Chapter 6
A New Era of Cancer Treatment: Carbon Nanotubes as Drug Delivery Tools

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6.1 Introduction

Life-threatening diseases like cancer continue to increase despite substantial progress in modern medicine. Cancer treatment is limited to surgery, chemotherapy, hormone therapy, immune therapy, and radiation therapy (Faubert et al. 2020). These conventional treatment methods' significant drawbacks are their inability to distinguish normal cells from the tumor cells, leading to multiple side effects (Kilgallen et al. 2020). Exploring the efficient ways of targeted drug delivery and early detection of cancer cells is of utmost importance to revolutionize the existing cancer treatment (Ingle et al. 2020). The use of carbon-based nanomaterials in the range of 5–200 nm has emerged as a potential medical weapon for cancer therapy (Adrita et al. 2020). The commonly used carbon nanomaterials include carbon nanotubes (CNTs), nanodiamonds (ND), and graphene-based materials like graphene oxide (GO), reduced graphene oxide (rGO), and few-layer graphene (FLG), among others (Simon et al. 2019). Several recognized advantages of nanomaterial-based cancer treatment are increased systemic circulation lifetime, enhanced serum solubility, sustained drug release, and multiple drug molecules' codelivery (Kaur et al. 2019). Remarkably, the fabrication of CNT-based nanocarrier has shown

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tremendous promising results to overcome cancer treatment challenges, especially drug delivery.

CNTs consist primarily of a series of condensed benzene rings rolled up into a tubular structure and belong to fullerenes' family, the third allotropic form of carbon. CNTs exhibit a unique combination of desired properties such as biocompatibility and excellent mechanical properties (Yadav and Mohite 2020). However, the insolubility of CNTs in the aqueous medium was a significant challenge for its utility in biomedical applications (Naqvi et al. 2020). Nevertheless, a wide range of applications of CNTs emerged, including the delivery of drugs after the functionalization of the CNTs was reported to increase its solubility drastically. Moreover, the solubility of CNTs in different solvents can be modulated depending on the type of its surface functionalization (covalent or non-covalent) (Panigrahi and Nayak 2020). The various applications of CNTs as a drug delivery tool are depicted in Fig. 6.1. This chapter discusses the structure, synthesis, functionalization, and potential applications of CNTs in cancer theranostics.

6.2 Classification and Structure of CNTs

As mentioned earlier, CNTs are allotropes of carbon, comprising graphite sheets rolled into a tube of either single or multiple layers (Tiwari et al. 2016). The surface of the layers displays a honeycomb lattice structure originating from the sp^2

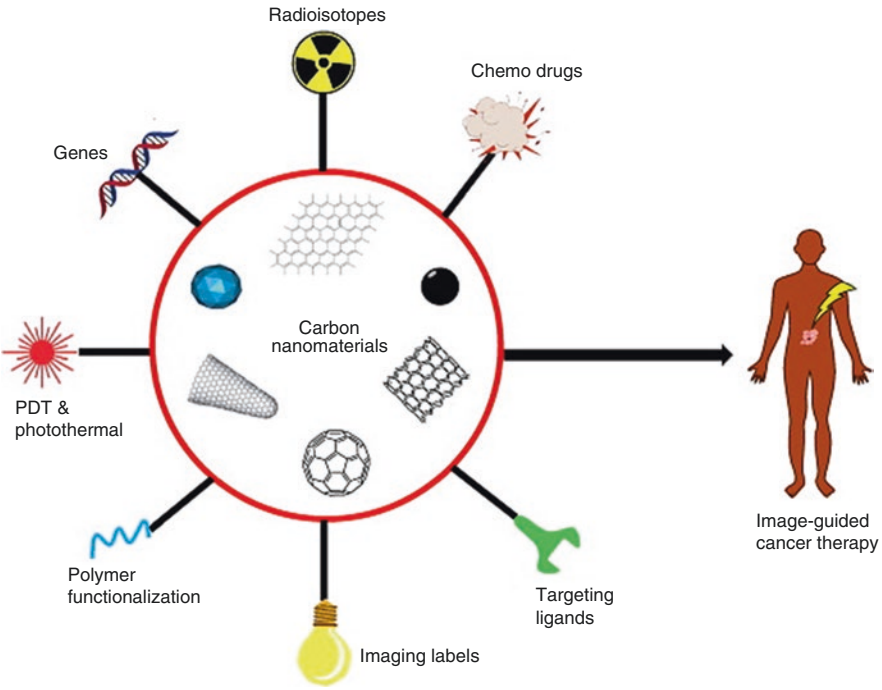

Fig. 6.1 Schematic presentation for the application of carbon-based nanomaterials for cancer theranostics

hybridization of graphite, where each atom is connected uniformly to three carbons (120°) in the *XY* plane. Additionally, the Z-axis harbors a weak π bond. Based on the number of layers, CNTs are classified into two major types: single-walled carbon nanotubes (SWCNTs) and multiple-walled carbon nanotubes (MWCNTs) (Wang et al. 2007; Li et al. 2002; Takamori et al. 2007).

