-walled carbon nanotube films. *Phys. Stat. Sol. B*, 2006, **243**, 3514–3518.
31. Fiorito, S., Serafino, A., Andreola, F. and Bernier, P., Effects of fullerenes and single-wall carbon nanotubes on murine and human macrophages. *Carbon*, 2006, **44**, 1100–1105.
32. Kagan, V. E. *et al.*, Direct and indirect effects of single walled carbon nanotubes on RAW 264.7 macrophages: role of iron. *Toxicol. Lett.*, 2006, **165**, 88–100.
33. Tian, F., Cui, D., Schwarz, H., Estrada, G. G. and Kobayashi, H., Cytotoxicity of single-wall carbon nanotubes on human fibroblasts. *Toxicol. in Vitro*, 2006, **20**, 1202–1212.
34. Yehia, H. N. *et al.*, Single-walled carbon nanotube interactions with HeLa cells. *J. Nanobiotechnol.*, 2007, **5**, 8–25.
35. Ding, L. *et al.*, Molecular characterization of the cytotoxic mechanism of multiwall carbon nanotubes and nano-onions on human skin fibroblast. *Nano Lett.*, 2005, **5**, 2448–2464.
36. Monteiro-Riviere, N. A., Nemanich, R. J., Inman, A. O., Wang, Y. Y. and Riviere, J. E., Multi-walled carbon nanotube interactions with human epidermal keratinocytes. *Toxicol. Lett.*, 2005, **155**, 377–384.
37. Warheit, D. B., Borm, P. J., Hennes, C. and Lademann, J., Testing strategies to establish the safety of nanomaterials: conclusions of an ECETOC workshop. *Inhal. Toxicol.*, 2007, **19**, 631–643.
38. Isobe, H. *et al.*, Preparation, purification, characterization, and cytotoxicity assessment of water-soluble, transition-metal-free carbon nanotube aggregates. *Angew. Chem. Int. Ed. Engl.*, 2006, **45**, 6676–6680.
39. Monteiro-Riviere, N. A. and Inman, A. O., Challenges for assessing carbon nanomaterial toxicity to the skin. *Carbon*, 2006, **44**, 1070–1078.
40. Wörle-Knirsch, J. M., Pulskamp, K. and Krug, H. F., Oops they did it again! Carbon nanotubes hoax scientists in viability assays. *Nano Lett.*, 2006, **6**, 1261–1268.
41. Casey, A., Davoren, M., Herzog, E., Lyng, F. M., Byrne, H. J. and Chambers, G., Probing the interaction of single walled carbon nanotubes within cell culture medium as a precursor to toxicity testing. *Carbon*, 2007, **45**, 34–40.
42. Casey, A., Herzog, E., Davoren, M., Lyng, F. M., Byrne, H. J. and Chambers, G., Spectroscopic analysis confirms the interactions between single walled carbon nanotubes and various dyes commonly used to assess cytotoxicity. *Carbon*, 2007, **45**, 1425–1432.
43. Chambers, G., Carroll, C., Farrell, G. F., Dalton, A. B., McNamara, M., in het Panhuis, M. and Byrne, H. J., Characterization of the interaction of gamma cyclodextrins with single walled carbon nanotubes. *Nanoletters*, 2003, **3**, 843–846.
44. Casey, A., Farrell, G. F., McNamara, M., Byrne, H. J. and Chambers, G., Interaction of carbon nanotubes with sugar complexes. *Synth. Met.*, 2005, **153**, 357–360.
45. Moulton, S. E. *et al.*, Biomolecules as selective dispersants for carbon nanotubes. *Carbon*, 2005, **43**, 1879–1884.
46. Salvador-Morales, C., Flahaut, E., Sim, E., Sloan, J., Green, M. L. H. and Sim, R. B., Complement activation and protein adsorption by carbon nanotubes. *Mol. Immunol.*, 2006, **43**, 193–201.
47. Puck, T. T. and Markus, P. I., Action of X-rays on mammalian cells. *J. Exp. Med.*, 1956, **103**, 653–666.
48. Rober, P. R. and Drewinko, B., Comparison of *in vitro* methods to determine drug induced cell lethality. *Cancer Res.*, 1976, **36**, 2182–2188.
