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Review

Carbon-Based Nanomaterials as Drug Delivery Agents for Colorectal Cancer: Clinical Preface to Colorectal Cancer Citing Their Markers and Existing Theranostic Approaches

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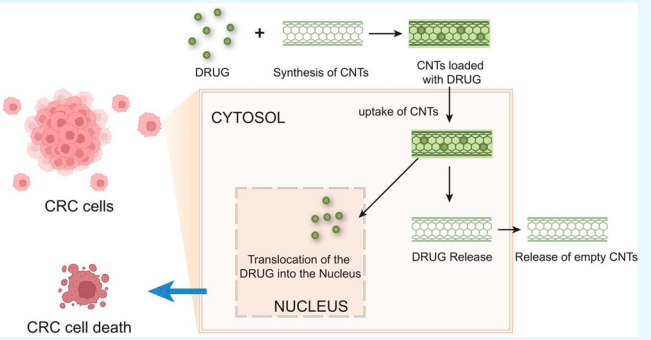
ABSTRACT: Colorectal cancer (CRC) is one of the universally established cancers with a higher incidence rate. Novel progression toward cancer prevention and cancer care among countries in transition should be considered seriously for controlling CRC. Hence, several cutting edge technologies are ongoing for high performance cancer therapeutics over the past few decades. Several drug-delivery systems of the nanoregime are relatively new in this arena compared to the previous treatment modes such as chemo- or radiotherapy to mitigate cancer. Based on this background, the epidemiology, pathophysiology, clinical presentation, treatment possibilities, and theragnostic markers for CRC were revealed. Since the use of carbon nanotubes (CNTs) for the management of CRC has been less studied, the present review analyzes the preclinical studies on the application of carbon nanotubes for drug delivery and CRC therapy owing to their inherent properties. It also investigates the toxicity of CNTs on normal cells for safety testing and the clinical use of carbon nanoparticles (CNPs) for tumor localization. To conclude, this review recommends the clinical application of carbon-based nanomaterials further for the management of CRC in diagnosis and as carriers or therapeutic adjuvants.



1. INTRODUCTION

The worldwide market for nanomaterials and their use was assessed to be 8.5 billion USD for the year 2019. The annual growth rate projections for the years 2020−2027 is 13.1%. The estimated global revenue for the year 2020 is USD 3000 billion which is projected at USD 30,000 billion for the year 2030 based on various sectors including medicine. The United States projections are USD 750 and 7500 billion, respectively, for the years 2020 and 2030.[1] In drug delivery and diagnostics, an emergent trend of using carbon nanotubes (CNTs) is observed.[2] Particularly, the CNTs have been classified into single, double, multiwalled, and functionalized materials based on their structure.[3]

Since cancer remains a global burden with a considerably limited remission rate, it is considered a primary cause for mortality across the globe. Colorectal cancer (CRC) is considered to be the foremost reason for mortality across the globe in the 21st century. It is one of the leading causes of deaths in elderly people across several countries, and this pattern is rising worldwide with complicated factors and limited understanding.[4] Explicitly, CRC remains a leading cause of death as the CANZUK countries and the United States remain high-risk countries. Some countries of South America and Africa along with Asian countries like China and India endure a low-risk for CRC.[5]

The current treatment options for early diagnosis and treatment of CRC lack precision and pose side effects in addition to occurrence of resistance among cancer cells. Nanoparticles can be useful in this regard as they are effective diagnostic and therapeutic agents and reduce side-effects as they can increase solubility of drugs being loaded into organized sequences. Specific targeting of tumor cells can be accomplished by using nanoparticles with limited accumulation in off-target organs such as liver, kidney, or spleen. Hence, nanomedicine must receive extensive consideration for the management of CRC.[6,7,7] Specifically, the particle size of nanoparticles must be around 200 nm to provide the desired effects, whereas materials with sizes of less than 10 nm are removed by the kidneys before the materials can reach the desired tumor site. Materials of sizes around 200 nm can enter

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and accumulate into the tumor microenvironment effectively as the interstitial spaces of tumor cells are approximately 400−800 nm wide.[8,9]

