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Carbon nanotubes in biology and medicine: An overview

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Rapid development in the field of nanomedicine is bringing novel opportunities for improved disease diagnosis and drug delivery. Among various nanomaterials involved in nanomedicine, carbon nanotubes (CNTs) possessing a unique one-dimensional structure with interesting intrinsic mechanical, physical, and chemical properties have been extensively explored for a wide range of applications in biology and medicine. This review article provides an overview of how CNTs are used in different aspects of biomedicine including drug delivery and cancer treatment, bio-sensing, biomedical imaging, as well as tissue engineering. The recent developments, future perspective, and major challenges in this field are discussed.

carbon nanotube, imaging, drug delivery, biosensor, tissue engineering

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Since Iijima first discovered carbon nanotubes (CNTs) in 1991 [1], intensive investigations of their structures and properties have been actively pursued for potential applications in various fields [2–7]. Carbon nanotubes are categorized into two structural forms, including single-walled carbon nanotubes (SWNTs) and multi-walled nanotubes (MWNTs), both of which have shown great application in many areas including composite materials, nanoelectronics, field effect emitters, energy research, as well as biomedicine [7–10].

Motivated by the unique one-dimensional (1-D) structure, and interesting physical and chemical properties, CNTs have been extensively explored in the field of biology and medicine. In this paper, we will provide an overview of the biomedical applications of CNTs in different applications, including drug delivery, cancer treatment, biosensors, biomedical imaging, and as composite materials for tissue engineering. CNT-based drug delivery has shown promise for the intracellular delivery of small drug molecules, DNA plasmids, short-interfering RNA (siRNA), and proteins, for applications in disease treatment such as cancer therapies *in vitro* and *in vivo* [11–17]. The unique optical and electrical properties of CNTs make them attractive platforms to detect various biological molecules [18,19]. SWNTs exhibit many intrinsic optical properties such as optical absorption and photoluminescence in the near-infrared region (NIR), as well as strong resonance Raman scattering, all of which can be used in different biological imaging modalities [20–22]. Moreover, CNTs with excellent mechanical properties have also found applications as potential tissue engineering scaffold materials [23,24]. CNT-based nanomaterials appear to be encouraging and may bring novel opportunities for future disease diagnosis and treatment (Table 1).

1 Drug delivery and cancer treatment

Functionalized CNTs are able to enter cells without obvious toxicity [11,12], and are able to shuttle small drug molecules and biological macromolecules, including plasmids, siRNA, and proteins into cells [13–17]. CNT-based drug delivery has not only been experimentally demonstrated at the cellular level, but also applied for the treatment of cancer in animal models [15,17,25,28,31]. The physical properties,

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Table 1 A summary of the use of carbon nanotubes for various biomedical applications

Applications		Examples
Drug delivery and cancer treatment	Delivery of small drug molecules	Covalently conjugated to CNTs (e.g. Pt (IV) pro-drug, paclitaxel) [25–27]
		Non-covalently conjugated to CNTs (e.g. doxorubicin) [15,17]
	Gene delivery	Plasmid DNA and siRNA [13,16,28–34]
	Delivery of proteins	Proteins covalently or noncovalently conjugated to CNTs [12,14]
Physical cancer therapies delivered by CNTs		Photothermal therapy [35–42]
		Radiofrequency (RF)-induced cancer ablation [43]
Biosensing	Optical sensors	NIR fluorescence biosensors (e.g. sensing of *β-D*-glucose, the conformational polymorphism of DNA, etc.) [20,44–48] Raman and SERS biosensors (e.g. protein detection) [49]
	Electronic and electrochemical sensors	Electronic biosensors (e.g. NH_3, NO_2, proteins, DNA, etc.) [50–55]
		Electrochemical biosensors (e.g. DNA, viruses, antigens, etc.) [56–61]
Biomedical imaging		NIR fluorescence imaging (drosophila larvae, cells, etc.) [22,62–65]
		Raman imaging (targeted *in viv*o and multicolor imaging) [20,21,66,67]
		Photoacoustic imaging in mice [68,69]
Scaffolds in tissue engineering		CNTs as scaffolds in bone regeneration [70–73]
		CNTs for neural applications [61,74,75]

especially the optical properties, have also been utilized for novel cancer therapies [35–43].

1.1 Delivery of small molecules

Small drug molecules may be loaded on CNTs via either covalent conjugation or non-covalent adsorption for drug delivery. Small anti-cancer drug molecules are able to be covalently conjugated to nanotubes. CNTs are covalently functionalized via the 1,3-dipolar cycloaddition of azomethine ylides containing amino groups that could be used to conjugate with drugs and/or fluorescent probes [27,76]. Furthermore, Dhar et al. [26] reported that the Pt (IV) pro-drug could be delivered into cells by SWNTs but was reduced to a cytotoxic Pt (II) compound after endocytosis resulting in cancer cell destruction.

SWNT-based *in vivo* drug delivery for cancer treatment in animal experiments was first reported by Liu et al. [25] in 2008. In this work, paclitaxel (PTX) was conjugated to branched polyethylene glycol (PEG) chains on SWNTs via a cleavable ester bond (Figure 1(a) and (c)). The blood circulation time of the SWNT-PTX complex was found to be much longer than that of free PTX, resulting in increased drug accumulation in the tumor due to the enhanced permeability and retention (EPR) effect of cancerous tumors, and thus, improved the therapeutic efficacy for the delay of tumor growth. Wu et al. [27] demonstrated that it was possible for MWNTs to be covalently conjugated to the antitumor agent, 10-hydroxycamptothecin (HCPT), using hydrophilic diaminotriethylene glycol as the spacer. The resulting MWNT-HCPT conjugates were found to be superior in antitumor activity both *in vitro* and *in vivo* compared with the clinical HCPT formulation. *In vivo* tumor targeted drug delivery with CNTs was also reported by Bhirde et al. [77]. In their work, SWNTs were co-conjugated with cisplatin and epidermal growth factor (EGF) to specifically target squamous cancer cells for *in vivo* treatment. The targeted SWNT-cisplatin-EGF conjugate exhibited improved tumor growth inhibition effect to the EGF receptor (EGFR) positive head and neck squamous cell carcinoma (HNSCC) tumors, owing to specific EGF-EGFR binding which enhanced the uptake of nanotube-delivered drugs in tumors.

Apart from covalent linking, aromatic anti-cancer molecules, such as doxorubicin (DOX), can robustly bind to the side walls of CNTs via non-covalent π-π stacking (Figure 1(a) and (b)); drug binding and release could be controlled by varying the pH [15]. The loading capacity of DOX on PEGylated SWNTs was shown to be as high as 400% by weight, owing to the ultra-high surface area of nanotubes compared other conventional drug nanocarriers. In a later study, the SWNT-DOX complex was further utilized for *in vivo* cancer treatment (Figure 1(d)) [17]. It was found that although the treatment efficacy of SWNT-DOX was only slightly higher than that of free DOX at the same drug dose, where SWNT-DOX exhibited remarkably reduced toxic effect to the treated animals, and was safe in mice, even at twice the drug dose (Figure 1(e) and (f)).