6.2.1 Classification of CNTs

6.2.1.1 Single-Walled Carbon Nanotubes

SWCNTs are a single roll of a graphene sheet with a length to diameter ratio of ~1000, making them almost one dimensional in structure. Generally, SWCNTs have a diameter of ~1 nm; however, nanotubes with a diameter of 0.4 nm have also been synthesized successfully (Wang et al. 2000). Nanotubes wider than 2.5 nm are rarely reported. Contrary, there is no such restriction on the nanotube length, which usually depends upon the adopted preparation method. Generally, the reported lengths of SWCNTs are in the range of micrometer to the millimeter. The structural feature of these nanotubes is extremely valuable in rendering the unique functions of these nanotubes. As reported, all the carbon atoms in SWCNTs are arranged in hexagonal rings except for the nanotube tips, where the carbon atoms are most likely placed in the pentagonal rings. Therefore, the nanotubes' chemical reactivity is thermodynamically favored at the ends of the nanotubes stretched with the pentagonal rings. Moreover, despite the involvement of the carbon atoms to form aromatic rings, the C=C bond angles are not always planar. Indicating the involvement of a certain degree of sp^3 character, which is inversely proportional to the tubes' radius of curvature, giving them overlapping energy bands and versatile electronic behavior. This feature of SWCNTs is believed to make the surface more reactive than planar graphene. There is potentially more than one way to roll a graphene sheet into a single-walled nanotube. These include rolling the sheet onto perpendicular, parallel, or helical symmetry planes to the nanotube axis, making SWCNTs zigzag type (angle of helicity = 0°), armchair-type (angle of helicity = 30°), and helical type, respectively (Rao et al. 2003; Hussain et al. 2020).

6.2.1.2 Multi-walled Carbon Nanotubes

Multi-walled carbon nanotubes involve numerous ways to fold and arrange graphene sheets into filamentary morphology and have diameters of up to 100 nm. Concentric-type MWCNTs (c-MWCNTs) are relatively simple to synthesize, where SWCNTs with increasing diameter are coaxially arranged. MWCNTs can also be synthesized by rolling a graphene sheet onto itself, resembling a scroll of paper. The interactions between adjacent walls of MWCNTs are primarily caused by weak van der Waals forces resulting in a dynamic shear property of MWCNTs (Filleter et al.

2014). Notable differences have been recognized between SWCNTs and MWCNTs in cancer therapy. MWCNTs are more useful for the thermal treatment of cancer than SWCNTs due to their higher vibrational energy dissipation capacity upon near-infrared light exposure (Hirsch et al. 2003). However, the efficiency of SWCNTs in drug delivery is more profound than MWCNTs owing to SWCNTs' unique features, including their one-dimensional structure and higher surface area (Feazell et al. 2007). Additionally, longer blood circulation time of anticancer drug conjugated to SWCNTs was also reported. Figure 6.2 represents the different structures of CNTs and their corresponding AFM images.

6.3 Synthesis of CNTs

Numerous methods are used for the synthesis of CNTs from different carbon sources. Synthesis methods include arc discharge, laser ablation, sonochemical techniques, hydrothermal techniques, chemical vapor deposition, and electrolysis. A few of the commonly used synthesis processes are described here.

6.3.1 Arc Discharge

Arc discharge method utilizes temperature above 1700 °C for CNT synthesis. This method uses a DC arc discharge between two graphite electrodes with diameters between 6 and 12 mm in a chamber filled with either helium, hydrogen, or methane at subatmospheric pressure to synthesize CNTs, with certain differences in the synthesis of SWCNTs and MWCNTs (Fig. 6.3a). A catalyst precursor such as a transition metal catalyst is often used for the synthesis of SWCNTs. A composite anode facilitates SWCNTs' growth in the presence of hydrogen or argon atmosphere. The anode comprises graphite and a metal such as Ni, Co, Fe, Ag, Pd, Pt, Fe-No, Ni-Cu, Co-Cu, Ni-Ti, etc. Contrary, MWCNTs are produced in the absence of a catalyst precursor. The yield and purity of the synthesized product largely depend upon the gas pressure in the reaction vessel. Besides, a higher yield of MWCNTs can be obtained by arc discharges in an organic atmosphere of ethanol, acetone, or hexane. Pulsed currents are also efficient in the production of vertically oriented MWCNTs. Synthesis of MWCNTs by arc discharge in liquid nitrogen shows promising results for their large-scale production (Prasek et al. 2011). This technique is reported to cause the least structural defects compared to other methods.