To address this issue, nanomaterials are ideal carriers (improving the therapeutic effects of the drug load) or therapeutic candidates by themselves as they possess sizes of less than 100 nm.[10−12] Among such materials, CNTs have been considered attractive candidates for nanomedicine and drug delivery due their intrinsic properties such as high surface area, structure that is flexible for contact with cargo and efficient loading, superior stability, biocompatibility, and targeted release of drugs at the directed sites due to their nanoneedle morphology. Based on these properties, CNTs are used extensively in monitoring of drug release, imaging, and diagnostics as well as in creating biosensors for related applications in medicine. CNTs can also change the near-infrared radiation into heat and are therefore used in lab-on-a-chip devices based on the previously mentioned properties.[13−19]

Since the use of nanomaterials remains critical for the management of tumors of the colon and rectum, the current review focuses on the preclinical studies performed toward the use of CNTs for drug delivery and CRC therapy. It also reviews the inherent properties of CNTs and the advantages of using CNTs for cancer therapy in comparison to other nanomaterials.

2. MATERIALS AND METHODS

The literature search strategy was predominantly based on PubMed searches. Initially, we found two recent articles related more to the epidemiology and pathophysiology of CRC. Similarly, to determine the global burden of CRC, we referred to the text of 4 multicenter studies and investigated a single review to elucidate the CRC burden in China. To represent the clinical features of CRC, 6 articles including a comparative study and a case report including a set of reviews were used. In addition, 2 review articles were used for determining the diagnostic modes and existing treatment methods for CRC. Also, 25 articles (including data from reviews, meta-analyses, book chapters, and research articles) were used to define the theranostic markers for CRC. The discussion for different categories of CNTs was prepared by means of referring to nine different articles. Similarly, the synthesis methods and characterization techniques for CNTs were presented in 6 articles, whereas their inherent properties were discussed in 9 articles. In addition, 26 different articles were discussed for elucidating the parameters involved in improving the tumor-targeting efficacy of CNTs.

With regard to discussing the research on the use of functionalized CNTs as drug delivery and cytotoxic agents, 10 research articles were used. Also, 7 different articles were used to reveal the internalization mechanisms for CNTs in addition to 12 research articles for determining the safety of CNTs for therapeutic purposes. Later, 5 different articles were used to determine the benefits of using CNTs in comparison to other nanomaterials for drug delivery, whereas, 13 different clinical trials were used to interpret the use of carbon nanoparticles (CNPs) in preoperative localization of colorectal tumors.

To summarize the methods adopted, 19 articles were used for preparing the Introduction, whereas 132 appropriate references related to reviews, meta-analyses, book chapters, and research articles were cited for preparing the core content of the Results and Discussion. Another 5 references were used as supportive references for the text in the Results and Discussion. Overall, 156 works were used and referenced for preparing this review.

3. RESULTS AND DISCUSSION

3.1. Epidemiology and Pathophysiology of CRC. The rate of mortality as a consequence of malignancies related to colon and rectal cancer is progressively on the rise in developed and developing countries based on diet and lifestyle modifications.[20] Based on these modifications, a set of mutations evolve into CRC over a period of 10−15 years following a progression model. In this typical CRC progression model, the neoplastic lesions termed as aberrant crypt foci lead to the formation of polyps which grow into early adenomas of less than 1 cm with tubulovillous histology. These early adenomas later progress into advanced adenomas with villous histology and size greater than 1 cm. Based on their structure and molecular differences, approximately 10% of polyps develop into CRC whereas others do not.[21]

According to the American Cancer Society, an estimated 1.8 million new cases of cancer and 0.61 million deaths are projected to happen in the United States alone.[22] The International Agency for Research on Cancer (IARC) produced an estimate through GLOBOCAN 2020 stating that 19.3 million new cases and 10 million deaths occurred globally in the year 2020. The prediction for 2040 is 28.4 million.[23] The predicted count of new CRC cases (colon and rectum, excluding anus) for 2021 in the United States is 149,500, whereas 52,980 deaths are projected.[24] Subsequently, 151,030 new cases and 52,580 deaths related to CRC are anticipated for 2022.[25]

As per the 2018 Chinese Cancer Registration Report based on National Cancer Center data, 387,600 new cases and 187,100 deaths were related to CRC in 2015 in China. Although the percentage of incidence and deaths are low in comparison to the global standards, China had a higher number of cases and deaths owing to its larger population. The incidence rate was higher in the male population over the age 50.[26] Yet, recent reports suggest that there is an increased incidence of CRC in younger adults in comparison to elderly adults.[27] Therefore, some cutting edge therapies are encouraged to manage CRC.