1.2 Gene delivery

Unlike small drug molecules which can enter cells by diffusion, biomacromolecules including DNA, RNA, and protein, are not able to penetrate the cell membrane and therefore require delivery vehicles for intracellular delivery. Pantarotto

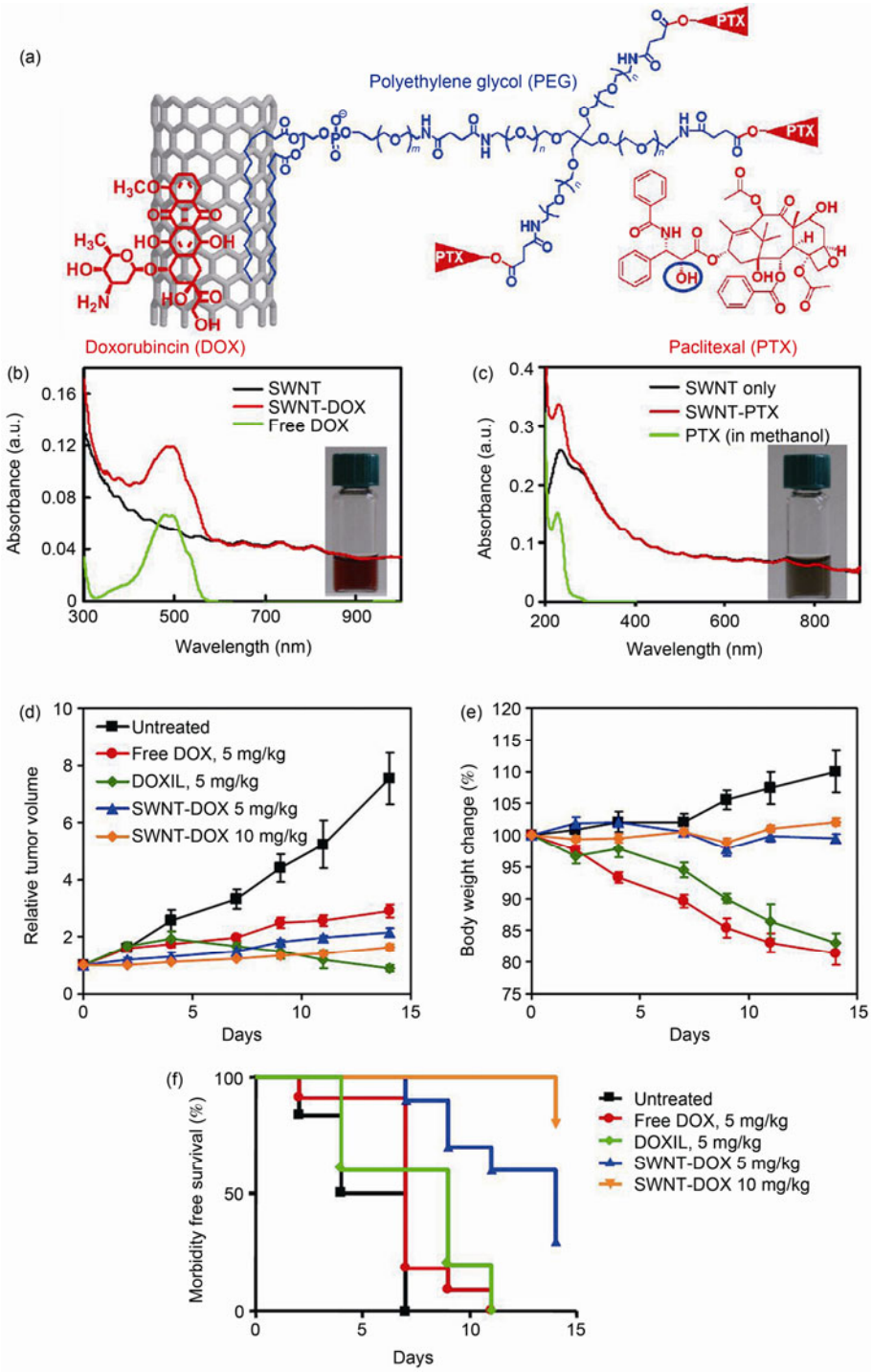

Figure 1 (Color online) Drug delivery with SWNTs. (a) A scheme showing non-covalent supramolecular π-π stacking of doxorubicin and covalent conjugation of paclitaxel on PEGylated SWNTs; UV-VIS-NIR spectra of DOX loaded SWNT (b) and PTX conjugated SWNT (c) solutions. Inset: photos of the corresponding solutions; Raji tumor bearing severe combined immune deficient (SCID) mice were treated with different DOX formulations once per week at day 0 and day 7. (d) Tumor sizes of untreated, 5 mg/kg free DOX treated, 5 mg/kg DOX treated, 5 mg/kg SWNT-DOX treated and 10 mg/kg SWNT-DOX treated mice were measured during treatment. Mean tumor volume was normalized to day 0. (e) SWNT-DOX resulted in far less weight loss than DOX and DOXIL. Mean body weight was normalized to day 0. (f) Kaplan-Meier analysis. *P*-values: DOX 5 mg/kg vs. SWNT-DOX 5 or 10 mg/kg, $P<0.001$; DOXIL 5 mg/kg vs. SWNT-DOX 5 mg/kg, $P=0.013$; DOXIL 5 mg/kg vs. SWNT-DOX 10 mg/kg, $P<0.001$. Error bars in (b) and (c) were based on the standard error of the mean (SEM) [15,17,25].

et al. [28] successfully delivered plasmid DNA into mammalian HeLa cells by using ammonium-functionalized CNTs with positive charges, although the efficiency was not satisfactory; plasmid DNA was loaded onto CNTs through electrostatic interactions. To improve the transfection efficiency of CNTs, cationic polymers including polyethylenimine

(PEI) were used to modify CNTs [29,31,33]. Ahmed et al. [31] developed cationic glycopolymer-modified SWNTs for *in vitro* gene transfer agent and found that the complex was biocompatible and exhibited transfection efficiencies comparable to the commercially available agent, lipofectamine 2000. Richard et al. [29] reported cationic amphiphiles functionalized SWNTs and MWNTs for *in vitro* gene transfection and showed that the efficiency of transfection was higher when using SWNTs instead of MWNTs, and that transfection efficiency was similar or slightly higher than that using lipoplexes and several orders of magnitude higher than that for naked DNA.

RNA interference (RNAi), a biological process within living cells that takes part in the control of gene expression, is expected to have great application in gene therapies. Two types of small RNA molecules, microRNA (miRNA) and short interfering RNA (siRNA), are key players in RNAi. Kam et al. [13] first successfully delivered siRNA into mammalian cells by linking siRNA to SWNTs via a cleavable disulfide bond. Liu et al. [16] further used this strategy to deliver siRNA into human T cells and primary cells, which were notoriously "hard-to-transfect" cells by conventional liposome-based agents (Figure 2). Further application of siRNA showed that it could also be non-covalently loaded onto CNTs. Bartholomeusz et al. [30] reported the use of positively-charged SWNTs for non-covalently binding siRNA resulting in the *in vivo* therapeutic silencing of hypoxia-inducible factor 1 alpha (HIF-1α) in animal experiments.

