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Review

A review of ligand tethered surface engineered carbon nanotubes

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ABSTRACT

Carbon nanotubes (CNTs) have emerged as fascinating materials, exhibiting promising potential in receptor based targeting owing to their unique physicochemical properties (cell membrane penetration, high surface area and drug payload, biocompatibility, easy surface modification, photoluminescence property, and non-immunogenicity etc). The hydrophilicity, a major constrain associated with the first generation of CNTs *i.e.* pristine CNTs, could be overcome using functionalization techniques. In the last two decades variety of functionalized CNTs (*f*-CNTs) *i.e.* oxidized, amidated, acylated, surfactant and biopolymer-assisted, and biomolecules modified have been developed and utilized as effective, safe, nano sized, and smart systems to deliver a wide range of bioactives in the biological system. The purpose of this review is to examine the various aspects of conjugation and associated conjugation chemistry of various targeting ligands to CNTs for their respective biomedical applications. The various biomolecules have been easily tethered to CNTs surfaces including proteins and amino acid, enzymes, nucleic acid (DNA and siRNA), aptamers, vitamins, monoclonal antibodies, peptides (NGR, RGD and Aniopep-2) and so on, for targeting purposes.

1. Introduction

During the last few decades, increasing attention has been paid to drug targeting concepts using nanotechnology to improve the overall therapeutic efficacy by reducing the side effects with minimizing degradation or elimination of drugs. It represents a viable option for cancer theragnostics owing to the size and spatial as well as temporal placement of drug at desired site(s).

In current scenario nanotechnology is a multidisciplinary, rapidly expanding scientific zone and has achieved breakthrough in molecular biology, diagnostics and imaging, bio-engineering, nanomedicines, and therapeutics. According to a statistical data nanotechnology will exceed the impact of the Industrial Revolution on society and is projected to become a US $2.6 trillion business in 2014 [1], while Global nanotechnology announced a comprehensive global outlook on the nanotechnology industry. According to this new report the global nanotechnology market to reach US $30.4 billion by 2015 [2,3]. Nanotechnology unfolds avenues to explore its impact on various fields and has certainly a great impact on the future of medical practice as well bio-nanomedicines [3–8]. Great progress has been made in the arena of nanomaterials such as liposomes [4,5], dendrimers [9–13], nanostructured lipid carriers (NLCs) [14], carbon nanotubes (CNTs) [4,15–25] and polymer-mediated therapeutic delivery strategies to target at the specific sites [26] for boosting the safety and therapeutic efficacy. These above mentioned nanomaterials have emerged as the most lucrative segment of which a large number of related nanotechnologies are already commercialized and earning revenues for the public sector [1].

2. Origin and historical perspective of carbon nanotubes

The CNTs were originally discovered and fully described by Sumio Iijima (Japanese Microscopist) in his TEM observation [27], while some scientists believed that it was earlier discovered by Bacon [28,29].

In 1952, two scientists L.V. Radushkevich and V.M. Lukyanovich published a clear TEM image of 50 nm diameter tubes made of carbon but unfortunately this discovery remained unnoticed worldwide because of langue as well as region restriction [30]. In 1970's, a different kind of CNTs were produced and imaged directly using high resolution transmission electron microscopy (HRTEM) and referred as single-walled carbon nanotubes (SWCNTs) [31]. The circular (armchair nanotubes), spiral, and helical arrangement (chiral tube) of the carbon nanotubes are shown in Fig. 1 [32].

In this sequence different methods for production of CNTs were come in existence like hyperion catalysis [33], capping with fullerene hemispheres [34] and arc discharge method [35]. In 1993, single-atomic layer walled carbon nanotubes (SWCNTs) was reported [36,37]. Since its discovery CNTs have been continuously

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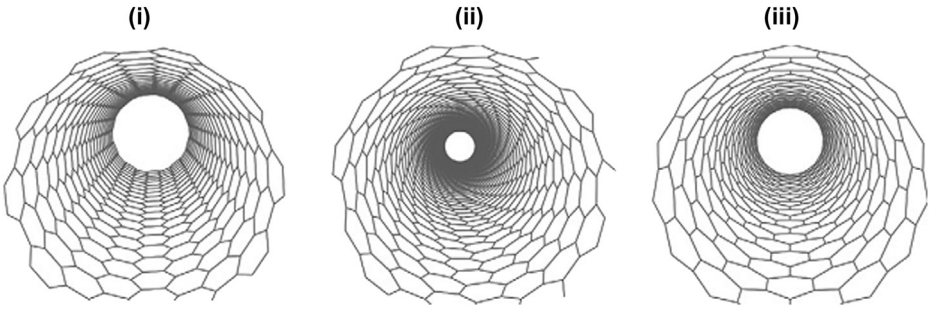

Fig. 1. Schematic representation of three typical types of SWCNTs. (a) Armchair (10, 10), (b) Chiral (13, 6), and (c) Zigzag (14,0) (Reprinted with permission from Ref. [21] Elsevier Pvt. Ltd.).

used in targeted/controlled drug after surface modification in biomedical applications [38–54]. Our group has made modest contribution in the field of pharmaceutical applications of CNTs.

3. Nature and classification of carbon nanotubes

In the existing circumstances, CNTs, the third allotrope of carbon and fullerene family member CNTs, comprised of thin graphite sheets of condensed benzene rings rolled up into the seamless tubular hollow cylinder, nanoneedle shape, have attracted great interest in biomedical and healthcare applications [1,4,5,16,17,20,55]. The ends of CNTs exhibit a resemblance to hemispherical buckyballs connected by a graphene cylinder and depending upon their atomic structure the properties of individual CNTs may vary [7]. Excellent electrical, mechanical and thermal properties including the special features like nanosize diameter, ultra light weight, ultrahigh surface area, aspect ratio (length/diameter) in the range of 1:1000, photoluminescence, rich surface chemistry, non-immunogenicity, biocompatibility and excretion by biliary pathway, neutral electrostatic potential and extremely high drug cargo ability are expected to make CNTs as attractive vehicle for drug delivery [4–6,16,17,56,57]. All types of CNTs materials are commercially available (Carbon Nanotechnologies; Cheaptubes; Sigma Aldrich, Nanoshell etc) and can also be easily synthesized by electric arc-discharge (EAD), laser ablation (LAB), electrolysis and high-pressure co-conversion (HiPCO), catalytic chemical vapor deposition (CCVD) and CoMoCat process. The properties of CNTs may vary depending upon the types of methods employed in the synthesis of CNTs [4,6,7,58–60].

CNTs are considered as promising nano drug delivery vectors, because these are observed to easily cross cell membranes and exhibit fair blood circulation half-lives in order of hours [4,6,7,49,55,57]. Three possible mechanisms of CNTs–drug interaction are: (1) absorption of the active components of drug within the CNTs mesh, (2) surface modification of drug molecules, peptides, nucleic acids on the exterior surface of the CNTs using covalent or non-covalent linkage, and (3) the use of CNT channels as catheters [55].

3.1. Classification of carbon nanotubes

Carbon nanotubes (CNTs) are mainly classified into four categories (Fig. 2) depending upon their diameter, lengths and presence of walls: (i) single-walled carbon nanotubes (SWCNTs), (ii) double-walled carbon nanotubes, (iii) triple-walled carbon nanotubes (TWCNTs) and (iv) multi-walled carbon nanotubes (MWCNTs) [4,6,7,22,23,61,63].

(i) Single-walled carbon nanotubes

Single-walled carbon nanotubes (SWCNTs) consist of only single graphitic sheet seamlessly wrapped into a cylindrical tube structure with a diameter between 0.4 and 2.5 nm. SWCNTs have diameter close to 1 nm, with a tube length that can be several million times longer because of their simplest geometry [1,4,6].

(ii) Double-walled carbon nanotubes

Double-walled carbon nanotubes (DWCNTs) belonging to second class of carbon nanotubes resemble SWCNTs due to the similarities in their morphology and properties. DWCNTs are coaxial nanostructures, containing exactly two concentric graphene cylinders. It is a synthetic blend of SWCNTs and MWCNs. Only the outer wall can be modified, while the properties of the inner tubes would remain unchanged and preserve its intrinsic properties. The smallest nanotubes possessing less than 1 nm diameter can never be DWCNTs [62,63].

(iii) Triple-walled carbon nanotubes

Triple-walled carbon nanotubes (TWCNTs) are characterized by the presence of three walls [61].

(iv) Multi-walled carbon nanotubes

Multi-walled carbon nanotubes (MWCNTs) consisting multiple rolled layers (concentric tubes) of graphene (2–10) are more than one atom thick with >10 nm external diameter. Generally two models, Russian Doll and Parchment models are used to describe the structure of MWCNTs. In Russian model, graphite sheets are arranged in concentric cylinders, while in Parchment model, a single graphite sheet is rolled in around itself, resembling a scroll of parchment or a rolled newspaper [1,4–7,22,56,64,65].

The pristine (raw synthesized) CNTs are not suitable for drug delivery due to their intrinsic hydrophobic nature, thus surface engineering is essential. In last two decade surface decoration of CNTs made a concrete foundation in the development of new drug products, which could be witnessed by many research papers continuously published every year. We believe that in coming years engineered CNTs will make a promising and alternative approach for the treatment of various diseases. Apart from drug delivery, surface engineered CNTs have also been used in the field of photodynamics, gene therapy, imaging and diagnostic, catalysis, sensor, and in nano-electronics etc. Jain and co-workers exhaustively reviewed the appraisal toxicities associated with the pristine and functionalized carbon nanotubes [7]. This research group also explored the safety and efficacy concerns of carbon nanotubes using cancer cell lines [56].

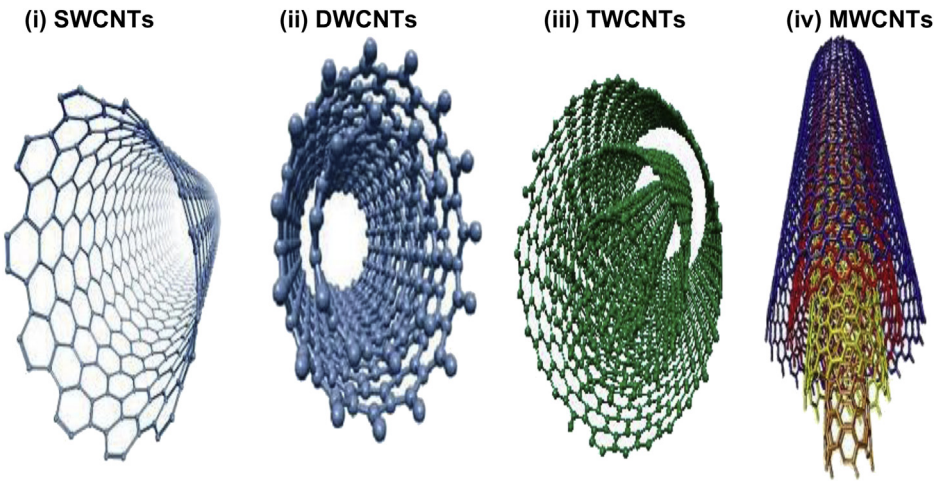

Fig. 2. The classification of the carbon nanotubes.