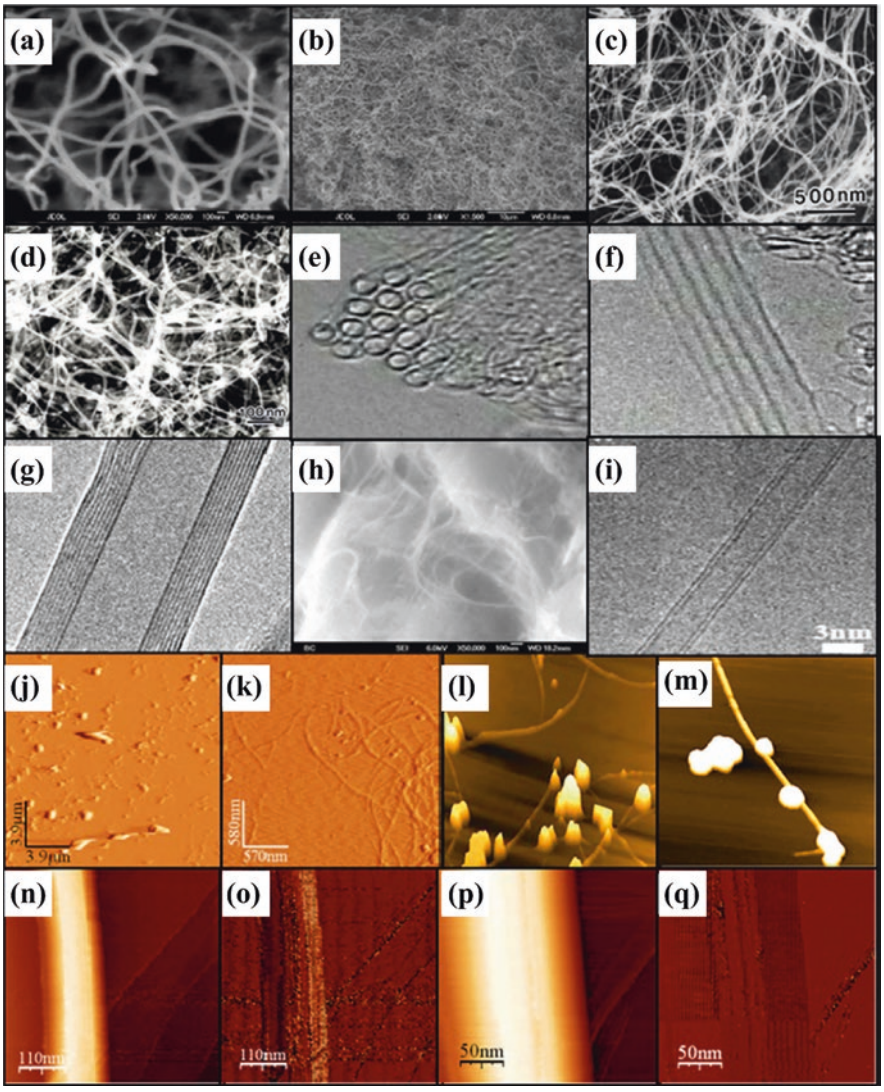

Fig. 6.2 Structure of various carbon nanotubes (CNTs). (**a** and **b**) FE-SEM morphology of MWCNTs with corresponding low and high magnification, (**c** and **d**) morphology of SWCNTs with corresponding low and high magnification, (**e** and **f**) FE-TEM morphology of the cross section of a bundle of SWCNTs, **(g)** an individual tube of SWCNTs, (**h** and **i**) morphology of the DWCNTs, (**j-k**) AFM surface topology of MWCNTs, (**l**) AFM morphology of the SWCNTs with corresponding high (**m**) magnification image, (**n**) STM photograph of the MWCNTs with corresponding high magnification images indicating the line of growth (**o, p,** and **q**). (Adopted from Dutta et al. 2020)

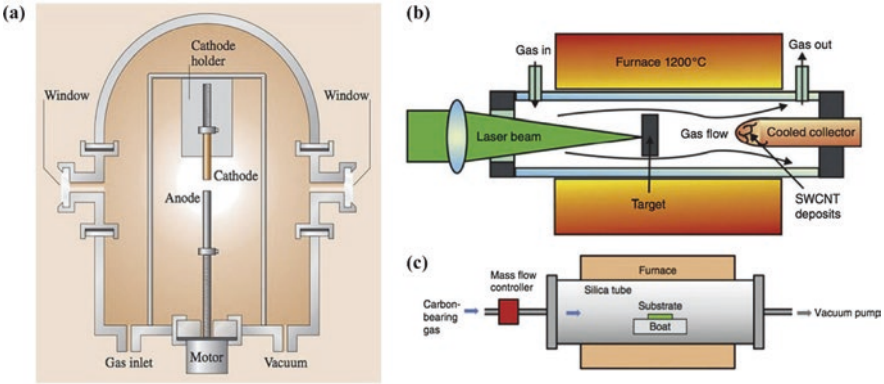

Fig. 6.3 Synthesis of CNTs. (**a**) Arc discharge process, (**b**) laser ablation method, and (**c**) chemical vapor deposition method (adopted from Dutta et al. 2020)