Nano-spearing or nano-injection by CNTs is another unique approach for controllable gene delivery. In a earlier work by Cai et al. [34], DNA plasmids encoding enhanced green fluorescence protein (EGFP) were prefunctionalized onto CNTs, and these nanotubes were embedded in nickel. Using magnetic force, the SWNT needle was speared into a specific cell, releasing the DNA plasmid inside cells and inducing expression of EGFP. In 2007, Chen et al. [32] reported the development of a nanoscale cell injection system that used CNTs to deliver nanoparticle cargo into cells. In their experiments, a single MWNT was attached to an atomic force microscope (AFM) tip and conjugate to cargo nanoparticles or molecules via disulfide linking. By controlling the AFM tip, the "nanoneedle" could penetrate the cell membrane. The subsequent reductive cleavage of disulfide bonds within the cell cytoplasm resulted in the release of the cargo inside cells. This method was successfully used to deliver protein-coated quantum dots into live human cells without significant cell damage. The above methods provide a controllable way to deliver molecules, genes, and nanoparticles into specific cells; however, these methods cannot be scaled-up to treat large numbers of cells.

1.3 Delivery of proteins

Proteins can be either covalently or non-covalently conjugated to CNTs for intracellular delivery [12,14]. Kam et al. [12] reported in 2004 that CNTs could act as molecular transporters to deliver proteins conjugated to nanotubes into human promyelocytic leukemia (HL60) cells and human T cells (Jurkat) via the endocytosis pathway. In 2005, the same group also reported that SWNTs were generic intracellular transporters for various types of proteins [14]. These proteins, including streptavidin (SA), protein A (SpA), bovine serum albumin (BSA), and cytochrome *c* (Cytc), could be non-covalently adsorbed onto the nanotube sidewalls by non-specific binding via hydrophobic interactions, and then shuttled into cells. However, protein delivery with CNTs has not yet been demonstrated in animal experiments thus far.

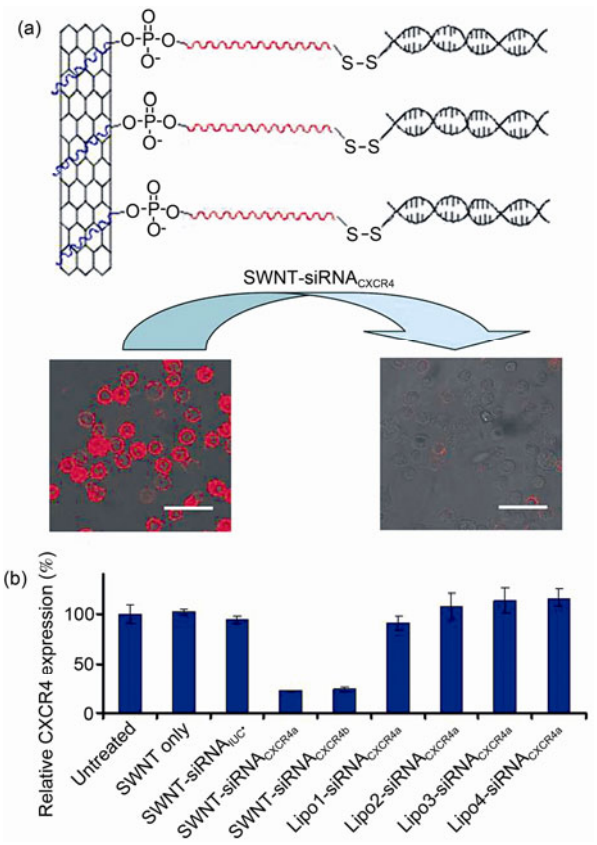

Figure 2 (Color online) siRNA delivery by carbon nanotubes. (a) A scheme of SWNT-siRNA conjugation via disulfide linkage. Below: confocal images of untreated cells (left) and SWNT-siRNA$_{CXCR4}$ treated cells (right) after PE-anti CXCR4 staining. Scale bars: 40 μm. (b) CXCR4 expression levels on CEM cells three days after various treatments, including four types of liposomes (Lipo1–4) and luciferase (Luc) siRNA control [16].

2 Physical cancer therapies delivered by CNTs

The unique physical properties of CNTs have been used for novel cancer therapies. Both MWNTs and SWNTs exhibit strong optical absorption in the NIR, and were used for photothermal treatment of cancer. In 2005, Kam et al. [42]

reported SWNTs as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction *in vitro*. Functionalized SWNTs were able to selectively target cancer cells, which were thermally destroyed after being exposed to an 808 nm NIR laser at a power density of 2 W/cm^2. Later work by Chakravarty et al. [41] used antibody-conjugated SWNTs for *in vitro* photothermal ablation of tumor cells. Kang et al. [38] demonstrated a unique approach using the photoacoustic effect of SWNTs for targeting and selective destruction of cancer cells, where, instead of simple heating, a "firecracker"-like explosion was triggered by the pulsed NIR laser to "bomb"cancer cells.

CNT-based *in vivo* photothermal therapy of cancer in animal models has been reported by in several studies [35–37,39,40]. Ghosh et al. [39] demonstrated that DNA-encapsulated MWNTs could be used to safely eradicate tumors growing on mice. A MWNT solution (100 μL of a 500 μg/mL solution) was directly injected to tumors of mice and after irradiation with a 1064 nm laser (2.5 W/cm^2), PC3 xenograft tumors in 8/8 (100%) of nude mice were completely eliminated. Tumors that received only MWNT injection or laser irradiation showed growth rates indistinguishable from control untreated tumors. SWNTs were also used for *in vivo* photothermal treatment of tumors. Moon et al. [37] showed that tumors injected with SWNTs and exposed to the NIR laser (76 W/cm^3) were completely eliminated, without harmful side-effects or recurrence of tumors for over six months. Most of the intratumorally-injected SWNTs were excreted from mice within two months through biliary and urinary pathways.