49. Horáková, K., Švičiková, A., Seemannová, Z., Syrová, D., Bušányová, K., Drobná, Z. and Ferenčík, M., Detection of drug-induced, superoxide-mediated cell damage and its prevention by antioxidants. *Free Radical Biol. Med.*, 2001, **3**, 650–664.
50. Herzog, E., Casey, A., Lyng, F. M., Chambers, G., Byrne, H. J. and Davoren, M., A new approach to the toxicity testing of carbon-based nanomaterials – the clonogenic assay. *Toxicol. Lett.*, 2007, **174**, 49–60.
51. Ozturk, S., Kaseko, G., Mahaworasilpa, A. T. and Coster, H. G. L., Adaption of cell lines to serum-free culture medium. *Hybridoma Hybridomics*, 2003, **22**, 267–272.
52. Poland, C. A. *et al.*, Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nature Nanotechnol.*, 2008, **3**, 423–428.
53. Sato, Y. *et al.*, Influence of length on cytotoxicity of multi-walled carbon nanotubes against human acute monocytic leukemia cell line THP-1 *in vitro* and subcutaneous tissue of rats *in vivo*. *Mol. Biosyst.*, 2005, **1**, 176–182.
54. Magrez, A. *et al.*, Cellular toxicity of carbon-based nanomaterials. *Nano Lett.*, 2006, **6**, 1121–1125.
55. Kang, S., Herzberg, M., Rodrigues, D. F. and Elimelech, M., Antibacterial effects of carbon nanotubes: size does matter! *Langmuir*, 30 May 2008 [Epub ahead of print].
56. Donaldson, K., Stone, V., Clouter, A., Renwick, L. and MacNee, W., Ultrafine particles. *Occup. Environ. Med.*, 2001, **58**, 211–216.
57. Oberdorster, G., Toxicology of ultrafine particles: *In vivo* studies. *Philos. Trans. R. Soc. London, Ser. A*, 2000, **358**, 2719–2740.
58. Nikolaev, P., Bronikowski, M. J., Bradley, R. K., Rohmund, E., Colbert, D. T., Smith, K. A. and Smalley, R. E., Gas-phase catalytic growth of single-walled carbon nanotubes from carbon monoxide. *Chem. Phys. Lett.*, 1999, **313**, 91–97.
59. Bronikowski, M. J., Willis, P. A., Colbert, D. T., Smith, K. A. and Smalley, R. E., Gas-phase production of carbon single-walled nanotubes from carbon monoxide via the HiPco process: A parametric study. *J. Vac. Sci. Technol.*, 2001, **A19**, 1800–1805.
60. Raja, P. M. V., Connolley, J., Ganesan, G. P., Ci, L., Ajayan, P. M., Nalamasu, O. and Thompson, D. M., Impact of carbon nanotube exposure, dosage and aggregation on smooth muscle cells. *Toxicol. Lett.*, 2007, **169**, 51–63.
61. Warheit, D. B., Webb, T. R., Colvin, V. L., Reed, K. L. and Sayes, C. M., Pulmonary bioassay studies with nanoscale and fine quartz particles in rats: toxicity is not dependent upon particle size but on surface characteristics. *Toxicol. Sci.*, 2007, **95**, 270–280.
62. Warheit, D. B., Webb, T. R., Reed, K. L., Frerichs, S. and Sayes, C. M., Pulmonary toxicity study in rats with three forms of ultrafine-TiO₂ particles: differential responses related to surface properties. *Toxicology*, 2007, **230**, 90–104.
63. Saxena, R. K., Williams, W., Mcgee, J. K., Daniels, M. J., Boykin, E. and Gilmour, M. I., Enhanced *in vitro* and *in vivo*

- toxicity of poly-dispersed acid-functionalized single-wall carbon nanotubes. *Nanotoxicology*, 2007, **1**, 291–300.
64. Chen, J., Hamon, M. A., Hu, H., Chen, Y., Rao, A. M., Eklund, P. C. and Haddon, R. C., Solution properties of single-walled carbon nanotubes. *Science*, 1998, **282**, 95–98.
65. Chen, J. *et al.*, Dissolution of full-length single-walled carbon nanotubes. *J. Phys. Chem. B*, 2001, **105**, 2525–2528.
66. Mickelson, E. T. *et al.*, Solvation of fluorinated single-wall carbon nanotubes in alcohol solvents. *J. Phys. Chem. B*, 1999, **103**, 4318–4322.