4. Recent advances in functionalization of carbon nanotubes

The creation of numerous functional groups on the surface of CNTs is termed as functionalization. The pristine CNTs are unsuitable for interaction with biomolecules because these have pronounced tendency to agglomerate or bundling and lack of solubility in aqueous media. These major hurdles can be sorted out by functionalization through chemical modification of the CNTs surface [4,20,24,66]. The various functional groups can easily be attached on the surface of CNTs by the chemical modification, which enhances its aqueous solubility with reduced tendency of agglomeration or bundling. The chemical modification can be accomplished by adsorption, and other interactions (electrostatic, hydrophobic, or covalent and non-covalent bonding). The considerable works have been carried out on the purification, carboxylation, acylation, amidation, esterification, PEGylation, and polymers wrapping (chitosan and alginic acid) as routine methodology for the functionalization of pristine CNTs [17,18,22,24,66]. Our laboratory has been continuously exploring the impact of chemical modifications of CNTs on biomedical applications, especially in targeted drug delivery of anticancer agents [1517,22]. Generally, CNTs functionalization can be achieved in two steps; first by non-covalent, and then covalent functionalization.

4.1. Non-covalent functionalization of CNTs

The non-covalent functionalization includes hydrophobic, π–π stacking, Vander-walls and electrostatic interactions among CNTs and biotherapeutics. Lipids and biopolymers show Vander-walls interaction; aromatic organic compounds and nucleic acids show π–π stacking, and fluorophores and proteins exhibit hydrophobic interaction [1,4,7,56].

4.1.1. Defect site chemistry–oxidation reactions

Among the various surface functionalization techniques, oxidation of pristine CNTs is most widely investigated. A gas-phase oxidation route, involving treatment with nitric acid vapor, is proved to be efficient enough in generating oxygen containing species on the sidewall of CNTs surfaces and avoids filtration, washing and drying steps [67,68]. Now-a days the treatment of CNTs with strong acids (nitric acid, sulfuric acid) and hydrogen peroxide are most extensively used functionalization approaches and involve, as primary step, for the generation of oxygen containing functional groups like carboxylic, phenolic, and lactone [7,16,18,69].

4.2. Covalent functionalization of CNTs

The 'ends and defects' and 'side walls' functionalizations are the two subcategories of covalent functionalization of CNTs, wherein the former technique is more specific and reactive than the later. The chemical treatment of pristine CNTs with most widely used strong oxidative agents (concentrated H_2SO_4:HNO_3::3:1) generate the oxygenated groups such as carboxylic, ketone, alcohol, and ester at 'ends and defects' sites of CNTs. This treatment not only generates the various functional groups, but also cut and shortens the CNTs into the smaller pieces.

Side wall functionalization introduces higher concentration of covalently attached functional groups on the CNTs surface with the trade-off of significant perturbation in the electronic structure [1,5,7,56]. A more detailed account about the progress on the chemical modifications of carbon nanotubes was reviewed by Karousis and Tagmatarchi [67].

5. Ligand-driven carbon nanotubes in targeted drug delivery

Ligand–receptor interactions play a crucial role in targeted drug delivery. Ligand is characterized as a biomolecule that specifically interacts with the receptors present on the surface of a cell, tissue or organ. It may bind with specific receptor site, and can be easily internalized inside the cell. Now-a-days it represents a diverse range of class of molecules that can be exploited in targeted drug delivery owing to the ligand–receptor interaction. The receptor expression and internalization are the two most crucial considerations, which govern the selection of ligand used in cancer cells targeting [4,70,71]. Recently, Jain and co-workers reviewed the various receptors engaged in targeting of therapeutics incorporated in dendrimers, liposomes, nanoparticles, micelles, carbon nanotubes and polymersomes etc. [4].

5.1. Biomolecules conjugated CNTs based nanoarchitecture

A full description of the ligand conjugation and their evaluation for targeting purposes on the ground of challenges and recommendations is elaborated under successive headings that will be beneficial for obtaining safer and reliable CNTs based drug products for biomedical applications. Apart from this we have also reviewed the latest researches in the field of CNTs with more emphasis on their targeting propensity at desired/specific cells or tissues and covered the most advanced fundamentals concerning the

conjugation of various targeting ligands in treatment of numerous diseases (cancer, diabetes, malaria, tuberculosis, HIV etc).

Due to their large surface area, CNTs may easily be conjugated with numerous biomolecules including proteins and amino acid, enzymes, nucleic acid (DNA and siRNA), aptamers, vitamins, monoclonal antibodies, peptides (NGR, RGD and Aniopep-2) and so on. The targeted delivery conjugated with the numerous biologic targeting ligands can be designed to dimensionally approach at sub-cellular scale providing a new generation of nanohybrid materials with potential applications in diagnostics and targeted therapy. Such nanohybrid materials have key functions like (i) specific targeting to desired/specific diseased sites/tissues, (ii) ability to deliver a high therapeutic "payload" at the site, (iii) ability to report information from the site, and (iv) tolerability to the host [72].

5.1.1. Aptamer conjugated CNTs

Some virus encoded small structured RNA showed the binding affinity and specificity with viral or wide range of biomedically relevant molecules like drug or host proteins etc. These small structured RNAs, termed as aptamer, are single stranded DNA and RNA materials containing 20 to 80 nucleotides and are currently used as molecular agent for targeting purposes [50,73–75]. These oligonucleic acid molecules have the ability to bind with target proteins or other biological molecules with high affinity and specificity. Small size, flexibility, chemical simplicity, and reversible denaturation make aptamers superior as compared to other receptors like antibodies [76]. Additionally, aptamer may confront few challenges like susceptibility to nuclease-mediated degradation of DNA and RNA. Further, aptamers as chemicals are unable to cross the biological barriers like cell membrane to perform target-specific recognition to entry into the cell. Aptamers are the biological molecules with more potential for CNT modifications. Moreover, these aptamers could easily bind with the CNTs *via* covalent and non-covalent interaction and stabilized aptamers against nuclease-mediated degradation with increasing solubility and binding affinity [50,73]. Szostak and his research group described a methodology, called *in vitro* selection of Systemic Evolution of Ligands by Exponential Enrichment (SELEX), to enrich aptamers with high binding affinity towards target molecules and used to isolate cell-specific aptamers. Till date numerous aptamers have been generated by this methodology for cancer cells like liver and lung cancers [77].

Moreover, in 2003 a selection strategy termed as Cell-SELEX was also designed to target the entire cells, which allowed isolated aptamers to recognize cells without prior knowledge of the target molecules. However a counter-selection process was further integrated into the conventional Cell-SELEX technique in which selection process itself could differentiate various types of cells to obtain cell-specific aptamers [78]. Anti-thrombin aptamers, highly specific to serine protein thrombin, were immobilized on the surface of the SWCNT-FET using CDI-Tween linking molecules [79]. Mi and co-investigators conducted an *in vivo* selection approach into tumor bearing mice for isolating aptamers, which were capable to localize in the tumors [80]. Recently Xiao and co-workers adopted the cell-uptake selection strategy for the targeted intracellular delivery of therapeutics to enrich cancer-cell specific internalization of aptamers [73].

Easy conjugation of aptamers to the various nanocarriers like dendrimers, solid lipid nanoparticles and carbon nanotubes may open a new path in biomedical applications. The Sgc8c aptamer were used as targeting ligand to leukemia biomarker protein, tyrosine kinase-7 and were recognized with high affinity (kd = 1 nM) among normal human bone marrow aspirates. Sgc8c aptamer, 5′-ATC TAA CTG CTG CGC CGC CGG GAA AAT ACT GTA CGG TTA GA-3′ was synthesized using a Polygene DNA synthesizer and purified by polyacrylamide denaturating gel. Cell specific internalization study of Sgc8c aptamer conjugated SWCNTs showed that Sgc8c were easily internalized in all T-cells. SWCNTs containing highly delocalized π electrons rich structure could easily be functionalized *via* π–π interaction. The π–stacking interactions between the nucleotide bases and the sidewalls played an important role in forming a stable complex. Briefly, sgc8c aptamer (40 μM) and SWCNTs (350 mg/l) were mixed in Tris–HCl buffer [tris(hydroxylmethyl)aminomethane] at pH 7.4 and sonicated for 2 h followed by centrifugation for 2 h at 21,000 g. The obtained pellet comprising of impurities, aggregates and bundle of nanotubes at the bottom was discarded and supernatant was collected and again centrifuged. The concentration of the solubilized SWCNTs *i.e.* aptamer-SWCNTs complex was estimated at 808 nm. Then daunorubicin (Dau) was loaded into functionalized SWCNTs at 37 °C in PBS pH 7.4. Dau loading efficiency (defined as the weight ratio of Dau to the functionalized SWCNTs) of nano-tubes was found to be more than 157% [81].

Recently Das et al. demonstrated the highly sensitive and selective detection of ATP by displacement of the ssDNA anti-ATP aptamer hybridized to a small capture oligonucleotide by covalently attached SWCNTs [82]. Fu et al. reported a simple and efficient post-labeling strategy based on dye-induced peeling of the aptamer molecules from SWCNTs for electrochemical aptasensing of thrombin with 3 pM detection limit [83]. Ouyang et al. reported CNT-based technique for label-free and time-resolved luminescent assay of lysozyme by engineering an anti-lysozyme aptamer and luminescent europium (III) (Eu^{3+}) complex based on the exceptional quenching capability of nanotubes for the propensities of ssDNA and DNA/protein complex. The mixture of chlorosulfonylated tetradentate β-diketone-Eu^{3+} and anti-lysozyme aptamer was easily quenched by nanotubes unless the aptamer interacted with the lysozymes. It showed the good selectivity and high sensitivity for lysozyme due to the highly specific recognition capability of the aptamers for the target and powerful quenching property [84]. Lee et al. reported the aptamer sandwich-based nanotubes sensor strategy for molecular detection in which aptamers used as targeting agent to capture target molecules with enhanced sensor signals, which helped for the onsite monitoring of various environmental pollutants, food toxicants and disease related metabolites. This sandwich assay will open up a new dimension in small molecular detection enabling a broad range of applications like environmental protection and food safety [85]. Maehashi et al. fabricated label-free protein biosensors by covalent immobilization of 5′-amino-modified 45-mer aptamers on the CNT channels for the detection of immunoglobulin E (IgE) with 250 pM detection limit [86].

Aptamers have the capacity to bind with specific target molecules. A simple and quick detection of the pathogens like *Salmonella typhi* have been developed by anchoring the aptamer to SWCNTs, which were deposited onto an electrode in an ultrathin layer that interacted selectively with micro-organism. When the biosensor interacts with *Salmonella* enriched samples, the microbes stick to the aptamers like flies to flypaper by interaction between aptamers and nanotubes. This interaction changes the electrode voltages noticeable within seconds and detects a bacterial concentration about 1000 *Salmonella*/ml. The conventional procedure usually takes approximately two days in laboratory, which is very tedious and time consuming, however it is very simple, precise, fast and error free as the measurement of pH value [2,87].

5.1.2. Peptides conjugated CNTs

The peptide conjugation is another alternative and attractive technique in targeted drug delivery based on peptide conjugation

to the nanotubes surface through specific interactions mechanism. Till date several peptides have been available like angiopep (TFFYGGSRGKRNNFKTEEY, molecular weight 2.4 kDa) [19], RGD (arginine–glycine–aspartic acid) [88], NGR (asparagine–glycine–arginine) [89,90] etc for conjugation to nanotubes surface.