In vivo photothermal therapy of tumors has been described in animal experiments using CNTs [35,36,40]. Apart from direct intratumoral injection, our group has achieved photothermal ablation of tumors in mice by well-functionalized SWNTs via systemic intravenous injection [36]. After a systematical studying the relationship between polymer surface coatings and *in vivo* behaviors of SWNTs, it was found that SWNTs with long blood circulation half-life (12–13 h) that showed high passive tumor uptake owing to the EPR effect and relatively low accumulations in reticuloendothelial systems (RES) and skin, were ideal for *in vivo* photothermal treatment of cancer (Figure 3(a) and (b)). Tumors in mice intravenously injected with SWNTs were almost completely eliminated after irradiation with the NIR laser (808 nm, 1 W/cm^2, 5 min) (Figure 3(c) and (d)). Further studies described by Robinson et al. [35] showed that

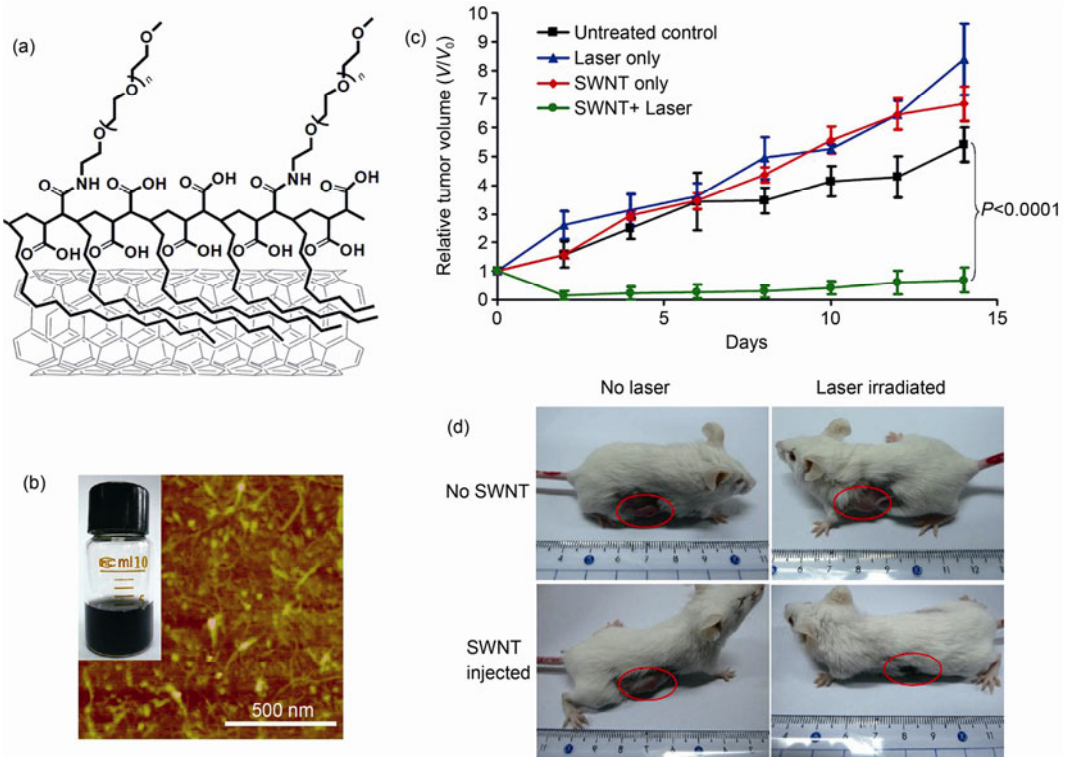

Figure 3 (Color online) SWNTs with the optimized PEG coating for *in vivo* photothermal therapy of cancer. (a) Scheme of a SWNT with PEGylated amphiphilic polymer coating. (b) An AFM image of PEGylated SWNTs. Inset: a solution of SWNTs in saline. (c) Tumor growth curves of different groups after treatment. The tumor volumes were normalized to their initial sizes. The *P*-value between the SWNT + Laser group and untreated control group was calculated by student's *t*-test. (d) Representative photos of tumors on mice after various treatments indicated. The laser irradiated tumor on a SWNT injected mouse was completely destructed. Error bars in (a) were based on SEM. (c) and (d) SWNTs with PEG coating were intravenously injected into seven BALB/c mice bearing 4T1 tumors (two tumors per mouse). Another seven control mice were not injected. One tumor of each mouse was exposed to an 808 nm laser at the power of 1 W/cm^2 while the other tumor was not irradiated [36].

SWNTs could be used for combined high performance *in vivo* NIR (>1 μm) fluorescence imaging and photothermal therapy, highlighting the promise of utilizing SWNTs for highly effective *in vivo* imaging-guided photothermal therapy of cancers. More interestingly, the NIR laser power applied was only 0.6 W/cm^2, which was much lower than that needed for other light-absorbing nanomaterials used in the photothermal therapy of cancer.

The major disadvantage of phototherapies is the limited light penetration depth in biological tissues, even when NIR light is used. In 2007, Gannon et al. [43] reported that functionalized, water-soluble SWNTs exposed to a noninvasive, 13.56 MHz radio frequency (RF) field could generate heat. They further utilized this property of SWNTs for RF-induced thermal destruction of cancer cells *in vitro* and *in vivo*. This is significant since RF waves have excellent tissue penetration that allows the heating of large or internal tumors.

3 Biosensing

As 1-D quantum wires with a sharp electronic density of states at the van Hove singularities, SWNTs exhibit many unique intrinsic properties, including semiconducting behaviors and band gap fluorescence for nanotubes with certain chiralities, as well as strong resonance Raman scattering, making them excellent candidates as novel nano-probes in biosensors [7]. Tailoring hybrid systems consisting of CNTs and biomolecules has been rapidly expanded recently and are still attracting substantial research efforts [18,19].

3.1 Optical sensors

The band-gap fluorescence of SWNTs is highly sensitive to the environment and undergoes shifts when the nanotube surface is in contact with other molecules. In 2004, Barone et al. [78] reported the synthesis and successful testing of solution-phase, NIR SWNTs optical sensor for *β-D*-glucose sensing as a model system. In their experiments, non-covalent functionalized SWNTs suspended using glucose oxidase (GOx) in solution was first prepared. Electroactive species, such as potassium ferricyanide, $K_3Fe(CN)_6$, could irreversibly adsorb to the surface and quenched the SWNT fluorescence emission. Hydrogen peroxide (one of the target analytes) could then reduce ferricyanide, resulting in the restoration of both absorption and emission features of SWNTs. The synthesized optical sensor was sensitive in the general range of blood glucose levels with a detection limit of 34.7 μmol/L. *In vivo* fluorescence detection of blood glucose by implanting the SWNT-based sensor beneath the skin was further proposed by this team in their later work [48].

Heller et al. [20] successfully detected the conformational polymorphism of DNA using SWNT-based optical sensors. Certain DNA oligonucleotides will transform from the native, right-handed *B*-form to the left-handed *Z*-form as the adsorption of some cations (for example, Hg^{2+} and Co^{2+}) onto the double-stranded DNA and screen the negatively-charged backbone. The dielectric environment of SWNT changed as the conformation of a 30-nucleotide dsDNA adsorbed onto SWNTs changed from an analogous B- to Z-form, decreasing the SWNT NIR emission energy up to 15 meV. The thermodynamics of the conformational change for DNA both on and off the SWNT are nearly identical. With this mechanism, they demonstrated the detection of the B-Z transition in whole blood, tissue, and from within living mammalian cells. Many other SWNT-based NIR fluorescence biosensors developed by different teams have also found their application for the detection of single nucleotide polymorphisms [47], fluorescent detection of cellular ATP [45], single-molecule H_2O_2 signaling from epidermal growth factor receptor (EGFR) [46], and nitric oxide [44].