6.3.2 Laser Ablation

In the laser ablation process, a pulsed laser vaporizes a graphite target in a high-temperature reactor (Fig. 6.3b). The principles and mechanisms are similar to the arc discharge; however, the energy is provided by a laser hitting a graphite pellet. The laser ablation method yields around 70% and produces SWCNTs primarily with controllable diameter, highly determined by the reaction temperature. Uniform SWCNTs can be made in the presence of transition metal catalysts like Co, Ni, and Fe. The diameter distribution can be tuned by altering the chemical composition of the target and the gaseous atmosphere. The properties of the CNTs prepared by the laser deposition process strongly depend on the laser properties like energy fluence, peak power, repetition rate, and oscillation wavelength. Additionally, the structural and chemical composition of the target material, the chemical composition and chamber pressure, the flow and pressure of the buffer, the substrate and the ambient temperature, and the distance between the target and the substrate also contribute to the property of the CNTs produced. Besides, the relationship between the excitation wavelength and the growth mechanism of CNTs is recently being studied extensively.

6.3.3 Chemical Vapor Deposition

The chemical vapor deposition (CVD) technique is the most standard method used to produce CNTs. In this method, CNTs are generated onto the surface of a catalyst by cleaving a carbon atom-containing gas that flows continuously through the catalyst nanoparticle (Fig. 6.3c). Usually, a mixture of hydrocarbon as the carbon source (methane, ethane, ethylene, xylene, acetylene, isobutene, ethanol), a metal catalyst, and an inert gas is introduced into the reaction chamber at a temperature of 700–900

°C and atmospheric pressure. CNTs are formed on the substrate by the decomposition of hydrocarbon. The catalyst used in the CVD process helps in the decomposition of the carbon source. The frequently used catalysts are the transition metals like Fe, Co, and N. Alternatively, gaseous carbon sources are also utilized. Other CVD techniques are water-assisted CVD, oxygen-assisted CVD, hot-flame CVD, microwave plasma CVD, or radiofrequency CVD. CVD is considered to be an economically viable process for large-scale and pure CNT production. The main advantage of CVD is the easy to control of the reaction course and the high purity of the obtained material.

6.3.4 Vapor Phase Growth

In this method, CNT synthesis is aided by a reaction gas and an organometallic catalyst in a reaction furnace without the involvement of a substrate. This is typically a four-step method, involving the use of a laser or arc discharge source, hydrocarbon gas, catalyst, and deposition of carbon nanotubes in the reaction chamber. Figure 6.4 indicates the vapor phase growth mechanism of CNTs' formation. The vapor phase growth is mostly used commercially for bulk manufacturing of CNTs.

6.4 Functionalization of CNTs

The pristine CNT is mostly hydrophobic due to the presence of sp^2 carbon nanostructure. Therefore proper functionalization is required to make it water-soluble for biomedical applications. There are two main strategies for CNTs' functionalization, namely, covalent and non-covalent type. Table 6.1 depicts an overview of the various functionalization techniques of CNTs for cancer theranostics. Generally,

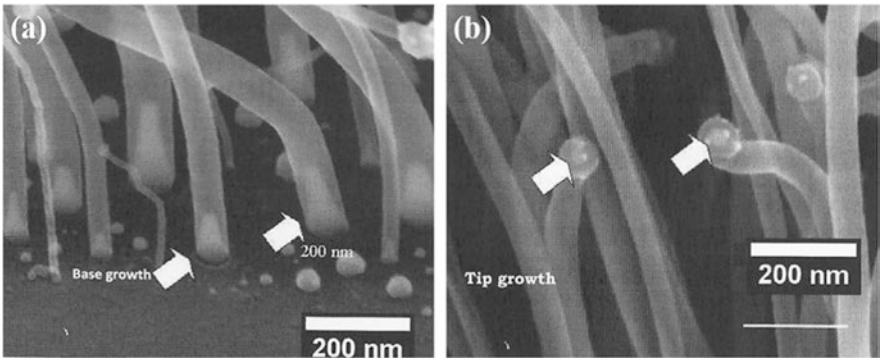

Fig. 6.4 FE-SEM images of CNTs indicating the (**a**) tube growth and (**b**) tip growth during vapor phase growth (adopted from Dutta et al. 2020)

Table 6.1 An overview of functionalization strategies for CNTs as drug delivery vehicle for cancer therapy

Type of interactions	Functionalization molecules	Purpose(s)	Toxicity
Covalent	Polyethylenimine (PEI)	Stabilization and gene delivery	Mild toxicity and immune response
	Radioisotopes and chelators	Imaging label	Radiation exposure
	Gadolinium (Gd)	Imaging label	Toxicity from premature release
	Chemo drugs	Tumor therapy	Toxicity from premature release
	Antibodies, proteins, or peptides	Tumor targeting	Potential immune response
Non-covalent	Polyethylenimine (PEI)	Stabilization and gene delivery	Mild toxicity and immune response
	M13 phage	Stabilization and tumor targeting	Potential immune response
	DNA	Stabilization	Potential immune response
	DNA/siRNA	Cancer therapy	Gene inhibition in normal tissues
	Nanostructures (IONP, QDs, gold NPs, etc.)	Imaging label, PDT, and PTT	MPS capture and potential metal contamination and serious toxicity
	Gadolinium (Gd)	Imaging label	Toxicity from premature release
	Photosensitizers	PDT	Damage to normal tissues by ROS