RGD is a tripeptide composed of L-arginine, glycine and L-aspartic acid. The RGD peptide sequence is a common element used as biochemical tool in cellular recognition. The doxorubicin (DOX) loaded RGD peptide conjugated phospholipids (PL)-polyethylene glycol (PEG) SWCNTs (PL-SWCNTs-RGD-DOX) selectively binds to the integrin $\alpha_v\beta_3$ receptor on cancer cells. The PL-SWCNTs-RGD-DOX showed an enhanced cell-killing effect to U 87 MG cell by lowering IC_{50} value owing to specific RGD-integrin recognition with enhanced cellular uptake [88].

The NGR peptide motif is known to bind CD13 isoforms over-expressed in tumor vessels and is widely used for tumor targeting. The NGR sequences play an important role in modulating the binding affinity and specificity of NGR to the CD13 receptor. In current scenario NGR peptides are considered to be promising new targeting ligands for developing tumor vasculature targeted bioactives and theragnostics agents with reduced systemic toxicity [91]. The NGR peptides were anchored onto the docetaxel (DTX) conjugated SWCNTs, where NGR played as angiogenesis targeting peptides. The overall tumor targeting efficiency of SWCNTs-NGR-DTX was found to be higher in tumor and liver as compared to the DTX control group. The maleimide group at the end of 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)-2000] (DSPE-PEG2000) onto the surface of SWCNTs reacted covalently with the double bond of sulfhydryl groups with cysteine in $CNGRCK_2HK_3HK_{11}$ [89]. In the continuation of this recently, a new neovascularity targeting anti-tumor drug delivery system *i.e.* SWCNTs loaded with 2-methoxyestraiol (2-ME) *via* π–π accumulation and linked with NGR peptide was prepared. This neovascularity targeting drug delivery system NGR-SWCNTs-2-ME revealed stronger tumor inhibition effect and improved targeting efficiency as compared to 2-ME alone and SWCNTs-2ME without NGR peptide [92].

Despite the availability of several peptides for angiogenesis, RGD and NGR peptides are the two most famous ones with their targeting receptors. RGD peptide specifically binds to the $\alpha_v\beta_3$ integrin, while NGR peptide primarily binds to amino-peptidase N (APN/CD13). RGD peptide is three-fold less in binding affinity as compared to NGR peptide. A rapid development has been reported with great tumor selectivity of NGR peptide and NGR-based drug delivery [89,90].

A ligand, Angiopep-2 (19-amino acid sequence), the low-density lipoprotein receptor-related protein (LRP) present on the blood brain-barrier (BBB), exhibits the higher LRP-1 receptor-mediated transcytosis mechanism and parenchymal accumulation. It is considered for the selective targeting to the brain *via* receptor-mediated targeting. Angiopep-2 (ANG) can specifically combine to the lLRP receptor over-expressed on the BBB and glioma cells. The terminal MAL groups of O-MWCNTs-PEG-MAL reacted with the thiol group of angiopep (ANG) in PBS and purified by Millipore ultrafiltration and re-dispersed in PBS (Fig. 3). The developed O-MWCNTs-PEG-ANG formulation was evaluated for its biological safety by cytotoxicity test on the BCEC and C6. The CD68 immunohistochemical analysis proved better biocompatibility and low toxicity of DOX-O-MWCNTs-PEG-ANG. Fig. 4 shows the intracellular fluorescence distribution of the FITC loaded developed MWCNTs formulation in BCEC cells. On the basis of data it could be concluded that DOX-O-MWCNTs-PEG-ANG was a promising dual targeting carrier, which efficiently delivered doxorubicin (DOX) to the brain tumor with decreased cardiac toxicity as compared to free DOX [18].

5.1.3. Chitosan/Alginic acid conjugated CNTs

The surface modification of pristine CNTs can be achieved by the synthetic hyperbranched polymers *i.e.* poly (phenyl acetylene), poly (propylene imine) (PPI) and poly amidoamine (PAMAM) dendrimers as well as natural polymers such as polysaccharides [sodium alginate (ALG) and chitosan (CHI)] and glycated chitosan *via* non-covalent interactions with improved compatibility in physiological environment [24,55,59,93,94].

Chitosan is a naturally occurring polymer derived from molluscs and prawns. It is an amino-glucopyran composed of repeating unit *i.e.* N-acetylglucosamine (GlcNAc) and D-glucosamine (glcN) with one amino group and two hydroxyl groups. This renewable polysaccharide has wide applications in controlled/targeted drug delivery due to its safe and non-toxic nature [24,55]. Chitosan possesses various attractive characteristics. The presence of reactive amine groups in chitosan facilitates ligand attachment for targeted drug delivery. The chelation and cationic properties of chitosan do not require chemical bonding with the nanoparticulate matter due to convenient means of electrostatic interactions. Further, cationic chitosan-based nanocarriers can effectively adhere to negatively charged phospholipid bilayer of cellular membranes. The presence of lysosomes in cellular endocytosis helps in degradation of chitosan to release the encapsulated drug with higher efficiency upon intracellular uptake of nanoparticles. The solubility of chitosan at endosomal pH 5.3 and insolubility at physiological pH 7.4 prevents untimely release of encapsulated drug before the target site is reached [24].

The chitosan can be conjugated on the surfaces of modified CNTs for attaining the improved dispersibility, release, and targeting efficiency as compared to chitosan-free CNTs conjugates. Despite the excellent progress in projecting the surface modified CNTs as drug delivery systems, much research is still needed for further optimizing the ability for selective targeting to specific tissues/sites and to release the toxic payload in a controlled fashion. Till date only few studies are available on chitosan, alginic acid-CNT conjugates for targeted drug delivery. The polysaccharide like ALG and CHI modified SWCNTs were used for controlled release of anticancer agent, DOX. Folic acid (FA), a targeting agent can additionally be tethered to the SWCNTs for selective delivery of DOX into the lysosomes [24]. The modifications of the oxidized SWCNTs with ALG or CHI were done by a two-step process involving initial treatment with ALG followed by CHI, wherein the SWCNTs core could be 'doubly' wrapped by both CHI and ALG. After coating with ALG or CHI, the sidewalls of SWCNTs seemed to be smooth and without impurities (metal particle and amorphous carbon) with size $<$500 nm (Fig. 5). The developed drug delivery exhibited excellent stability under physiological conditions, but at lower pH, typical of the tumor microenvironment, the DOX was efficiently released and entered the nucleus where it induced cell death more selectively and found to be more effective than free DOX in terms of reduced general toxicity. Similarly the several strategies for CHI conjugation to SWCNTs have been reported in the form of films, hydrogels and fibers [95–97]. A simple biopolymeric composite matrix was processed from CHI and SWCNTs dispersion and loaded with dexamethasone phosphate (DEX) [95].

The folate-decorated chitosan-CNTs (DOX-CNT-CHI-FA) nanocarrier for targeted delivery of DOX was synthesized and evaluated by Huang and co-workers in 2011. The relatively superior controlled release of DOX from the developed DOX-CNT-CHI-FA nanocarrier, in contrast to non-encapsulated, was attributed to: (i) degradation of CHI and diffusion through the CHI shell, and (ii) FA-DOX hydrogen bonding. The acidic microenvironment facilitated the higher DOX release due to the reduced interaction between DOX and CNTs [55]. In the allied of the chitosan conjugation to nanotubes, a new type of drug delivery systems involving CHI modified SWCNTs for

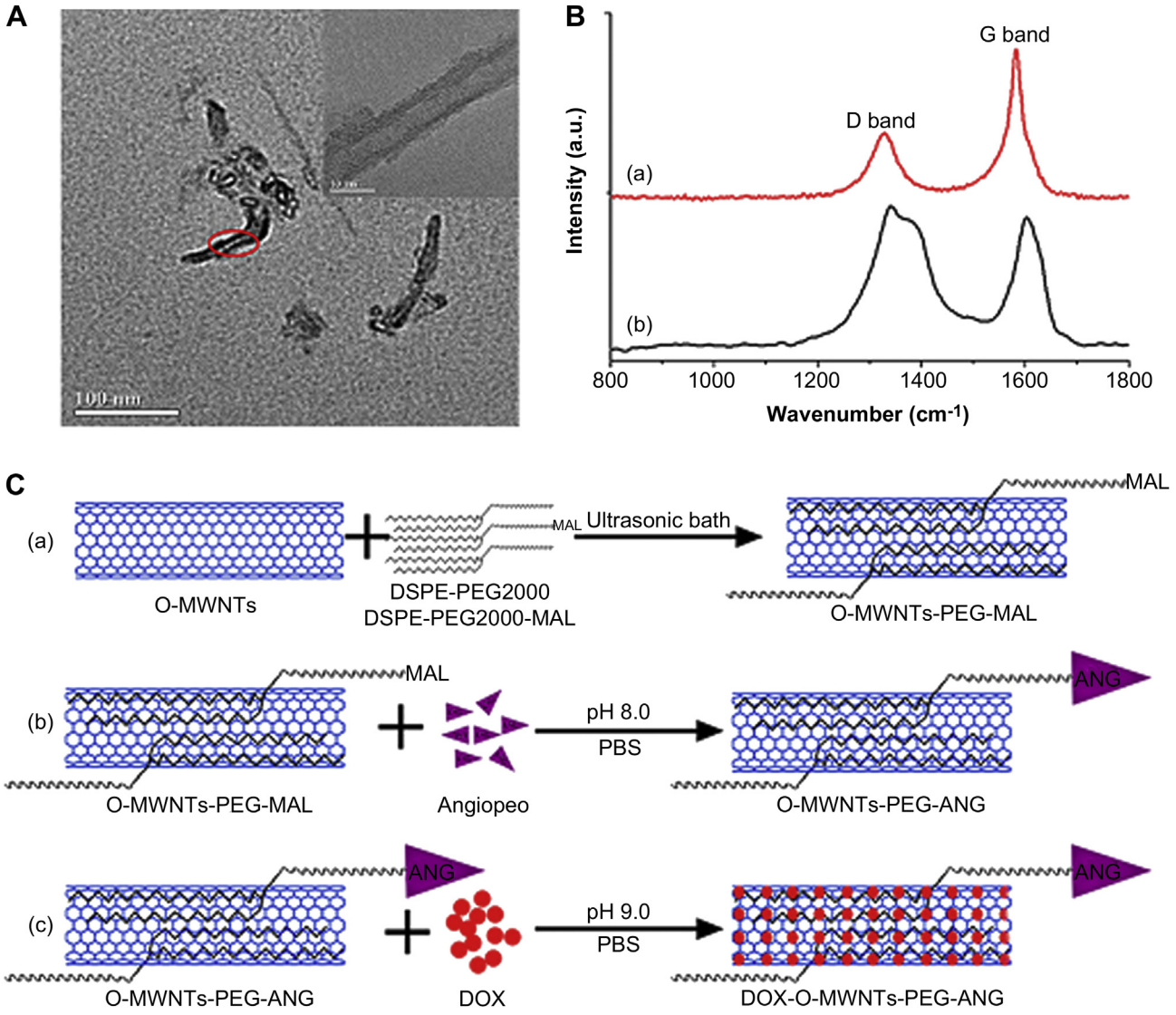

Fig. 3. (A) The TEM image of oxidized MWNTs. (B) The Raman spectra of raw MWNTs (a) and oxidized MWNTs (b). (C) Synthetic scheme to DOX-O-MWNTs-PEG-ANG (Reprinted with permission from Ref. [18] Elsevier Pvt. Ltd.).

controlled loading and release of DOX was constructed. CHI was non-covalently wrapped around the nanotubes, which imparted the water-solubility and biocompatibility to the nanotubes. FA was also used to bind on the outer CHI layer to obtain a tumor targeting vehicle (FA/CHI/SWCNTs) and the pharmaceutical efficiency was examined on SMMC-7721 cells. The zeta potential of the FA/CHI/SWCNTs and DOX/FA/CHI/SWCNTs was found to be -3.78 ± 0.12 and 5.53 ± 0.15, respectively. The DOX/FA/CHI/SWCNTs showed the high therapeutic payloads and efficiently released DOX at reduced pH in the tumor microenvironment as well as intracellular lysosomes and endosomes. The developed DOX/FA/CHI/SWCNTs effectively killed the HCC SMMC-7721 cell and depressed the growth of liver cancer in nude mice [66].