The Raman scattering property of SWNTs can also be used in biosensing. In a study by Chen et al. [49] the strong resonance Raman scattering of SWNTs was further enhanced by the gold substrate via surface enhanced Raman scattering (SERS), for ultra-sensitive detection of biomolecules (Figure 4(a) and (b)). PEGylated SWNTs with different isotopic compositions (^{12}C and ^{13}C) were conjugated to antibodies and used as multicolor Raman labels for multiplexed protein detection in an arrayed format. The detection limit achieved in this study was down to 1 fmol/L, a three-order-of-magnitude improvement over most reports using fluorescence-based detection (Figure 4(c) and (d)).

3.2 Electronic and electrochemical sensors

The molecules that bind to the surface of nanotubes are able to act as the "gating molecules" to modulate the conductance of semi-conducting SWNTs in a field effect transistor (FET) device. This property is used in various SWNT-based electronic biosensors [79].

Kong et al. [55] presented one of the first SWNT-based chemical sensors. Because of the sensitivity increase or decrease of the electrical resistance of SWNTs when exposed to gaseous molecules such as NH_3 and NO_2, the lowest detectable limit was down to concentrations as low as 1 ppm. Similar to SWNTs, aligned multi-wall carbon nanotubes (MWNT) grown on a platinum substrate were also developed into an amperometric biosensor [80]. The first CNT-FET showing real-time biological and pH sensing capabilities was reported by Besteman et al. [54] in 2003 where the changes in nanotube conductance were measured and then transformed the data to the glucose oxidase activity.

Chen et al. [53] first overcame the problem of nonspecific binding of proteins on nanotubes, by the immobilization of PEG chains on the sidewall surface of SWNTs. The selective detection of proteins in solution is highly sensitive, as the limit of detection was found to be 340 ng/mL

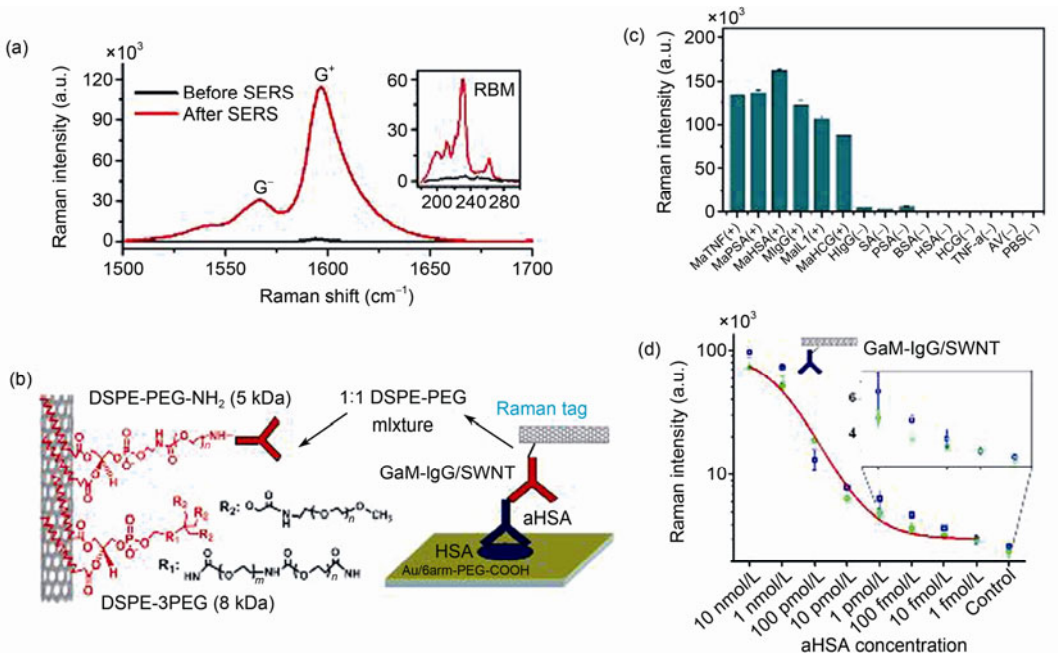

Figure 4 (Color online) SWNT-antibody conjugates may be utilized in conjunction with surface-enhanced Raman scattering (SERS) as bright Raman tags for sandwich assay protein detection. (a) Preparation of a spatially uniform SERS substrate for SWNT Raman tag detection of biomolecules significantly increases Raman scattering intensity, thus improving signal-to-noise and reducing assay time. (b) SWNT-antibody conjugates were formulated by first suspending SWNTs in aqueous media via PEGylated surfactants, which provide functionality and prevent non-specific interactions to the hydrophobic SWNT surface, and subsequently coupled to antibodies via bifunctional cross-linkers. Such antibody-Raman tags have been used in direct and indirect immunoassays. (c) SWNT-anti-mouse IgG conjugates were applied to direct detection of mouse IgGs, demonstrating excellent signal-to-noise and minimal cross-reactivity. (d) SWNT-anti-mouse IgG conjugates were used as Raman tags for the indirect detection of mouse anti-human serum albumin (aHSA) IgG captured onto a SERS active substrate by HSA. A limit of detection of 1 fmol/L analyte was reproducibly observed (two separate trials are shown). The data are well fit by a logistic regression (solid red curve, fit to data shown in green) allowing accurate quantitation of analyte over eight orders of magnitude [49].

(approximately 2.3 nmol/L). Ultrasensitive electrical biosensing of proteins and DNA based on carbon nanotubes were achieved by Wang et al. [52] in 2004. The sandwich hybridization technique dramatically enhances the sensitivity of these assays, and shows a remarkably low detection limit of around 1 fg/mL (54 amol/L). Except for proteins and enzymes, the first SWNT-FET-based biosensor comprising DNA aptamers as the molecular recognition elements was reported by So et al. [51] in 2005. Maehashi et al. [81] developed label-free protein biosensors also based on aptamer-modified CNT-FETs for the detection of immunoglobulin E (IgE).

In addition to the single-mode biosensors, Oh et al. [50] reported a carbon nanotube based dual-mode biosensor for electrical and surface plasmon resonance (SPR) measurement. When DNA hybridization occurred on the Au top gate, it could be detected immediately by the change in electrical conductance and surface plasmon resonance (SPR). Using this kind of dual-mode measurement, the sensor-to-sensor variation of conductance measurements could be minimized by calibration using SPR data.

Electrochemical detection offers several advantages over conventional fluorescence measurements, such as portability, higher performance with lower background, less-expensive components, and the ability to perform measurements in turbid samples [56]. CNT-based electrochemical biosensors for the detection of diverse biological structures such as DNA, viruses, antigens, and disease markers have been recently reported by many groups [56,57,82]. The key to using CNTs for these applications is their ability to promote electron transfer in electrochemical reactions [59,83].