Adopted from Chen et al. (2015)

covalent modification at the surface of CNTs involves the introduction of hydrophilic functional moieties, such as hydroxyl groups (-OH), carboxylic groups (-COOH), and amino groups ($-NH_2$) along with some biocompatible polymers, such as polyethylene glycols (PEG), ethylene glycols (EG), polyethylenemine (PEI), or drug/gene cargos (Yang et al. 2014). The covalently functionalized CNTs are structurally stable and can be easily dispersed in any polar solvents. However, problems regarding covalent modification are the rapid change of functional properties of CNTs, such as photothermal properties. Thus, non-covalent modification is comparatively mild and retains the original structural properties of CNTs, therefore making it more suitable for drug delivery applications. Non-covalent interaction can be achieved by electrostatic interactions, π–π stacking, and hydrogen bonding, among others. A lot of knowledge and expertise should be needed before choosing the appropriate functionalization method for CNt-based materials. CNT-based nanohybrids are the most effective cancer theranostics if chosen as the proper functionalization strategy (Chen et al. 2015).

6.5 Applications

A wide variety of drug delivery system are currently available that promotes the pharmacological profile and therapeutic properties of drugs. Functionalized CNTs (fCNTs) are highly emerging nanovectors for the delivery of cancer therapeutic drug owing to their capacity to penetrate into the cells via two established mechanisms, via passive diffusion across the lipid bilayer without causing membrane damage or via the process of endocytosis (Pantarotto et al. 2004; Cai et al. 2005; Kam and Dai 2005; Shi Kam et al. 2004). The conjugation of drugs to CNTs is achieved through several ways of surface functionalization which includes covalent linkages, hydrophobic interactions, π-stacking interaction, and capillary-induced filling, among others (Lay et al. 2011). This section discusses a few selected CNT-loaded drug as a nanocarrier.

6.5.1 CNTs-Paclitaxel Conjugates

One of the initial studies of CNTs-based in vivo tumor treatment in mice came up with the use of CNT-paclitaxel complex. Paclitaxel (PTX) conjugated to the branched polyethylene glycol (PEG) chains via ester bonding on the surface of SWCNTs has been used in the synthesis of water-soluble SWCNTs-PTX conjugates to suppress tumor growth in murine 4T1 breast cancer model. Moreover, the SWCNT-PTX complex was found to have tenfold higher uptake rate by the targeted tumor, much likely due to enhanced cell permeability and retention. Additionally, this SWCNT-PTX formulation was much safer compared to Cremophor containing Taxol owing to the higher efficiency of SWCNTs as the drug carrier. The reported amount of SWCNTs required to deliver 5mg/kg PTX is ~4 g/kg, contrary to ~420 mg/kg Cremophor in the case of Taxol for the same PTX dose (Liu et al. 2008). Dual-functionalized MWCNTs with ethylenediamine and phenylboronic acid groups showed promising results of PTX loading through non-covalent π-π stacking within the CNTs interior to target colon cancer cells. Higher water dispersity and biocompatibility were noted as a result of the dual functionalization and drug loading of 30.85% achieved (Rathod et al. 2019). CNT-PTX conjugates can also be used for ultrasound imaging of certain tumors. Due to low xenotoxicity, PTX is commonly used in clinical and preclinical application for ovarian and breast cancer (Högberg et al. 2001). A study conducted by Zhang et al. reported that PEGylated folic acid (FA)-CNT conjugates have superior potential for in vivo high-resolution ultrasound imaging of breast cancers on live Kunming mice (Fig. 6.5). Therefore, functionalized CNTs in combination of PTX can be used as multifunctional toolkit for anticancer therapy as well as bioimaging applications (Zhang et al. 2019).