67. O'Connell, M. J. *et al.*, Reversible water-solubilization of single-walled carbon nanotubes by polymer wrapping. *Chem. Phys. Lett.*, 2001, **342**, 265–271.
68. Pompeo, F. and Resasco, D. E., Water solubilization of single-walled carbon nanotubes by functionalization with glucosamine. *Nano Lett.*, 2002, **2**, 369–373.
69. Georgakilas, V., Kordatos, K., Prato, M., Guldi, D. M., Holzinger, M. and Hirsch, A., Organic functionalization of carbon nanotubes. *J. Am. Chem. Soc.*, 2002, **124**, 760–761.
70. Huang, W. *et al.*, *Nano Lett.*, 2002, **2**, 311.
71. Feng, L., Li, H., Li, F., Shi, Z. and Gu, Z., Functionalization of carbon nanotubes with amphiphilic molecules and their Langmuir–Blodgett films. *Carbon*, 2003, **41**, 2385–2391.
72. Peng, H., Alemany, L. B., Margrave, J. L. and Khabashesku, V. N., Sidewall carboxylic acid functionalization of single-walled carbon nanotubes. *J. Am. Chem. Soc.*, 2003, **125**, 15174–15182.
73. Matsuura, K., Hayashi, K. and Kimizuka, N., Lectin-mediated supramolecular junctions of galactose-derivatized single-walled carbon nanotubes. *Chem. Lett.*, 2003, **32**, 212–213.
74. Lin, Y., Allard, L. F. and Sun, Y.-P., Protein-affinity of single-walled carbon nanotubes in water. *J. Phys. Chem. B*, 2004, **108**, 3760–3764.
75. Qin, S., Qin, D., Ford, W. T., Resasco, D. E. and Herrera, J. E., Functionalization of single-walled carbon nanotubes with polystyrene via grafting to and grafting from methods. *Macromolecules*, 2004, **37**, 752–757.
76. Wang, Y., Iqbal, Z. and Mitra, S., Rapidly functionalized, water-dispersed carbon nanotubes at high concentration. *J. Am. Chem. Soc.*, 2006, **128**, 95–99.
77. O'Connell, M. J. *et al.*, Band gap fluorescence from individual single-walled carbon nanotubes. *Science*, 2002, **297**, 593–596.
78. Zhang, M., Yudasaka, M., Miyawaki, J., Fan, J. and Iijima, S., Isolating single-wall carbon nanohorns as small aggregates through a dispersion method. *J. Phys. Chem. B*, 2005, **109**, 22201–22204.
79. Monteiro-Riviere, N. A., Inman, A. O., Wang, Y. Y. and Nemanich, R. J., Surfactant effects on carbon nanotube interactions with human keratinocytes. *Nanomedicine*, 2005, **1**, 293–299.
80. Wick, P. *et al.*, The degree and kind of agglomeration affect carbon nanotube cytotoxicity. *Toxicol. Lett.*, 2007, **168**, 121–131.
81. Soto, K., Garza, K. M. and Murr, L. E., Cytotoxic effects of aggregated nanomaterials. *Acta Biomater.*, 2007, **3**, 351–358.
82. Ikeda, A., Tanaka, Y., Nobusawa, K. and Kikuchi, J., Solubilization of single-walled carbon nanotubes by supramolecular complexes of barbituric acid and triaminopyrimidines. *Langmuir*, 2007, **23**, 10913–10915.
83. Ogoshi, T., Inagaki, A., Yamagishi, T. and Nakamoto, Y., Defection-selective solubilization and chemically-responsive solubility switching of single-walled carbon nanotubes with cucurbit[7]uril. *Chem. Commun.*, 2008, 2245–2247.
84. Huang, W., Fernando, S., Lin, Y., Zhou, B., Allard, L. F. and Sun, Y. P., Preferential solubilization of smaller single-walled carbon nanotubes in sequential functionalization reactions. *Langmuir*, 2003, **19**, 7084–7088.
85. Fei, B., Lu, H., Hu, Z. and Xin, J. H., Solubilization, purification and functionalization of carbon nanotubes using polyoxometalate. *Nanotechnology*, 2006, **17**, 1589–1593.
86. Nobusawa, K., Ikeda, A., Kikuchi, J., Kawano, S., Fujita, N. and Shinkai, S., Reversible solubilization and precipitation of carbon nanotubes through oxidation–reduction reactions of a solubilizing agent. *Angew. Chem., Int. Ed. Engl.*, 2008, **47**, 4577–4580.