5.1.4. Antibody conjugated CNTs

The conjugation of targeting antibodies to the CNTs surface to yield prototype nanoconstructs is a major advancement in the targeted delivery. Generally, nanotubes based antibody targeting is a promising nanomodality in therapeutics and diagnostic oncology. Till date only few studies have been reported for conjugation of antibody to functionalized CNTs. All of the antibodies, which are clinically used today for cancer cell targeting are mammalian IgG monoclonal antibodies [72]. Recently, there has been renewed interest in using IgY antibodies as IgG substitutes in clinical applications because IgYs are distinct from IgGs in molecular structure and biochemical features. The IgYs have many attractive advantages like biochemical, immunological and production over IgGs and are most suitable for product development [72,98,99].

The conjugation of antibodies to CNTs is generally performed into two ways: (i) direct, and (ii) indirect conjugation. In direct conjugation, the direct adsorption of antibodies to CNTs is performed, but the weak interaction between the nanotubes and antibody raises the possibility of loss of the targeting function of the antibodies while in indirect conjugation an additional step *i.e.* Streptavidin–biotin interaction was utilized for the preparation of antibody–biotin conjugate [100].

The binding of an anti-fullerene IgG monoclonal antibody to SWCNTs was reported by Erlanger and co-workers. This monoclonal antibody specifically recognized C60 fullerenes and bound exclusively to SWCNTs. Induced fit mechanism was involved in the binding of fullerenes [101].

The tumor targeting CNT nanoconstructs were synthesized from sidewall-functionalized, water-soluble CNT by covalently attaching multiple copies of tumor-specific monoclonal antibodies, radiometal

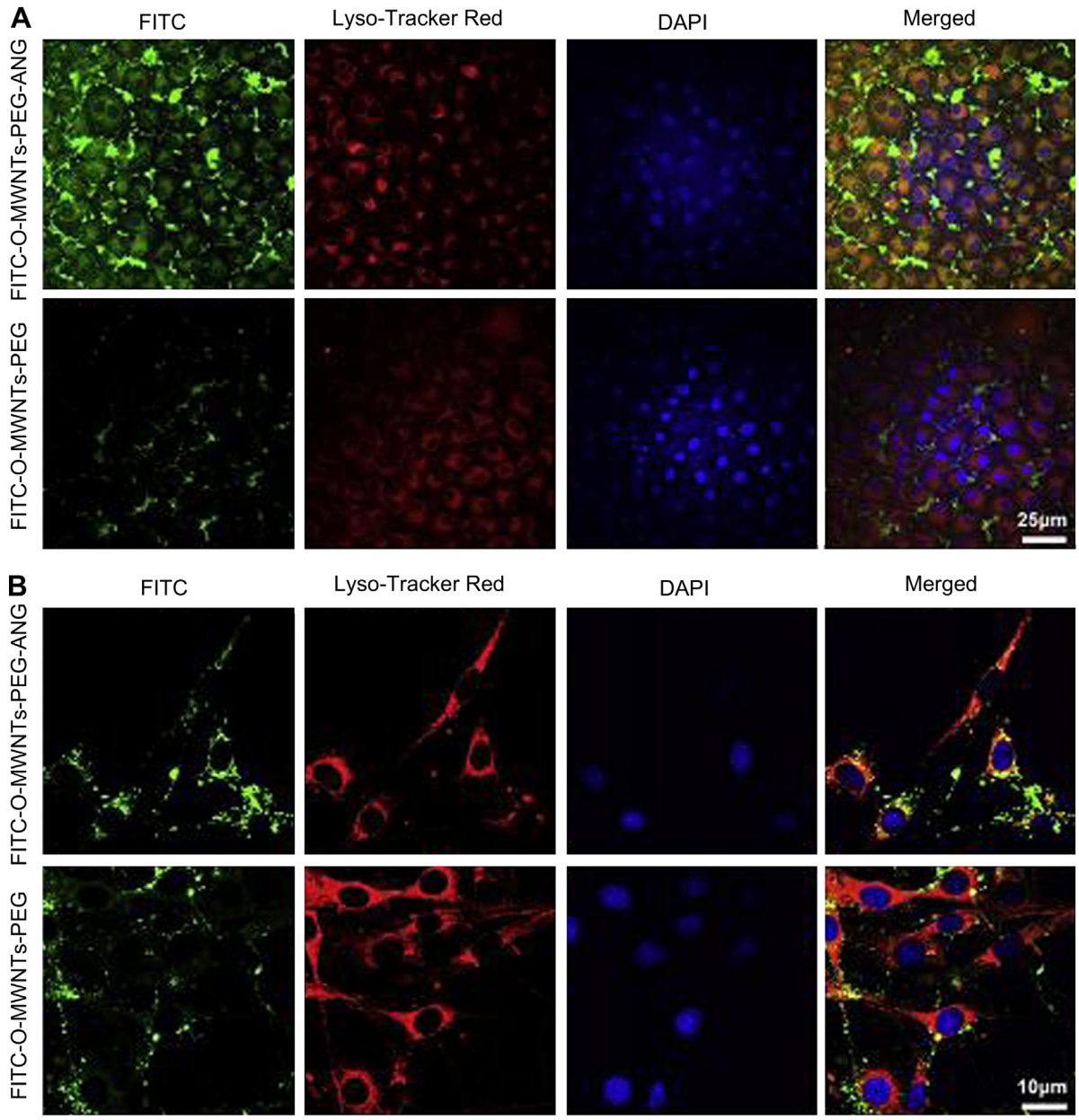

Fig. 4. Intracellular fluorescence distribution in BCEC cells (A) and C6 cells (B) after incubated with FITC-O-MWNTs-PEG or FITC-O-MWNTs-PEG-ANG (containing 10 ug/mL FITC) for 2 h, the nuclei were stained with DAPI and the lysosomes were stained with Lyso-tracker Red. (Reprinted with permission from Ref. [18] Elsevier Pvt. Ltd.).

ion chelates as well as fluorescent probes and characterized spectroscopically, chromatographically and electrophoretically [72]. The anti-HER2 chicken IgY antibody, is found more specific and sensitive than the mammalian IgGs. In an interesting study anti-HER2 chicken IgY antibody was covalently conjugated to carboxylated HiPco SWCNTs using a microwave-assisted functionalization method. The synthesized HER2 IgY–SWCNTs complexes demonstrated very high specificity for HER2-expressing cancer cell during the selective targeting of cancer cells [100].

5.1.5. Nucleic acid conjugated CNTs

The DNA modified or wrapped nanotubes have also been investigated as building blocks for self-assembled nanodevices and mostly used in nanoelectronics and biosensing [102]. In a research study amine terminated DNA strand was conjugated onto the open ends and defect site of oxidative functionalized SWCNTs (Fig. 6) [103]. Villa and co-workers synthesized the covalently modified SWCNTs bearing single stranded oligonucleotide analogs, radio tracing moieties and targeting peptides. The developed SWCNTs-oligonucleotide conjugates were proved as nanoscale platforms capable of specifically recognizing complementary sequences and as targeted self-assembled complex therapeutic nanostructures *in vivo* [102].

5.1.6. Human serum albumin (HSA) protein conjugated CNTs

The specific human serum albumin (HSA) receptors are over expressed on the liver cancer cells and have the ability to internalize huge amount of albumin through caveolae-mediated endocytosis mechanism. Till date only one report is available for selective targeting *via* Gp60 receptors located on the membrane of malignant liver cancer cells using HSA-FITC and CNTs. The HSA was conjugated on CNTs *via* non-covalent functionalization method. Briefly, the oxidized MWCNTs and HSA-FITC were mixed with the de-ionized water at a concentration of 0.25 and

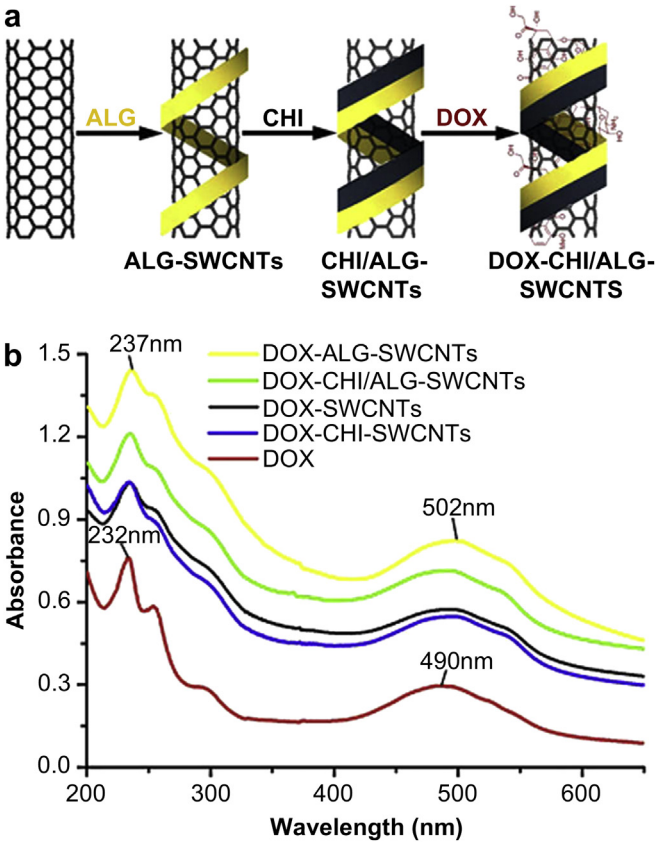

Fig. 5. Preparation of modified SWCNTs. (a) Modification of SWCNTs (derivatized with-CO_2H groups) with ALG, CHI and DOX, and (b) UV–Vis absorption spectra of DOX and DOX loaded SWCNTs (Reprinted with permission from Ref. [24] Elsevier Pvt. Ltd.).

1.25 mg/mL, respectively. The mixture was sonicated for 1 h with tip sonicator in an ice bath and centrifuged for 5 min at 12,000 rpm. The solid fraction settled down at the bottom of the centrifuge tube and resultant supernatant was collected and again centrifuged. The HSA-MWCNTs conjugate was purified using gel chromatography. The post irradiation apoptotic rate of HAS-MWCNTs treated HepG2 cells for 50 mg/L was found to range from 88.24% at 60 s to 92.34% at 30 min with lower necrotic rates. Authors claimed that HAS-MWCNTs conjugate selectively attached to albondin (aka Gp60) receptor located on HepG2 cell membrane, followed by uptake through a caveolin-dependent endocytosis process but on the other hand authors suggested that the further research was required for better understanding of the mechanism involved in selective binding of HSA-MWCNT conjugate in malignant cells and also assessment of the unexpected toxicities [104].