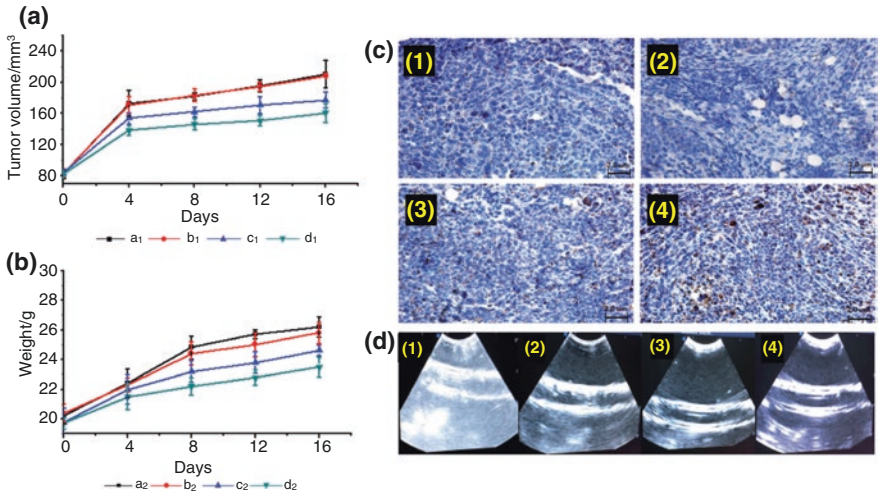

Fig. 6.5 (**a**–**b**) Tumor volume and weight measurements after administration of the Span-PEG (**a1**), Span-PEG-FA-CNTs (**b1**), Span-PEG-CNT@PTX (**c1**), and Span-PEG/FA-CNT@PTX, respectively, (**c**) in vivo apoptosis study in different formulations, and (**d**) in vivo ultrasound imaging of tumor after administration of formulated drugs. (Adopted from Zhang et al. 2019)

6.5.2 CNT-Doxorubicin Conjugates

Folic-acid (FA)-modified MWCNTs have shown promising results in the development of pH-responsive Dox delivery system, including exceptional colloidal stability and biocompatibility. The FA-CNTs often exhibited a substantially high drug loading and encapsulation efficiency of 70.4% besides drug release in an acidic environment. Moreover, FA-CNTs loaded with Dox have been shown to inhibit the tumor cells overexpressing FA receptors (Yan et al. 2018). Non-covalent MWCNT-Dox supramolecular complex is also a promising candidate to kill breast cancer cells. In this regard, MWCNTs coated with tri-block copolymer (Pluronic F127) were found to possess notably higher cytotoxic activity in comparison to Dox alone and Dox-pluronic complexes (Ali-Boucetta et al. 2008). DOX-loaded CNTs incorporated into the poly(lactic-co-glycolic acid) (PLGA) electrospun nanofibers have high antitumor efficiency against HeLa cells. Such electrospun mats render a uniform distribution and trigger sustained and prolonged release of the drug, making PLGA/Dox-CNTs' electrospun mats a long-term drug-releasing platform for potential chemotherapy (Yu et al. 2015). CNTs can also be used for encapsulating nanoparticle-DOX conjugates for effective delivery in tumor cells. Seyfoori et al. reported a pH-responsive chitosan nanogel coated with $MnFe_2O_4$ nanoparticles and grafted it on the surface of MWCNTs to enhance the delivery of Dox in U-87 glioblastoma cells (Seyfoori et al. 2019). The chitosan/$MnFe_2O_4$-grafted MWCNT