87. Wang, J., Musameh, M. and Lin, Y., Solubilization of carbon nanotubes by nafion toward the preparation of amperometric biosensors. *J. Am. Chem. Soc.*, 2003, **125**, 2408–2409.
88. Wang, D. and Chen, L., Temperature and pH-responsive single-walled carbon nanotube dispersions. *Nano Lett.*, 2007, **7**, 1480–1484.
89. Banerjee, S., Kahn, M. G. C. and Wong, S. S., Rational chemical strategies for carbon nanotube functionalization. *Chem. Eur. J.*, 2003, **9**, 1898–1908.
90. Chen, J., Liu, H., Weimer, W. A., Halls, M. D., Waldeck, D. H. and Walker, G. C., Noncovalent engineering of carbon nanotube surfaces by rigid, functional conjugated polymers. *J. Am. Chem. Soc.*, 2002, **124**, 9034–9035.
91. Wu, Y. *et al.*, Coating single-walled carbon nanotubes with phospholipids. *J. Phys. Chem. B*, 2006, **110**, 2475–2478.
92. Karajanagi, S. S., Yang, H., Asuri, P., Sellitto, E., Dordick, J. S. and Kane, R. S., Protein-assisted solubilization of single-walled carbon nanotubes. *Langmuir*, 2006, **22**, 1392–1395.
93. Liang, Z. *et al.*, Solubilization of single-walled carbon nanotubes with single stranded DNA generated from asymmetric PCR. *Int. J. Mol. Sci.*, 2007, **8**, 705–713.
94. Cheng, F. and Adronov, A., Noncovalent functionalization and solubilization of carbon nanotubes by using a conjugated Zn-porphyrin polymer. *Chem. Eur. J.*, 2006, **12**, 5053–5059.
95. Ortiz-Acevedo, A. *et al.*, Diameter-selective solubilization of single-walled carbon nanotubes by reversible cyclic peptides. *J. Am. Chem. Soc.*, 2005, **127**, 9512–9517.
96. Kim, O. K., Je, J., Baldwin, J. W., Kooi, S., Pehrsson, P. E. and Buckley, L. J., Solubilization of single-wall carbon nanotubes by supramolecular encapsulation of helical amylose. *J. Am. Chem. Soc.*, 2003, **125**, 4426–4427.
97. Murakami, T., Fan, J., Yudasaka, M., Iijimaand, S. and Shiba, K., Solubilization of single-wall carbon nanohorns using a PEG-doxorubicin conjugate. *Mol. Pharm.*, 2006, **3**, 407–414.
98. Tomonari, Y., Murakami, H. and Nakashima, N., Solubilization of single-walled carbon nanotubes by using polycyclic aromatic ammonium amphiphiles in water – strategy for the design of high-performance solubilizers. *Chem. Eur. J.*, 2006, **12**, 4027–4034.
99. Liu, L., Qin, Y., Guo, Z. X. and Zhu, D., Reduction of solubilized multi-walled carbon nanotubes. *Carbon*, 2003, **41**, 331–335.
100. Ishibashi, A. and Nakashima, N., Individual dissolution of single-walled carbon nanotubes in aqueous solutions of steroid or sugar compounds and their Raman and near-IR spectral properties. *Chem. Eur. J.*, 2006, **12**, 7595–7602.
101. Singh, R. *et al.*, Tissue biodistribution and blood clearance rates of intravenously administered carbon nanotube radiotracers. *Proc. Natl. Acad. Sci. USA*, 2006, **103**, 3357–3362.
102. Sayes, C. M. *et al.*, Functionalization density dependence of single-walled carbon nanotubes cytotoxicity *in vitro*. *Toxicol. Lett.*, 2006, **161**, 135–142.
103. Zhang, L. W., Zeng, L., Barron, A. R. and Monteiro-Riviere, N. A., Biological interactions of functionalized single-wall carbon nanotubes in human epidermal keratinocytes. *Int. J. Toxicol.*, 2007, **26**, 103–113.
104. Borm, P. J. A. *et al.*, The potential risks of nanomaterials: A review carried out for ECETOC. *Part. Fibre Toxicol.*, 2006, **3**, 11–46.
105. Alexandra, P., Direct imaging of single-walled carbon nanotubes in cells. *Nature Nanotechnol.*, 2007, **2**, 713–717.