5.1.7. *Epidermal growth factor (EGF) conjugated CNTs*

The epidermal growth factor receptor (EGFR) is a transmembrane receptor consisting of: (i) a transmembrane domain, and (ii) an extracellular ligand-binding and intracellular domain with tyrosine kinase activity. Among all cancer cells it is over-expressed especially on squamous cancer cells. The EGF has six endogenous ligands *i.e.* EGF, transforming growth factor-α (TGF-α), betacellulin, amphiregulin, heparin-binding EGF (HB-EGF), and epiregulin. The EGFR is generally over expressed in number of malignancies that are related to angiogenesis, proliferation, invasion, and metastasis of the tumor cells. Thus it has been increasingly used as a potential cancer targeting ligand with high binding ability as well as selectivity [4,71].

EGFR-mediated SWCNTs bioconjugates targeted delivery was reported by Bhirde and co-workers [105,106]. Cisplatin, first line anticancer agent and EGF were attached to SWCNTs surface for specific targeting of the squamous cancer, while SWCNTs-cisplatin without EGF was used as non-targeted control. The cisplatin loaded

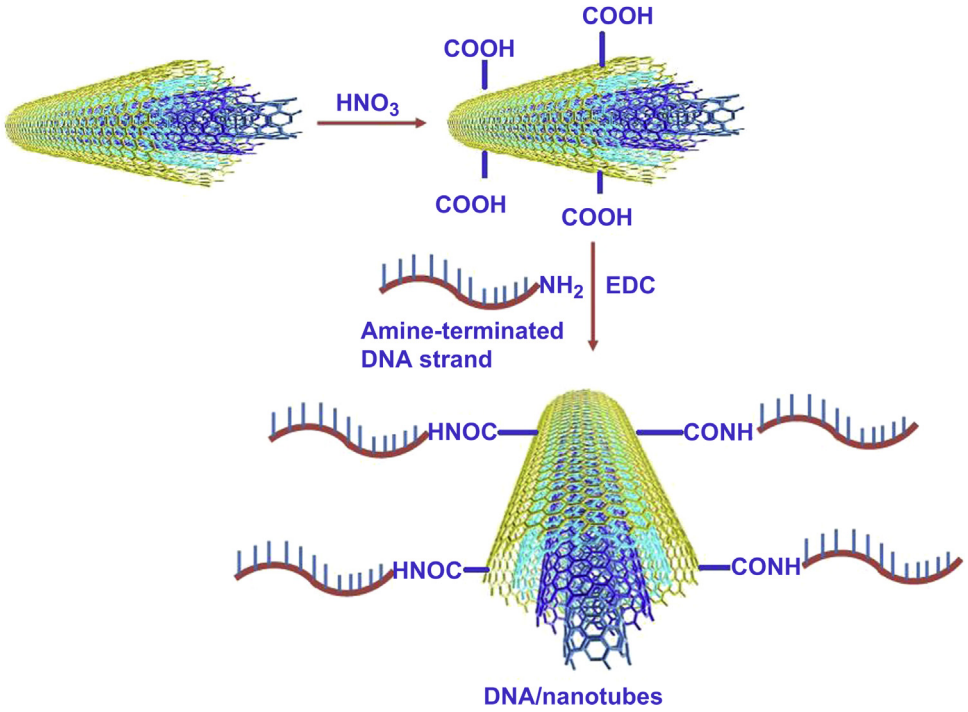

Fig. 6. Modified schematic representation of nucleic acid conjugated carbon nanotubes [103].

EGF–SWCNTs conjugates selectively targeted, entered and killed cancerous cells utilizing EGF–EGFR interactions in HNSC cells. The biodistribution and blood clearance studies of PEGylated SWCNT in mice demonstrated the effectiveness and lower toxicity of the aqueous dispersible PEG–SWCNTs in HNSC cells.

An anticancer drug, Etoposide (ETO), the semi-synthetic derivative of podophyllotoxin, acts in the late S and early G2 phases of the cell cycle. The mode of action of ETO is based on the interaction of ETO with DNA topoisomerase II and breakdown of DNA. Its current therapeutic uses are limited owing to its side-effects *i.e.* hair loss, myelosuppression and low blood pressure. To enhance the therapeutic potential of ETO, a targeted drug delivery system (TDDS) of epidermal growth factor-chitosan-carboxyl single-walled carbon nanotubes-ETO (EGF/CHI/SWCNTs-COOHs/ETO) using modified SWCNTs (m-SWCNTs) carrier was employed. The loading efficiency of CHI/SWCNTs-COOH and EGF/CHI/SWCNTs-COOHs was found to be 27.6 ± 2.25 and 25.2 ± 2.18%, respectively because ETO molecules were easily bound *via* π–π stacking and electrostatic interactions. The ETO was subsequently released from the developed TDDS at the lower pH and was taken up by the tumor cells through energy-dependent endocytosis mechanism using A-549 tumor cell line due to active targeting by EGF overexpressing EGFR [107]. These research studies have proved the targeting potential of EGFR-CNT-mediated targeted delivery of bioactive.

5.1.8. Protein conjugated CNTs

The protein can be attached to the substrate in two ways, firstly protein can directly be adsorbed onto the substrate surface, wherein the portion of the protein in contact with the surface is enzymatically inactive, and accessing of the active functional site is spatially hindered. Secondly, it can be tethered to the substrate using flexible polymer chain (PEG or DNA). A modified Staudinger–Bertozzi ligation has been used to couple an end of CNTs with an azide group, which is site-specifically incorporated into a protein of interest. Yoshimura et al. successfully attached the calmodulin, Ca^{2+}-sensor protein to the end of nanotubes without affecting its enzymatic function (calcium-dependent substrate binding). In this study authors also found that as the diameter of the nanotubes increased, the number of bound protein molecules also increased and this can be controlled by the controlling the size of the nanotubes end [108]. The conjugation of various biomolecules to CNTs has been depicted in the Fig. 7.

5.1.9. Miscellaneous conjugated CNTs

The conjugates of carbohydrates (galactose, lactose, mannose etc) and the functionalized nanotubes are called as "*glyco-nanotubes*" similar to *glycodendrimers* (conjugates of carbohydrates and dendrimers) [11,22,65]. Our laboratory has published two research articles in context with glyconanotubes involving sequential steps (purification, carboxylation, acylation and

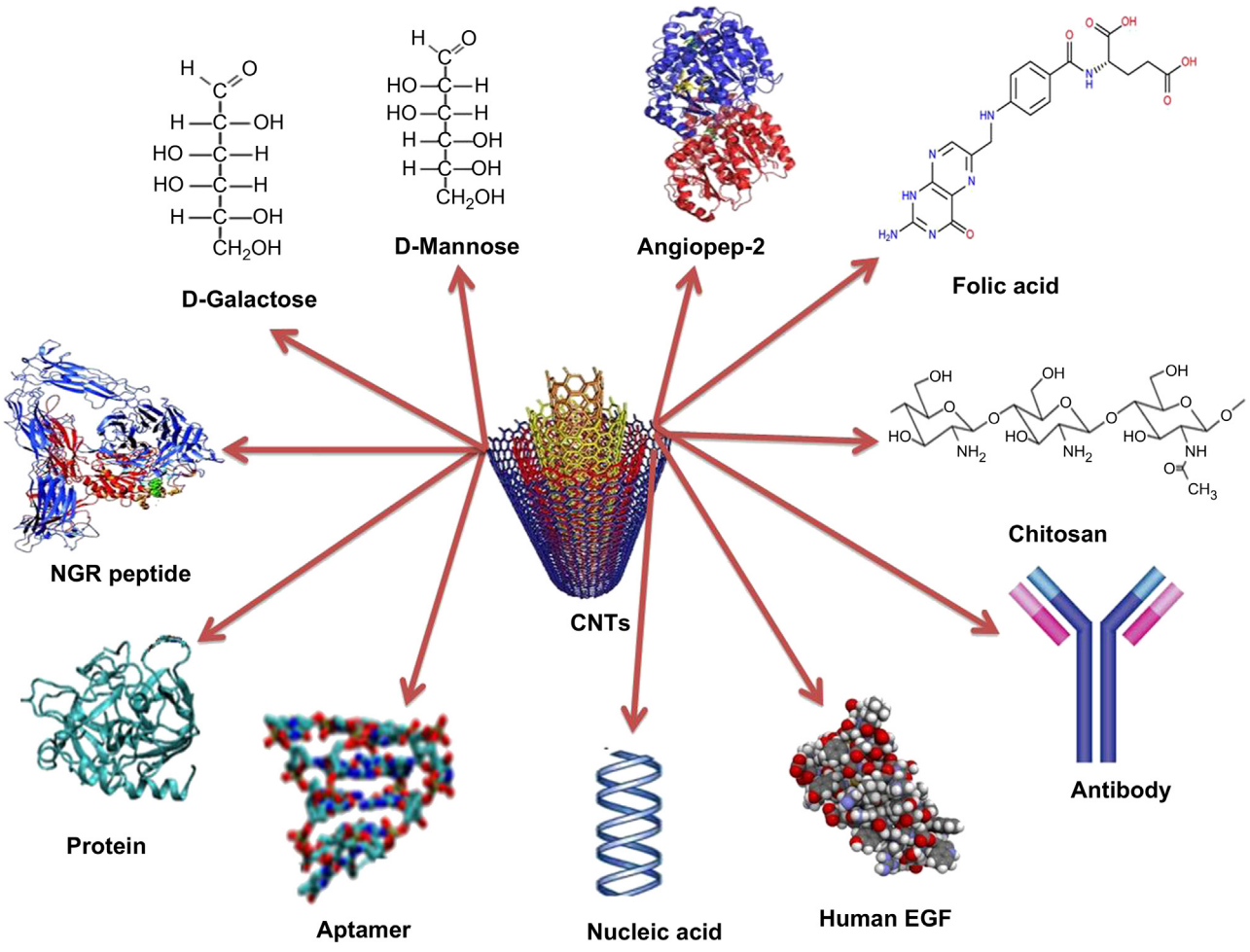

Fig. 7. The various biological molecules conjugated to carbon nanotubes.

amidation) in the development and characterization of engineered nanotubes [9,22,65].

For the development of galactose-conjugated MWCNTs (Gal-MWCNTs) the MWCNTs (5 mg; 7.9 mmoL of $-NH_2/g$) were taken in the sodium acetate buffer (pH 4–5) and D-galactose (13.5 mg; 15 mmoL) and stirred for 3 days at the ambient temperature. The resultant solution was dialyzed in a dialysis tubing (MWCO, 12 kDa) against distilled water followed by the removal of the excess D-galactose. The resultant dispersion of the developed Gal-MWCNTs was again centrifuged for 15 min at 20,000 rpm and settled Gal-MWCNTs were dried overnight at room temperature under vacuum (Fig. 8) [65].