An emerging trend is the sensing of whole cells (for example, neural biosensing), and interactions between different cells using CNTs [58,61,84]. Keefer et al. [60] investigated the use of nanotube-coated electrodes in preparing brain-machine interfaces. The carbon nanotube coating on the electrodes were biocompatible and enhanced both recording and electrical stimulation of neurons *in vitro* in cell culture, as well as *in vivo* in rats and monkeys, by decreasing the electrode impedance and increasing charge transfer. Lin et al. [85] reported an electrochemical sensor devised for the continuous and simultaneous monitoring of glucose and lactate in rat brain tissue. This study included a complex electroanalytical system in which SWNTs were loaded with glucose dehydrogenase or lactate dehydrogenase were prepared for real-time monitoring of the metabolic intermediate, glucose, or the circulatory impairment molecule, lactate.

4 Biomedical imaging

CNTs have also been widely used in a variety of biomedical imaging modalities, including optical imaging [7,22,42,66,

68,69], magnetic resonance imaging [86–88], and nuclear imaging [89] (Figure 5); the latter two mainly rely on the external labels or impurities in the CNT samples for imaging contrast. Here, we will focus on the utilization of the inherent physical properties of SWNTs for optical imaging in biological systems.

4.1 NIR fluorescence imaging

Semi-conducting SWNTs exhibit bad-gap photoluminescence when excited by the NIR light and have been used in fluorescence imaging of biological samples. Cherukuri et al. [65] found that macrophage cells could actively take up significant quantities of SWNTs by detecting NIR fluorescence signals (>1100 nm) from nanotubes inside macrophage cells. Subsequently, the first report of *in vivo* NIR imaging of SWNTs in drosophila larvae was achieved by Leeuw et al. [63] in 2007.

Welsher et al. [22] reported the use of SWNTs as NIR fluorescent tags for selective probing and imaging of cells. In this study, they first conjugated Rituxan (anti-CD20 antibody) onto biocompatible SWNTs, and incubated these with two types of cells: Raji B-cell lymphoma ($CD20^+$) and CEM T-cell lymphoma ($CD20^-$). Strong NIR photoluminescence of SWNTs could be detected from the positive Raji cells after labeling with the nanotube-antibody conjugate.

Low quantum yield (QY) is a major limitation for SWNT-based fluorescence imaging [64] . It has been found that the photoluminescence QY is closely related to the length and surface coating of nanotubes. Although PEGylated SWNTs

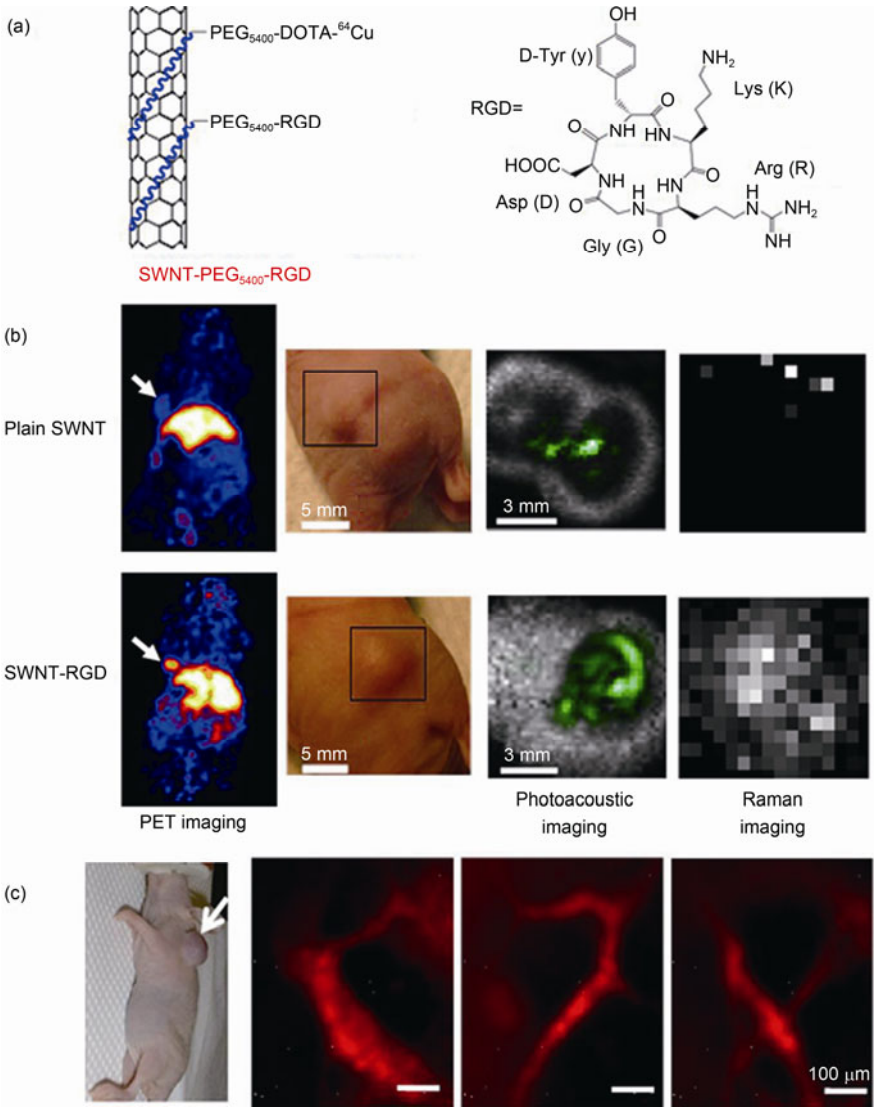

Figure 5 SWNT based *in vivo* cancer imaging. (a) A scheme of PEGylated SWNT conjugated to RGD peptide and DOTA-^{64}Cu, for tumor targeting and radiolabeling, respectively. (b) Integrin $\alpha_v\beta_3$ targeted *in vivo* tumor imaging by whole mouse micro-PET imaging, tumor local photoacoustic imaging and Raman imaging. Nude mice bearing U87MG tumors (integrin $\alpha_v\beta_3$ positive) were intravenously injected with either plain SWNTs or SWNT-RGD. (c) In-travital high resolution photoluminescence imaging of tumor vasculature using SWNTs as the contrast agent [62,67,69,89].

exhibit excellent biocompatibility, their QY was significantly lower than that of surfactant-suspended nanotubes, the latter however were not biocompatible and thus not suitable for applications in bio-imaging. In 2009, Welsher et al. [62] revealed that the surface coating of SWNTs presonicated in sodium cholate could be replaced by PEGylated phospholipid (PL-PEG) to gain biocompatibility with retained high QY. It was reported that the QY of the exchanged-SWNTs prepared by this method was more than one order of magnitude enhanced over SWNTs directly suspended in PL-PEG. These exchange-SWNT conjugates were then used as contrast agents for *in vitro* and *in vivo* NIR imaging with outstanding performance (Figure 6(c)). More recently, Hong et al. [90] observed the first metal-enhanced fluorescence of SWNTs. In their experiments, SWNTs modified by gold showed enhanced fluorescence resulting from radioactive lifetime shortening by >10-fold through resonance coupling of SWNT emission to plasmonic modes in the metal. A latest study by the same laboratory further showed that the Au substrate could be used for NIR-fluorescence-enhanced (NIR-FE) cellular imaging by using both SWNTs and organic fluorescent labels [91]. The noble metal nanostructure-enhanced SWNT photoluminescence provides a novel approach for improved sensitivity in SWNT-based fluorescence imaging and detection.