106. Kateb, B., Handel, M. V., Zhang, L., Bronikowski, M. J., Manohara, H. and Badie, B., Internalization of MWCNTs by microglia: possible application in immunotherapy of brain tumors. *NeuroImage*, 2007, **37**, S9–S17.
107. Saxena, R. K., Gilmour, M. I. and Hays, M. D., Isolation and quantitative estimation of diesel exhaust and carbon black particles ingested by lung epithelial cells and alveolar macrophages. *BioTechniques*, 2008, **44**, 799–805.
108. Verdejo, R., Jell, G., Safinia, L., Bismarck, A., Stevens, M. M. and Shaffer, M. S., Reactive polyurethane carbon nanotube foams and their interactions with osteoblasts. *J. Biomed. Mater. Res. A*, 7 February 2008 [Epub ahead of print].
109. Shi, X. *et al.*, *In vitro* cytotoxicity of single-walled carbon nanotube/biodegradable polymer nanocomposites. *J. Biomed. Mater. Res. A*, 2008, **86**, 813–823.
110. Kawaguchi, M. *et al.*, Preparation of carbon nanotube–alginate nanocomposite gel for tissue engineering. *Dent. Mater. J.*, 2006, **25**, 719–725.
111. Zhu, L., Chang, D. W., Dai, L. and Hong, Y., DNA damage induced by multiwalled carbon nanotubes in mouse embryonic stem cells. *Nano Lett.*, 2007, **7**, 3592–3597.
112. Garza, K. M., Soto, K. F. and Murr, L. E., Cytotoxicity and reactive oxygen species generation from aggregated carbon and carbonaceous nanoparticulate materials. *Int. J. Nanomed.*, 2008, **3**, 83–94.
113. Zhou, H. *et al.*, A nano-combinatorial library strategy for the discovery of nanotubes with reduced protein-binding, cytotoxicity, and immune response. *Nano Lett.*, 2008, **8**, 859–865.
114. Warheit, D. B., How meaningful are the results of nanotoxicity studies in the absence of adequate material characterization? *Toxicol. Sci.*, 2008, **101**, 183–185.
115. Nimmagadda, A., Thurston, K., Nollert, M. U. and McFetridge, P. S., Chemical modification of SWCNT alters *in vitro* cell-SWCNT interactions. *J. Biomed. Mater. Res.*, 2006, **A76**, 614–625.
116. Meng, J. *et al.*, Using single-walled carbon nanotubes nonwoven films as scaffolds to enhance long-term cell proliferation *in vitro*. *J. Biomed. Mater. Res. A*, 2006, **79**, 298–306.
117. Zanello, L. P., Zhao, B., Hu, H. and Haddon, R. C., Bone cell proliferation on carbon nanotubes. *Nano Lett.*, 2006, **6**, 562–567.
118. Khang, D., Sato, M. and Webster, T. J., Directed osteoblast functions on micro-aligned patterns of carbon nanofibers on a polymer matrix. *Rev. Adv. Mater. Sci.*, 2005, **10**, 205–208.
119. Balani, K., Anderson, R., Lahaa, T., Andaraa, M., Terceroa, J., Crumpler, E. and Agarwala, A., Plasma-sprayed carbon nanotube reinforced hydroxyapatite coatings and their interaction with human osteoblasts *in vitro*. *Biomaterials*, 2007, **28**, 618–624.
120. Li, A., Sun, K., Dong, W. and Zhao, D., Mechanical properties, microstructure and histocompatibility of MWCNTs/HAP biocomposites. *Mater. Lett.*, 2007, **61**, 1839–1844.
121. Shi, X. *et al.*, Fabrication of porous ultra-short single-walled carbon nanotubes nanocomposite scaffolds for bone tissue engineering. *Biomaterials*, 2007, **28**, 4078–4090.
122. Supronowicz, P. R., Ajayan, P. M., Ullmann, K. R., Arulandam, B. P., Metzger, D. W. and Bizios, R., Novel current-conducting composite substrates for exposing osteoblasts to alternating current stimulation. *J. Biomed. Mater. Res.*, 2002, **59**, 499–506.
123. Gilmore, K. J., Moulton, S. E. and Wallace, G. G., Incorporation of carbon nanotubes into the biomedical polymer poly (styrene-*b*-isobutylene-*b*-styrene). *Carbon*, 2007, **45**, 402–410.

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