Mannose receptor is found on liver endothelial cells, macrophages and hepatic sinusoidal cells. It is 175 KDa membrane molecule expressed by tissue macrophages. The mannose receptors play an important role in various physiological activities of mononuclear phagocyte system like growth, differentiation, activation, migration, antigen recognition and receptor-mediated endocytosis for cellular internalization. Generally, mannose receptors are highly expressed on the Kupffer cells, alveolar, splenic, peritoneal macrophages, monocyte-derived dendritic cells and brain macrophages *i.e.* astrocytes and microglia [5].

Till date mannose conjugation to nanotubes has been reported exclusively from our laboratory [22]. A debut report on mannose conjugation on the surface functionalized MWCNTs for macrophages targeting was reported by Jain and co-workers. Briefly, D-mannose (8 μM) was dissolved in sodium acetate buffer (pH 4.0; 0.1 M) and added to lyophilized amine modified MWCNTs, followed by heating at 60 ± 0.5 °C for an hr. The mixture was continuously stirred at ambient temperature for 72 h to ensure the completion of reaction. Mannosylated MWCNTs were purified through a dialysis membrane (MWCO

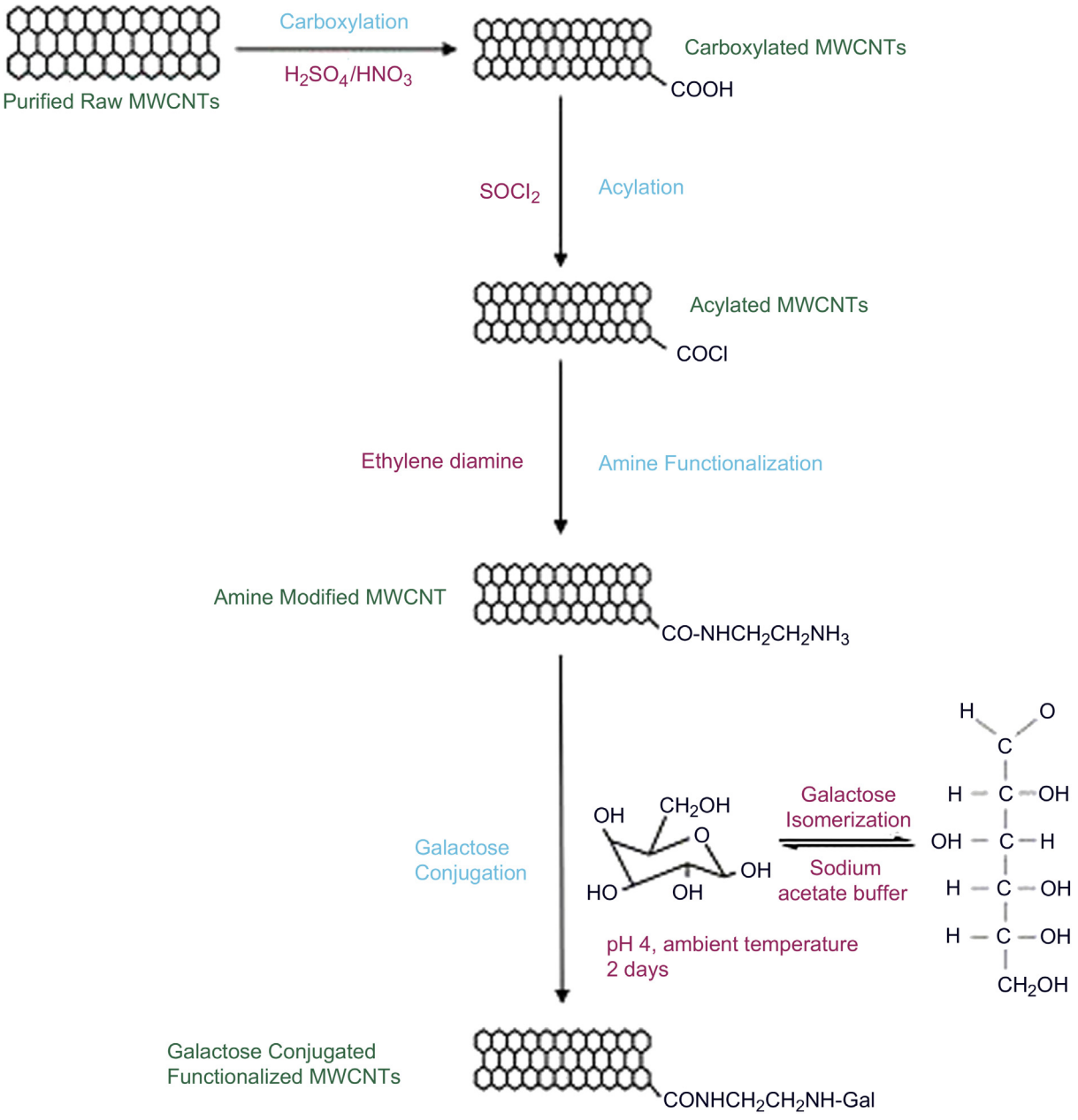

Fig. 8. Schematic representation of various conjugation sequences of galactose conjugated MWCNTs (Reprinted with permission from Ref. [65] Elsevier Pvt. Ltd.).

12–14 kDa) against triple-distilled deionized water for 12 h to remove unreacted mannose along with other impurities, followed by lyophilization (Fig. 9) [22].

5.1.10. Carbon-nanotubes monohybrid: a multifunctional contour

Despite the great contribution of the conjugation chemistry in surface engineered carbon nanotubes for biomedical applications the modification of CNTs with dendrimers (CNTs-DENs) [93,109–114], liposomes (CNT-liposomes) [98,115–117], nanoparticles (CNTs-NPs) (Fig. 10) [40,118–122] and quantum dots (CNTs-QDs) [114,123] shows enormous potential as CNTs-nano-hybrids that find applications in diverse arenas including nano-biomedicines, diagnostic and imaging purposes, mechanical engineering, and nanocatalysts. Still there is much room for the creation; designing and development of the CNTs based new products by the combined efforts of physicists, chemists, and biologists [93]. Table 1 summarizes the targeting ligand conjugated CNTs for biomedical purposes [15–18,21,22,40,55,57,65,66,72,81,88,89,92,100,106,107,113,124–126].

6. Carbon nanotubes in drug delivery

Since their rediscovery, carbon nanotubes are continuously being explored as nano-vectors in targeted and controlled drug delivery due to their unique physicochemical properties [4,15–19]. Surface modified CNTs are able to carry the numerous cargo biomolecules and deliver them to specific or target sites claiming only as valuable candidate for potential delivery of proteins, peptides, nucleic acid and drugs including DOX [15–19,21,24,55,57,126], mitoxantrone [127], paclitaxel (PTX) [53,111], docetaxel (DTX) [89], cisplatin (Cis) [106], amphotericin B (AmB) [22,49,128], gemcitabine (Gem) [17],cyclosporine A [129], epirubicin (Epi), daunorubicin (Dau) [81] and paracetamol [130] in diverse range of biomedical applications. The pristine CNTs and its conjugates are shown in Fig. 11.

Doxorubicin (DOX) is an anthracycline antibiotic, chemotherapeutic drug. It is usually administered intravenously in the salt form as doxorubicin hydrochloride, which results in its inefficient distribution, low selectively and inability to cross cellular barriers along with various severe associated toxic effects like acute cardio-

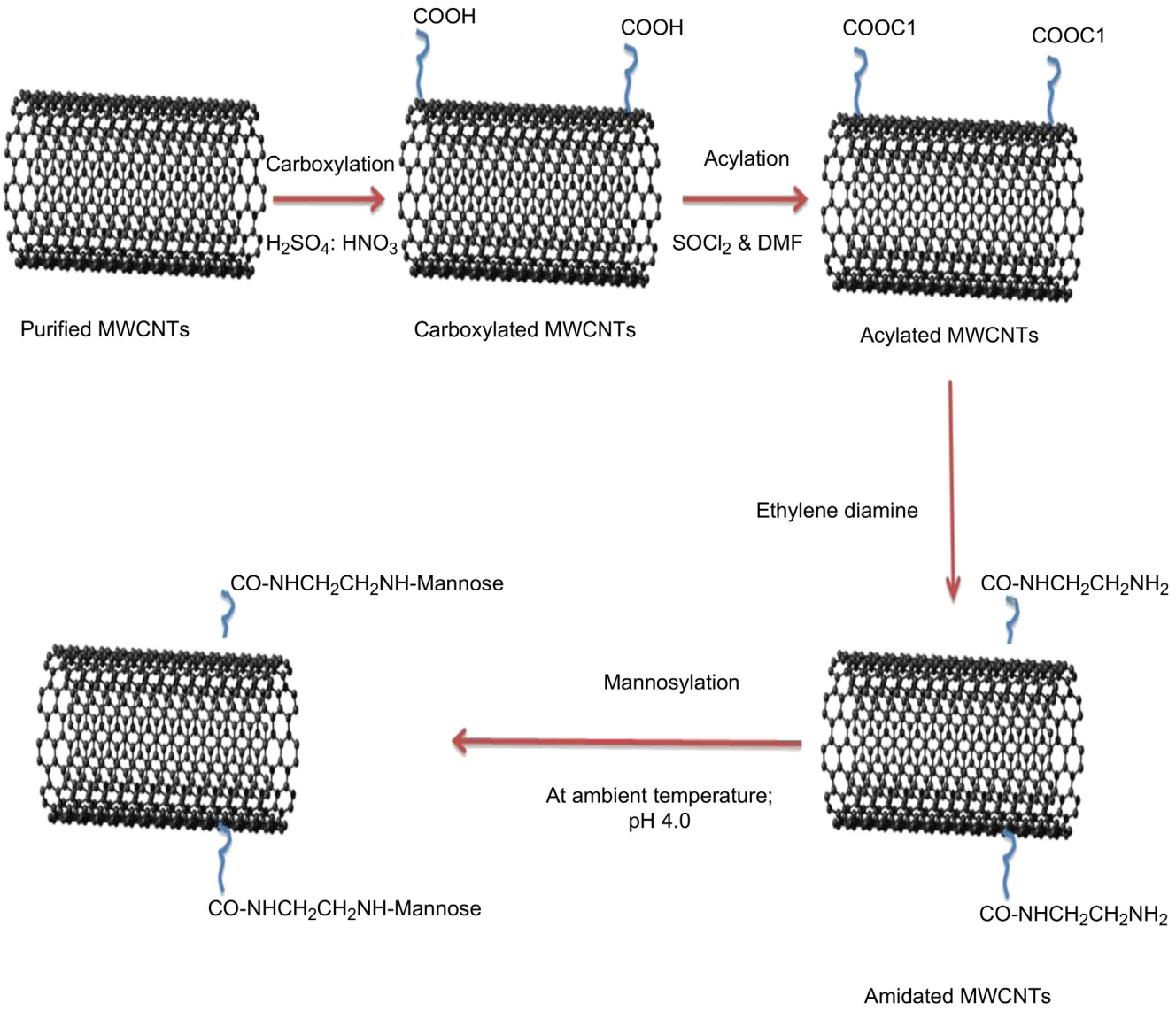

Fig. 9. Modified schematic representation of mannosylation of MWCNTs [22].