4.2 Raman imaging

Raman spectroscopy is a very important and noninvasive tool to analyze vibration in molecules. Raman imaging of SWNTs in live cells was first reported by Heller et al. [92] in 2005. DNA functionalized SWNTs were incubated with 3T3 fibroblast and myoblast stem cells and Raman spectroscopic mapping was conducted under 785 nm laser excitation, showing high SWNT Raman signals inside cells. It was found that the photostability of SWNTs was many orders of magnitudes better than that of organic fluorescent dyes and NIR quantum dots.

Targeted *in vivo* Raman imaging was conducted by Zavaleta et al. [67]. In their experiment, mice bearing U87MG

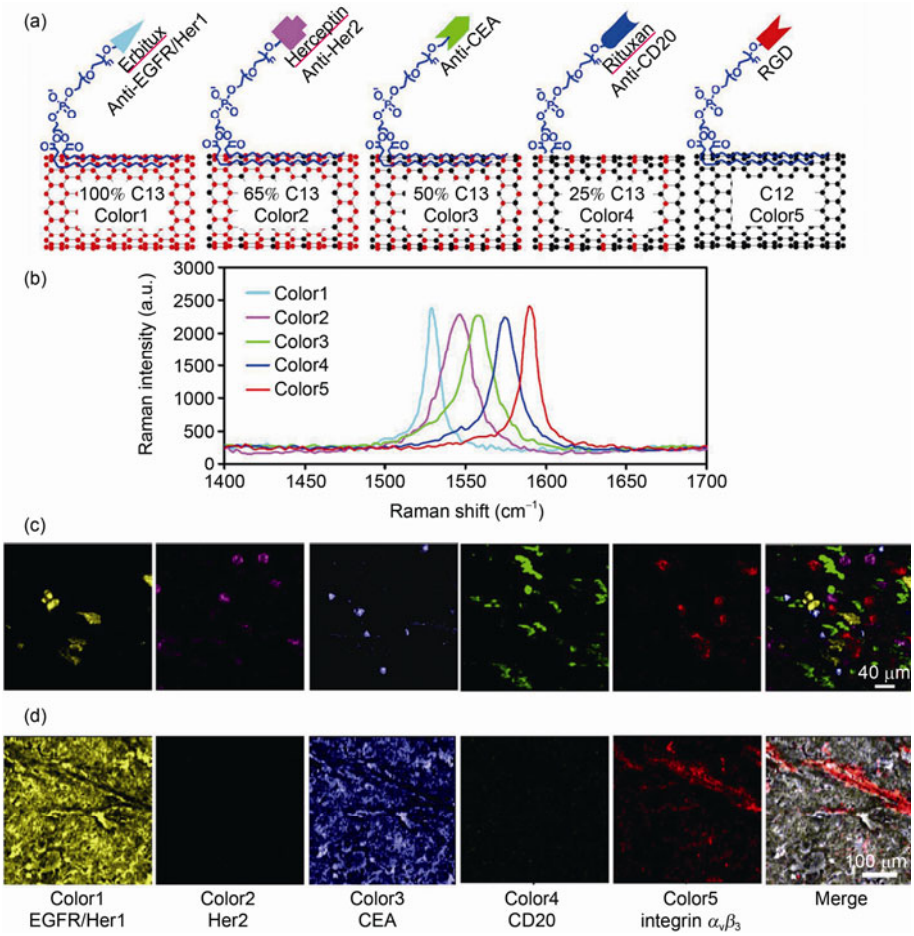

Figure 6 (Color online) Mutli-color Raman imaging with isotopically modified SWNTs. (a) A scheme showing isotopically modified SWNTs conjugated with different targeting ligands. Color1, 2, 3, 4, and 5 represent SWNTs with ^{13}C percentages of 100%, 65%, 50%, 25%, and 0%, respectively. (b) Raman spectra of the five different SWNT samples in aqueous solutions. The shift of SWNT Raman G-band peak is clearly dependent on the $^{12}C/^{13}C$ ratio in SWNTs. (c) Five-color Raman imaging of cancer cells. (d) Five-color Raman images of a LS174T human colon tumor slice from a mouse model. Separate images represent five protein expression levels [21,66].

tumors were intravenously injected with PEGylated SWNTs conjugated with a RGD peptide to target integrin $\alpha_v\beta_3$ up-regulated on tumor vasculature and tumor cells. Strong SWNT Raman signals were detected from tumors receiving targeting SWNTs but not plain SWNTs without RGD conjugation (Figure 6(b)).

SWNTs with different isotope compositions show shifted G-band peaks [93], thus can be used as different colors for Raman imaging under a single laser excitation. Multiplexed three-color imaging of tumor cells and tissues with CNTs was achieved by Liu et al. [21]. Pure ^{12}C, pure ^{13}C and mixed $^{12}C/^{13}C$ SWNTs with different Raman G-band peaks at positions of 1590, 1528, and 1544 cm^{-1}, respectively, were used as three different Raman "colors" for multiplexed Raman imaging. By varying $^{12}C/^{13}C$ ratios during nanotube synthesis, as many as five SWNT Raman "colors" were obtained in in subsequent studies by the same group [66] (Figure 6).

Compared with fluorescence dyes, Raman spectroscopy of SWNTs are resistant to autofluorescence and photobleaching, but its inherently weak effect has limited its application to some extent. The full width at half-maximum (FWHM) of the SWNT G peak is very narrow, allowing for high degrees of multiplicity [21,66]. In the Raman imaging, excitation (785 nm) and scattered photons (892–897 nm) are all in the NIR window which allows excellent tissue absorption [8] and low autofluorescence background.

4.3 Photoacoustic imaging

Photoacoustic imaging of living subjects allows deeper tissue penetration compared with most optical imaging techniques [38,68,69]. CNTs with strong light absorption in the NIR absorb optical energy from a pulsed laser and turn it into acoustic waves, and thus can serve as photoacoustic contrast agents. De la Zerda et al. [69] showed that PEGylated SWNTs conjugated with RGD were also useful for targeted *in vivo* tumor photoacoustic imaging in mice (Figure 6(b)). An improved SWNT-based photoacoustic imaging contrast agent was reported by the same laboratory [68]. In this work, indocyanine green dye-SWNTs (SWNT-ICG) gave a 300-fold higher photoacoustic contrast compared with plain SWNTs, while the size and targeting ability of the nanotube contrast agent did not significantly vary.

5 Scaffold in tissue engineering

Tissue engineering focuses on the improvement, repair, or replacement of tissues and organs [94]. CNTs are becoming increasingly attractive tissue engineering materials as they can be modified to be integrated into biological systems with excellent biocompatibility [23]. In addition to high aspect ratios, CNTs also show many intrinsic mechanical, electrical, and physical properties useful for applications in tissue scaffolds [24,95–97].