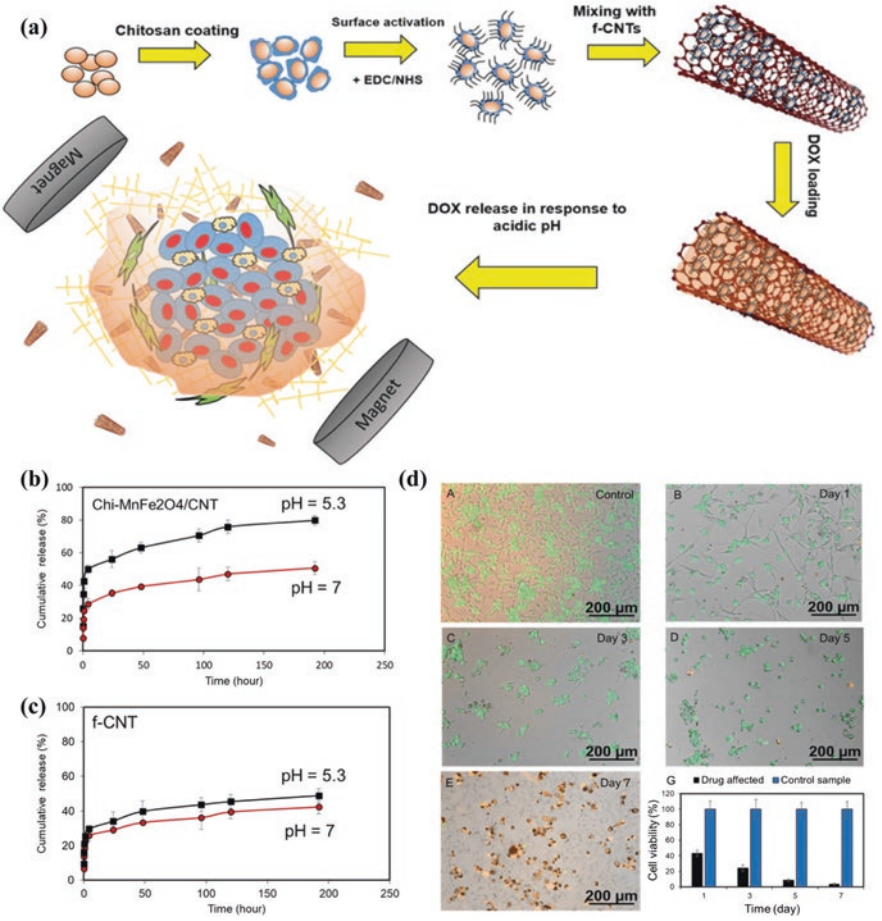

Fig. 6.6 (**a**) Schematic illustration of chitosan/$MnFe_2O_4$ (Chi-$MnFe_2O_4$) nanoparticle synthesis and grafting onto the surface of CNTs, (**b-c**) cumulative release profile of Chi-$MnFe_2O_4$ and Chi-$MnFe_2O_4$/CNT at different pH, and (**d**) cytotoxicity evaluation of Chi-$MnFe_2O_4$/CNTs on U-87 glioblastoma cells. (Adopted from Seyfoori et al. 2019)

exhibited greater pH responsiveness in acidic environment compared to MWCNTs alone (Fig. 6.6). CNTs can also be used as strong photothermal agent owing to its strong absorption at near-infrared region-I (NIR-1). A study conducted by Oh et al. showed NIR-I responsive SWCNT-Dox conjugates as promising treatment strategy for breast cancers. Therefore, the CNT-based nanomaterials could be used to encapsulate Dox for minimizing the high cytotoxic effect of Dox.

6.5.3 CNT-Methotrexate Conjugates

A comparative study of drug release between carboxylated MWCNTs conjugated with methotrexate (MTX) and free MTX was reported to favor slow release of the drug in the conjugated form (Karimi et al. 2018). Furthermore, covalently attached MTX onto MWCNTs through cleavable linkers has been reported to increase its cellular uptake and cytotoxic efficiency to the target cells. The design of such linkers is the critical factor determining the efficiency of such conjugate systems (Samorì et al. 2010). Controlled delivery of MTX against MCF-7 breast cancer cells was achieved by CNT-loaded MTX-injected hydrogel of chitosan and β-glycerophosphate (Saeednia et al. 2019). Besides, hybrid system of MWCNTs coated with chitosan was fabricated as a pH-responsive carrier for MTX delivery to lung cancer cells. The MTX release from such hybrid system is favored at an acidic pH of H1299 cancer cells (5.0), as compared to the neutral pH of the noncancerous cells (7.4) (Cirillo et al. 2019). CNTs functionalized with interpolymer complexes of polyethylene glycol (PEG) and polyacrylic acid (PAA) have also been used for the delivery of MTX to increase the biocompatibility of CNTs itself (Azqhandi et al. 2017). Moreover, CNTs functionalized with both folic acid and MTX showed enhanced anticancer activity against folate receptor-positive (FR) lung epithelial carcinoma (A549) and breast cancer cells (MCF-7) (Das et al. 2013). The endolysosomal trafficking in the presence of folate-modified MWCNT-MTX conjugates was found significantly higher than MWCNTs alone (Fig. 6.7). In another study, the amine-modified MWCNTs were conjugated with MTX via esterification reaction which favors the controlled delivery of MWCNT-MTX conjugates inside cells as well as high cytotoxicity in cancer cells (Joshi et al. 2017). Therefore, functionalized CNTs have several merits over pure CNTs and could be used as a potential delivery vehicle for MTX.

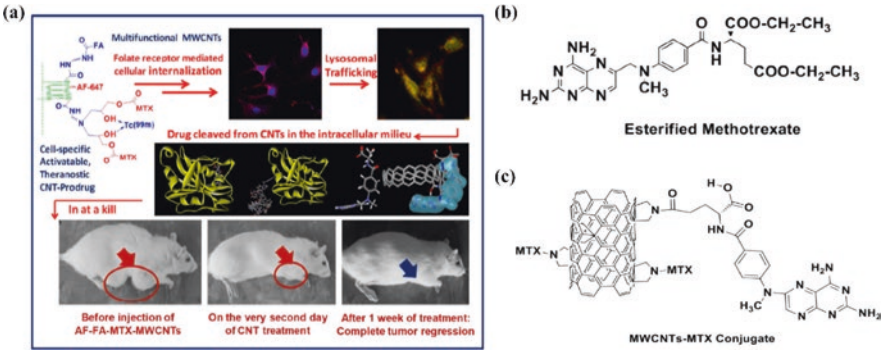

Fig. 6.7 (**a**) Schematic illustration for the synthesis of MTX-loaded folic acid-modified MWCNTs with enhanced anticancer properties (Das et al. 2013), (**b–c**) synthesis of esterified MTX and its loading onto aminated MWCNTs. (Adopted from Joshi et al. 2017)

6.5.4 *Biomolecule-Functionalized CNTs*

Targeted delivery of doxorubicin to HeLa cancer cells is often enhanced by the synthesis of programmable drug delivery system, DNA-functionalized CNTs. For instance, the incorporation of GC/CG-rich stem loop DNA and specific aptamer facilitates selective binding of the intercalating drug and the cell surface receptors, respectively (Hu and Niemeyer 2020). CNT-DNA conjugates are structurally stable. The DNA helix can bind tightly with SWCNTs either by covalently or non-covalently. An overview of various functionalization strategies of CNTs by using different biomolecules is shown in Fig. 6.8.