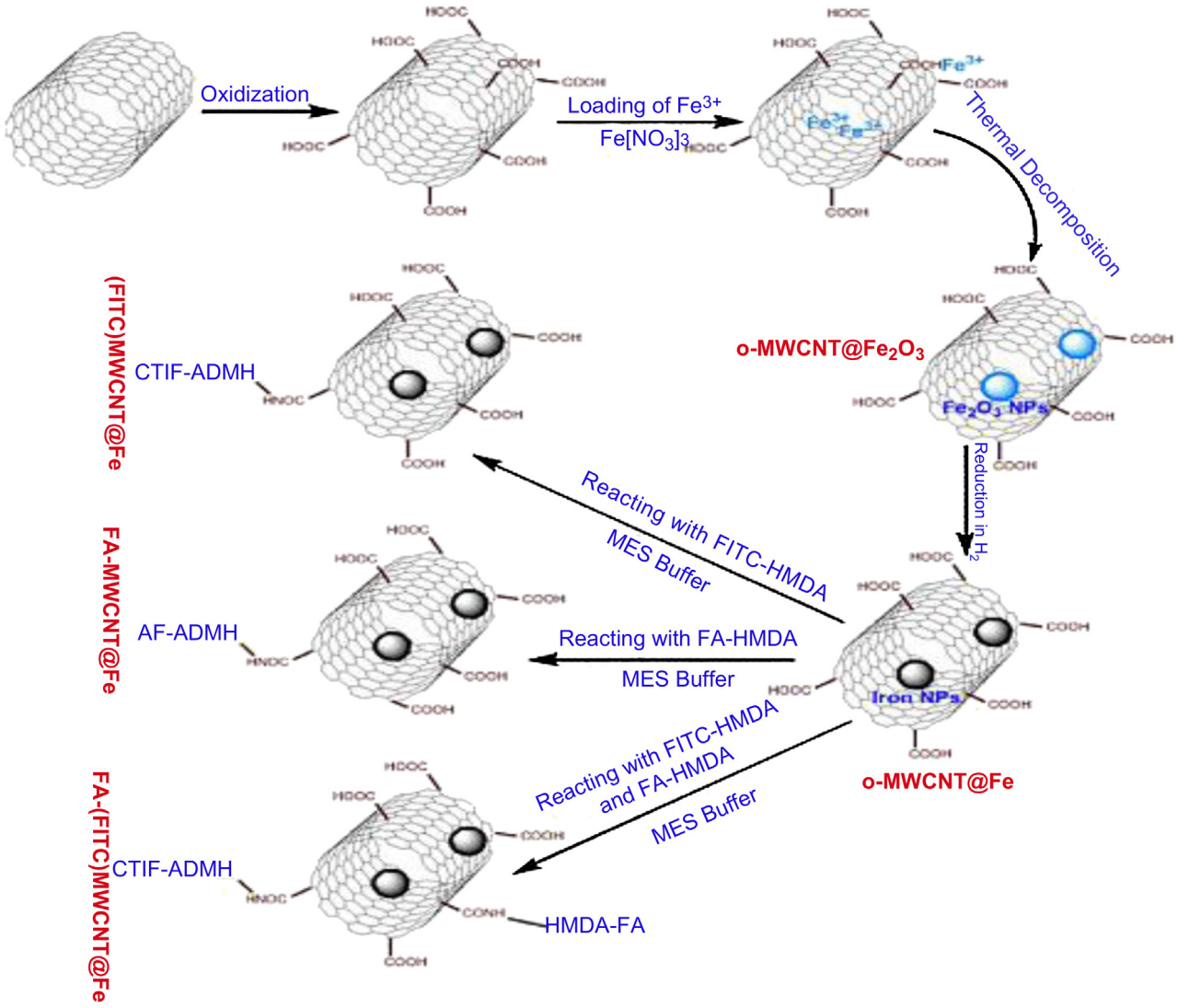

Fig. 10. Synthesis scheme for NPs of FA-MWCNT@Fe, FA-(FITC)MWCNT@Fe and (FITC)MWCNT@Fe, including the oxidization of MWCNT, loading of $Fe(NO_3)3$onto o-MWCNT, thermal decomposition of $Fe(NO_3)3$, reduction of Fe_2O_3NPs, and diimide-activated amidation reaction between o-MWCNT@Fe and the intermediates (Reprinted with permission from Ref. [40] Elsevier Pvt. Ltd.).

toxicity. It intercalates the DNA fractions functions and most widely used in treatment of various cancers [24,131]. The DOXIL® is a US Food and Drug Administration (FDA) approved liposomal formulation with reduced DOX toxicity [16]. The potential side effects can be counteracted by the surface modified CNTs, molecular transporters, due to their capability of adsorption of DOX on the CNTs surface *via* π−π stacking, hydrophobic and electrostatic interactions mechanism [16,24,88].

The DOX can be loaded on the folic acid and polysaccharide materials like sodium alginate and chitosan wrapped functionalized SWCNTs and tested on human cervical carcinoma cells. The developed FA and polysaccharide wrapped SWCNTs selectively accumulated in the cancerous tissues and efficiently released DOX in controlled manner; and after entering in the nucleus, induced the cell death [24]. Various research groups explored the use of DOX-CNTs conjugate for efficient and targeted delivery of DOX for the treatment of cancer cells. Similarly, the supramolecular stacking of DOX on CNT was reported through π−π stacking and the DOX-CNTs were found to be more effective and less toxic as compared to the free DOX in equimolar concentration [16,18,21,24,40,51,52,55,88]. Very recently, Mehra and Jain assessed *in vitro* and *in vivo* potential of doxorubicin loaded folic acid (FA) appended PEGylated MWCNTs (DOX/FA-PEG-MWCNTs) for efficient tumor targeting. The developed nanoconjugates afforded higher tumor growth suppression efficacy due to its stealth nature and preferential uptake by the MCF-7 human breast cancer cells through caveolae-mediated endocytosis mechanism, as compared to free DOX solution. The amount of DOX released from DOX/FA-PEG-MWCNTs formulation was found to be remarkably increased at tumor site over time. The median survival time for tumor bearing rats treated with DOX/FA-PEG-MWCNTs (30 days) was found to be extended very significantly [15].

Paclitaxel (PTX) is one of the most effective broad-spectrum, mitotic inhibitor chemotherapeutic agent mainly used in the treatment of cancer including ovarian cancer, breast cancer, small and non-small-cell lung cancer, colon cancer, ovaries cancer, bladder cancer, esophagus cancer, head and neck cancer, multiple myeloma cancer and Kaposi's sarcoma. It is also used in the prevention of restenosis. PTX was originally obtained from the needle and bark of the pacific yew tree in the late 1960s. But its clinical application is limited due to its poor aqueous solubility (<0.03 mg/mL) because it does not contain any functional groups to be ionized,

Table 1
Targeting ligands used in the delivery of various bioactives using surface modified CNTs.

CNTs scaffold	Targeting ligand	Receptors	Bioactive used	Cell lines used	Ref.
SWCNTs	RGD	Integrin $\alpha_v\beta_3$ receptor	Doxorubicin	MCF-7 and U87 cells	[88]
	Rituximab and Lintuzumab	–	–	HL 60 cells	[72]
	Folic acid	Folate receptor	Doxorubicin	HeLa cells	[21]
			Doxorubicin	HCC SMMC-7721 cells	[66]
			Cisplatin and Carboplatin	KB cells	[124]
	EGF	Epidermal growth factor receptor	Cisplatin	HN 13 cells	[106]
			Etoposide	A-549 lung epithelial cancer cells	[107]
	HER2 IgY	HER-2		MCF-7 and SK-BR-3 cells	[100]
	Aptamer	–	–	Molt-4 ad U266 cells	[81]
	NGR peptide	–	Docetaxel	PC 3 and S 180 cancer cells	[89]
		CD 13 receptor	2-methoxyestradiol (2-ME)	S 180 cancer cells	[92]
	Hydrazinobenzoic acid	–	Doxorubicin	HepG2 cells	[125]
	Angiopep-2	Low-density lipoprotein receptor	Doxorubicin	BCEC and C6 cells	[18]
CNTs	Folic acid	Folate receptor	Doxorubicin	MCF-7 breast cancer cells	[22]
				–	[55]
MWCNTs	Folic acid	Folate receptor	Gemcitabine	MCF-7 cells	[17]
			Doxorubicin	MCF-7 cells	[15]
				U 87 cells	[57]
				HeLa cells	[40]
			–	KB-HFAR cells	[113]
	Galactose	–	–	–	[65]
	Hyaluronic acid	Hyaluronate receptor	Doxorubicin	A-549 lung epithelial cancer cells	[126]
	Mannose	Lectin receptors	Amphotericin B	J 777.4 macrophage cells	[22]
	Dexamethasone mesylate	Nuclear receptor	Doxorubicin	A-549 cells	[16]

which may form salt by altering the pH to enhance the aqueous solubility. In 1992 the US FDA approved Taxol® for the treatment of drug-resistant ovarian cancer and then in 1994 for treatment of the breast cancer [132–134]. Apart from CNTs the recent developments in various PTX based delivery systems including liposomes, micelles, nanoparticles, solid lipid nanoparticles, dendrimer, nanohydrogel as well as PTX-eluting stents were exhaustively compiled by Zhang and co-workers [132].

In a study PTX was conjugated to the carboxyl functional groups of poly citric acid (PCA) *via* a cleavable ester bond for the preparation of MWCNTs-g-PCA-PTX conjugates. The drug content of this conjugate was found to be 38% *w/w*. The size of MWCNTs-g-PCA and MWCNTs-g-PCA-PTX was found to be approximately 125 and 200 nm, respectively. The MWCNTs-g-PCA showed an insignificant cytotoxic effect on A-549 and SKOV3 cell, while MWCNTs-g-PCA-PTX exhibited more cytotoxic effect than the free PTX over a shorter incubation time. Thus PTX conjugated MWCNTs-g-PCA proved itself as a promising candidate for cancer therapeutics [134]. Pastorin and co-workers attached fluorescein isothiocyanate (FITC) as well as methotrexate (MTX) onto the sidewall of MWCNT *via* 1, 3-dipolar cyclo addition reaction of azomethine ylides and observed that functionalized MWCNTs could be internalized into Human Jurkat cell, but no further anti-tumor and other biological activity were exhibited by the functionalized MWCNTs [48]. The 10-hydroxycamptothecin (HCPT) displays a prominent therapeutic role to a broad spectrum of tumors by inhibiting the DNA enzyme, topoisomerase. The HCPT was covalently linked through a cleavable ester linkage using hydrophilic diaminotriethylene glycol as the spacer between nanotubes and drug moiety. The developed MWCNTs-HCPT conjugates were found superior in anti-tumor activity both *in vitro* and *in vivo* as compared to clinical HCPT formulations. *In vivo* single photon emission computed tomography (SPECT) imaging and *ex vivo* γ-scintillation counting analysis revealed that the nanotubes-HCPT conjugates had relatively long blood circulation with high accumulation of HCPT in to tumor sites [135]. The cellular internalization of engineered CNTs through receptor-mediated endocytosis is shown in Fig. 12.

7. Appraisal on toxicity of carbon nanotubes

Pristine CNTs (first generation) are not suitable candidate due to the low solubility in most of the organic or aqueous solvents and the presence of impurities, therefore surface engineered CNTs have brought immense progress in the field of drug delivery. Still CNTs are at the very early stages of their clinical development. Their safety, efficacy and other major challenges must carefully be determined, addressed and resolved.