5.1 CNTs used as scaffolds in bone regeneration

In 2006, Zanello et al. [73] found the use of CNTs as scaffold materials for osteoblast proliferation and bone formation, and demonstrated that CNTs were suitable to promote osteoblast functions. After incubating cells with CNTs, there was a dramatic change in cell morphology of osteoblasts, which correlated with changes in plasma membrane functions.

Three-dimensional (3D) tubular MWNT composite (for example, PLGA/MWNT) could also serve as scaffolds for tissue engineering [71]. The tubular knitted scaffold was prepared from a MWNT yarn by electrospinning poly(lactic-co-glycolic acid) (PLGA)/MWNT nanofibers onto the knitted scaffold. It was shown that not only could the scaffold support cell growth throughout the culture period, but also had the potential to utilize the intrinsic electrical and mechanical properties of carbon nanotubes for various special applications. Biocompatible MWNT/chitosan (CHI) scaffolds were also developed, showing remarkable performance in both *in vitro* and *in vivo* experiments [72]. The adsorption properties of MWNT were used for incorporation of recombinant human bone morphogenetic protein-2 (rhBMP-2) to promote the ectopic bone formation at muscle tissue. The biocompatibility of the MWNT/CHI scaffold was high since no chronic inflammation occurred throughout the implantation period. Bone tissue regeneration was indeed observed three weeks after the implantation. *In vivo* experiments also showed that most of the MWNT/CHI migrated out from the implant zone, and was possibly gradually cleared from the body.

Another CNT-based composite containing poly(3-hydroxybutyrate) (P(3HB)) was able to serve as 3D porous scaffolds with multifunctionality for bone tissue engineering was reported by Misra et al. [70]. The microstructure, cytocompatibility, biocompatibility, bactericidal ability, and multifunctionality of the composite were studied systematically (Figure 7). Each additive of the scaffold composite was able to contribute to the overall performance, i.e. bioglass particles imparting bioactivity, vitamin E improving protein adsorption, and finally MWNT inducing electrical conductivity. This work presents the concept of "multifunctional scaffolds", which paves the way for next generation of advanced scaffolds for bone tissue engineering. These previous studies demonstrate that CNTs and their composite materials can serve as osteogenic scaffolds with good biocompatibility, reinforced mechanical properties, and improved electrical conductivity to effectively enhance bone tissue growth.

5.2 CNTs for neural applications

CNTs can also be utilized in the neuron axon regeneration

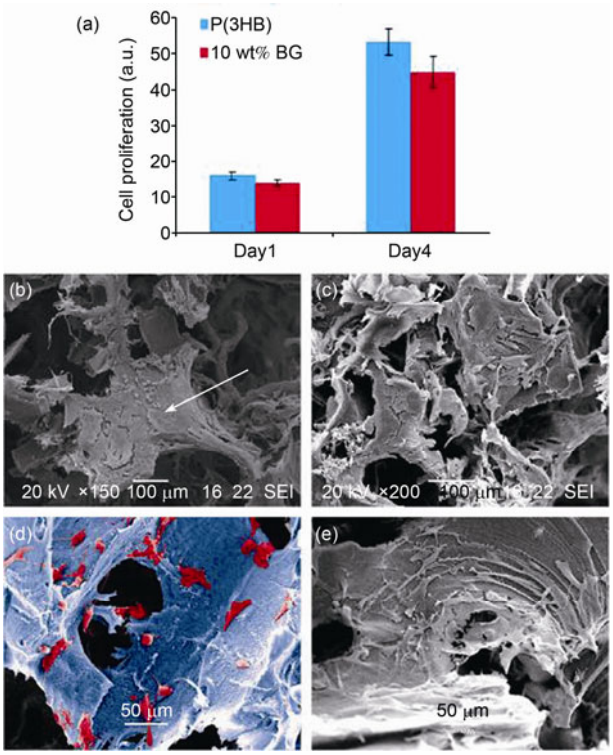

Figure 7 (Color online) An example of the CNT-based tissue engineering scaffold. (a) Cell proliferation study using Alamar blue assay for P(3HB) and P(3HB)/m- BG composite scaffolds on day 1 and 4. SEM micrographs of MG-63 cells grown for 4 days on (b) P(3HB) foams (arrow marks the MG-63 cell layer), (c) P(3HB)/m-BG foams, (d) P(3HB) foams highlighting the spread of the cells and bridging the pores (image enhanced by artificial colors) and, (e) P(3HB)/m-BG foams demonstrating the ability of the cells to bridge the pores and also to take up the contours of the substrate [70].

as biomimetic scaffolds. Hu et al. [61] used chemically functionalized CNTs as substrates for neuronal growth. Their results revealed that MWNTs with different surface charges resulted in different neurite outgrowth patterns. Compared with negatively charged MWNTs, positively charged MWNTs significantly increased the number of growth cones and neurite branches. Lovat et al. [75] reported the potentially boosted electrical signal transfer of neuronal networks by purified MWNTs where it was found that MWNTs were able to improve neural signal transfer while supporting neuron axon regeneration.

Neural stem cells (NSCs) are very plastic neural precursors and quite sensitive to environmental changes. Jan et al. [74] first demonstrated that mouse embryonic NSCs from the cortex could be successfully differentiated to neurons, astrocytes, and oligodendrocytes with clear formation of neurites on layer-by-layer assembled SWNT-composite. Biocompatibility, neurite outgrowth, and expression of neural markers were similar to those differentiated on poly-L-ornithine (PLO), one of the most widely used growth substrates for neural stem cells. The unique properties of CNTs rendered the SWNT composites high electrical conductivity, chemical stability, and physical strength with structural flexibility and showed no adverse affect on the differentiation of NSCs .

6 Conclusion

This review article summarizes the research of CNTs in a variety of biomedical applications, including drug delivery, cancer therapies, biosensing, bio-imaging, and tissue engineering. Recent years have witnessed the booming development of CNTs and their composites in biological and medicine. Although the future perspective of CNT-based nanomedicine is fascinating and encouraging, whether CNTs will bring a biomaterial revolution remains unknown. For applications that require the administration of CNTs into animals and hopefully finally into humans, the major challenge is the potential long-term toxicity of CNTs. It has been shown by many groups that the toxicology of CNTs is determined by the administration routes, doses, as well as the surface chemistry and sizes of nanotubes, and well-functionalized CNTs are not apparently toxic *in vitro* to cells and *in vivo* to mice at their tested doses [7,36,98–103]. However, many more studies are still required to optimize the surface chemistry and sizes of CNTs for accelerated excretion from biological systems, as nanotubes are not considered to be biodegradable. How CNTs affect the immune, nervous, and reproductive systems, especially in the long-term, are still not fully understood. On the other hand, for *ex vivo* detection in which the toxicology of CNTs is not a concern, reproducibility in the fabrication process may be a problem for CNT-based biosensors. Compared with other biosensors, how prominent the advantages of using CNTs-based biosensors needs to be further explored. Despite the obstacles towards further applications of CNTs in the clinic, the unique structure, size, shape, as well as highly enriched physical and chemical properties of CNTs make them extremely attractive nanomaterials and may enable novel applications in a variety of areas of biomedicine.

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