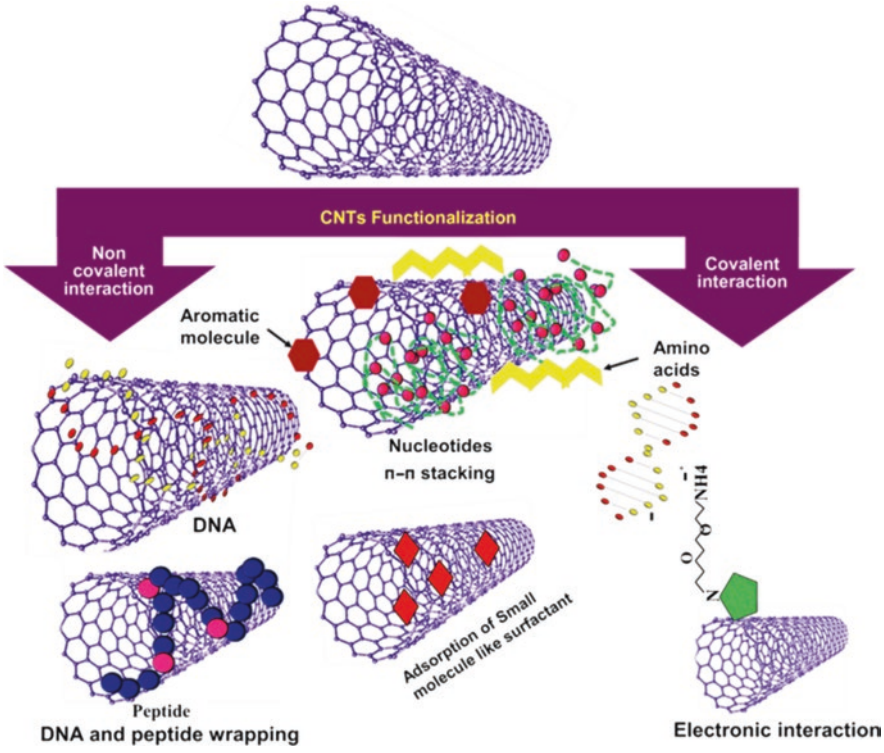

Fig. 6.8 Schematic illustration for functionalized CNTs using various biomolecules. (Adopted from Maheshwari et al. 2019)

6.5.5 Molecular Dynamic Simulation Studies

Simulation studies related to the interaction of drugs or a combination of drugs with functionalized CNTs have tremendously improved the selection of potential CNT-drug complexes. CNTs as a promising anticancer drug carrier are successfully determined through many computer simulations. A simulation study addressing the DOX adsorption efficiency on SWCNTs and MWCNTs at physiological pH of 7.0 revealed stronger adsorption of the drug than it is in acidic pH. This shows the suitability of CNTs as nanovehicles to target acidic cancerous tissues (Maleki et al. 2020). In a similar study, the adsorption of DOX onto covalently functionalized CNTs was studied by MD simulation and quantum mechanics calculations. The outcome of the simulations indicated carboxyl and amine-f-CNTs should primarily be the choice as a pH-sensitive drug carrier favored by the electrostatic interactions by the chemical moieties (Kordzadeh et al. 2019). Moreover, molecular dynamic (MD) simulation studies of DOX and PTX co-loading onto SWCNTs have shown favorable π-π stacking of DOX through conjugated aromatic rings, whereas PTX interacts with SWCNTs through x-π (x = C-H, N-H, and C=O) interactions along with π-π stacking. Upon functionalization of the SWCNTs with chitosan, the affinity of these drugs for the surface of SWCNTs was measured to decrease indicating a desired drug release strategy (Karnati and Wang 2018).

6.6 Conclusion

CNTs are invaluable nanomaterials for the targeted delivery of anticancer drugs. The exceptional physicochemical properties of CNTs based on their type, synthesis process, and numerous covalent and non-covalent functionalizations render them their unique cargo loading capacity. Functionalized CNTs have been used in the delivery of the commonly used drugs like paclitaxel, doxorubicin, methotrexate, DNA conjugates, peptide conjugates, and even small molecules. Several attempts have been made to improve the drug loading capacity of CNTs by enhancing their aqueous solubility and cell penetration ability. Despite such advantages of CNTs as nanocarriers, certain limitations prevail to be addressed which includes eliminating their limited biocompatibility, potential to trigger oxidative stress at higher concentrations, improved real-time imaging techniques, and biodegradability, which can help in the fabrication of smart CNT-based materials with improved pharmacokinetic behavior.

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