The toxicity issues of pristine and functionalized CNTs are under intensive debate and need the determination of the possible impact upon exposure of nanotubes in the biological environment. However, few studies supported that after functionalization CNTs exhibited lesser toxicities as compared to pristine CNTs. Thus we can conclude that the functionalization and standard procedure of synthesis, formulation development and purification of nanotubes should be developed to enable a move into large-scale multi-centre clinical trials [7,23,41]. The toxicity of MWCNTs with end defects critically depends on their density of functionalization. Jain and co-workers found that the pristine and acid functionalized MWCNTs were devoid of any obvious nephrotoxicity. The CNTs with larger dimension and lower degrees of functionalization cleared out from the body through the renal excretion pathway, and also excreted *via* biliary pathway through faeces. It was also clear that the oxidized CNTs with surface carboxyl density >3 μmol/mg were not retained in any RES organs [136].

8. Pharmacokinetics and biodistribution profile of pristine and surface modified CNTs

The pharmacokinetics and biodistribution aspects of CNTs have been continuously investigated on account of the safety issue of CNTs. It is very interesting that the CNTs can easily excreted out through biliary pathway with no chance of accumulation, but the long-circulating (stealth) CNTs accumulate more in tumor [88,137]. The surfactant (Pluronic F108, Tween 80) conjugated CNTs mainly accumulated in liver and spleen [138,139], while serum dispersed MWCNTs preferentially accumulated in lungs [19]. The polyethylene

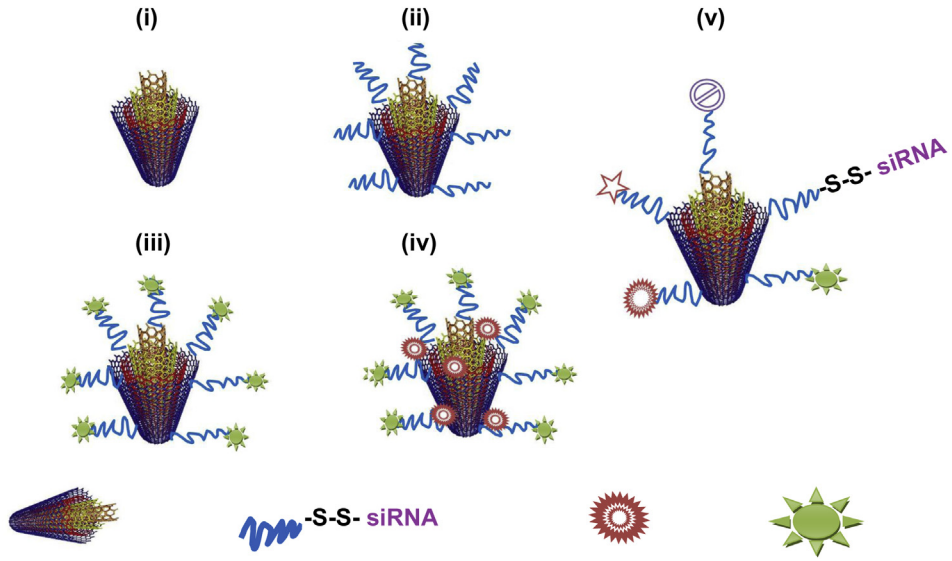

Fig. 11. Schematic representation of CNTs and its conjugates, (i) Pristine CNTs, (ii) PEGylated CNTs, (iii) Ligand conjugated PEGylated CNTs, (iv) Ligand conjugated drug loaded CNTs, and (v) various biomolecules conjugated CNTs.

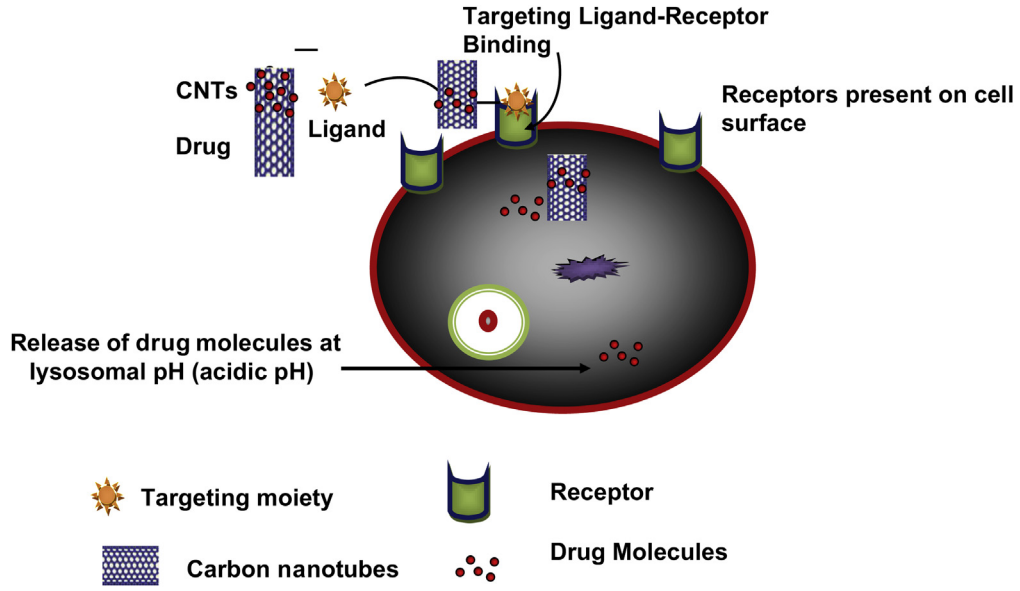

Fig. 12. The receptor-mediated endocytosis mechanism for cellular internalizations of surface engineered CNTs [15,16,56].

glycol-phospholipids (PEG-PL) suspended CNTs showed the prolonged blood circulation half-life with reduced RES uptake *in vivo*. The studies suggested the half-life of PEG2000 and PEG5000-PL-SWCNTs to be 1.2 and 5 h, respectively that could further be prolonged by the use of longer PEG chain [53,88]. The poly-(γ-glutamic acid)-pyrine and poly(ethylene glycol) methyl ethers in 30:70 (γPGA-Py-mPEG) dispersed SWCNTs were found to be very stable with prolonged circulation half-life upto 22.1 h [140]. Recently, Jain and co-workers evaluated and determined the pharmacokinetic parameters of gemcitabine loaded FA conjugated MWCNTs (GEM/FA-MWCNTs). The half-life of GEM/FA-MWCNTs was found to be increased upon functionalization from 0.766 (free GEM) to 5.677 h (GEM/FA-MWCNTs), which assisted the sustained release and prolonged circulation time of GEM/FA-MWCNTs [17]. The numerous reports have been available concerning the toxicological parameters and pharmacokinetics, which suggested that surface modified CNTs are devoid of any toxic effect, and are safer for biomedical and pharmaceutical fields [17,22,47,53,88,136,138–140].

9. Conclusions and future perspective

Over the past decades, a number of specific ligand driven targeted drug delivery have been clinically approved to target the overexpression on infectious cells for the treatment of specific infectious cells. Till date numerous attractive targeting motifs such as vitamins, peptide, antibodies, nucleic acid, and aptamers have been identified and are available, which can be tethered with nanocarriers to design, construct and tailor for specific diseases. The targeted therapeutics have emerged as one of the most rewarding strategies to overcome the lack of specificity for better management of diseases over conventional therapy, but only few target

based nanomedicines have successfully passed the clinical stages and will reach the market in due course of time.

In cancer chemotherapeutics, USFDA and European Medicines Agency (EPA) have clinically approved the specific liposomal formulations (Doxil®: Centocor Ortho Biotech, Horsham, Pennsylvania; Caelyx®: Schering–Plough, North Ryde, New South Wales, Australia; Myocet™: Enxon Pharmaceuticals, Piscataway, New Jersey; DaunoXome®, Diatos, Paris, France) and recently paclitaxel loaded human albumin nanoparticles (Abraxane®; Celgene, Summit, New Jersey). The DOXIL® (doxorubicin bearing PEGylated liposomes) is an approved liposomal product for the treatment of AIDS-related Kaposi's sarcoma [15,22,141].

The significant contributions of functionalized CNTs in delivery of DOX for cancer treatment have been visualized in last few years. In our opinion, surface *f*-CNTs has shown great targeting potential in DOX delivery as compared to the other available nanocarriers like liposomes, dendrimers, and nanoparticles etc. CNTs were found more efficient in DOX delivery with higher holding capacity, controlled/sustained release and targeting potential along with minimum or no toxicity.

The presence of multiple wall layers in MWCNTs minimizes the possibility of leakage of loaded drug hence may be preferred over SWCNTs.

SWCNTs-DOX conjugate was found superior in overall clinical efficacy as compared to DOX alone as well as DOXIL® [142]. Conflicting reports preclude conclusion which type of CNTs will show promising cancer targeting efficacy. However in terms of higher drug loading efficiency CNTs are proving themselves as better carriers as compared to other nanocarriers like polymeric as well as solid lipid nanoparticles, nanostructured lipid carriers, dendrimers, and liposomes etc. Again it is a matter of debate which types of CNTs either SWCNTs or MWCNTs is better in drugs loading efficiency! However, distinct loading propensity of drug molecules at the surface and inside the nanotubes is not reported so far. But on the basis of various reports authors conclude that in context to DOX loading efficiency of CNTs the following ascending order can be summarized *i.e.* Plain CNTs $<$ SWCNTs $\leq$ MWCNTs.

Several research groups investigated that the DOX laden *f*-CNTs were more significantly accumulated in the cancerous cell and destroyed them with minimal accumulation and side effects to healthy cells. The IC_{50} value of DOX loaded FA-magnetic nanoparticles conjugated MWCNTs (DOX/FA-MN-MWCNTs) was found to be ~15 μg/mL on U87 cell, which is much lower than that of DOX alone (~50 μg/mL). Thus DOX/FA-MN-MWCNTs showed superparamagnetic property and could be targeted and concentrated in a specific zone with the aid of magnetic field [57]. In another study, DOX loaded hydrazinobenzoic acid conjugated SWCNTs (DOX/HBA-SWCNTs) showed 4.8 μM half-inhibitory concentration [125]. Jain and coworkers studied the functionalized MWCNTs loaded with DOX and using MCF-7 and A-549 cells and found improved pharmaceutical therapeutic efficiency but devoid of unnecessary side effects [15,16]. Our research group is continuously exploring the ligand driven CNTs mediated targeted drug delivery employing various anticancer bioactives.

Lastly, stability is another important concern in the development of any safe, effective, and clinically meaningful formulation. In this regard *f*-CNTs show greater stability in terms of minimum chances for leaking of drug molecules as compared to liposomal as well as other nanoformulations.

In the current scenario, surface functionalized carbon nanotubes are attracting a great deal of attention in targeted drug delivery. The surface decoration can be achieved easily by exploiting the several targeting motifs, which may provide promising results for controlled and sustained drug delivery along with minimizing dose, side effects associated with the free drug and most importantly the improved patient compliance. This review highlights the designing, construction and development of safe and effective as well as economical CNTs based nanomedicines for the diagnostic as well as efficient management of various diseases, which is the foremost challenge in the nanomedicines.

Declaration of interest

The authors report no conflict of interest.

Conflict of interest

The authors declare no competing financial interests.

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