3.2. Clinical Presentation of CRC. Abdominal pain, constipation, and diarrhea are the most common symptoms associated with colon cancer prognosis in patients who visit a gastroenterologist.[28] Rectal bleeding, weight loss, rectal abnormalities, positive fecal occult blood test related to iron-deficiency anemia, and hyperglycemia are other major clinical symptoms.[29] Other acute symptoms of late-stage may include bowel obstruction and intussusception.[30,31] Bacteremia or infectious endocarditis associated with *Streptococcus bovis* (termed and called at present as *S. gallolyticus*) is a procarcinogenic or oncogenic factor. The progression may occur from normal tissue to precancerous lesions to adenomas, eventually resulting in malignant forms.[32] It is interesting to note that proximal colon tumors are characterized by delicate systemic symptoms such as microcytic anemia and weight loss. The distal cancers show local symptoms indicating altered bowel habits and rectal bleeding.[33]

3.3. Diagnosis and Existing Treatment Modes for the Management of CRC. Colonoscopy and biopsy followed by subsequent histology are used for the primary identification of CRC. After the diagnosis of carcinoma, computed tomography

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Table 1. List of Proteomic, Genomic, and Microbial Markers for CRC

type of markers	markers	ref
proteomic markers	calreticulin, transgelin, serotransferrin, triphosphate isomerase, carbonic anhydrase II	36
	CEA and carbohydrate antigen 19−9	37
	apolipoproteins	38
	cystatins	39
	haptoglobin, serum amyloid A, CRP, AAT	
	preoperative transferrin	41
	HSP27, HSP40, HSP60, HSP70, HSP90A, HSP110), MMP-1, 2, 3, 7, 9, 12 and 13, complement components C3 and C9, MRC1, S100A9	42
	human neutrophil peptides 1, 2 and 3	43
	uromodulin, gelsolin, MEP1A	44
	α-enolase	45
	transthyretin	46
	paraoxonase 1	47
	β-2 microglobulin	48
genomic markers	*KRAS*, *BRAF*, *NRAS*	49, 50
	PIK3CA	51
	APC	52
	TP53	53
	SMAD4	54, 55
	NF1	55
	ARID1A, *SOX9*, and *FAM123B*	56
fecal microbes	Bacteroides (*Alistipes*, *Prevotella*), Firmicutes (*Clostridium*, *Parvimonas*, *Peptostreptococcus*, *Roseburia*, *Oscillibacter*), Proteobacteria (*Campylobacter*, *Escherichia*, *Halomonas*, *Shewanella*), Verrucomicrobia, Fusobacteria, and Actinobacteria	57−59
proteomic stool markers	Human neutrophil peptides, S100 proteins, hemoglobin, haptoglobin, AAT, lactoferrin, CEA, dipeptidyl peptidases I and IV, cadherin-17, Selenium Binding Protein 1, tumor pyruvate kinase type M2, MMP-9	60

of the chest, abdomen, and pelvis is recommended for evaluation and staging of the tumor and determining the therapeutic plan for colon cancer. Magnetic resonance imaging is recommended for rectal cancer.[34]

After being diagnosed, the current treatment methods for management of CRC include surgery, chemotherapy, and radiotherapy. Targeted therapy using antibodies and tyrosine kinase inhibitors is a recently evolving approach. Although these screening techniques are available and advance every day, approximately 25% of CRC is diagnosed at late metastatic stages, thus preventing effective cure by surgical procedures. For patients who are diagnosed late and have limited chances for surgical treatment, radiotherapy and chemotherapy are viable options. The use of adjuvants can improve the chances of recovery and survival.[35]

3.4. Theragnostic Markers for CRC. Proteomic and genomic markers for early detection of CRC can prevent the disease from turning into metastatic forms. As an alternate technique, the early detection of precursors for CRC such as aberrant crypt foci can lead to a timely recognition of the cancer. Among such proteomic markers for this early CRC lesion, calreticulin, transgelin, serotransferrin, triphosphate isomerase, and carbonic anhydrase II are significant ones for successful management of CRC.[36] With regard to serum biomarkers, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 are established, well-studied, and extensively used. Other serum proteins like apolipoproteins, cystatins, haptoglobin, serum amyloid A, C-reactive protein (CRP), alpha1-antitrypsin (AAT), preoperative transferrin, β-2 microglobulin, heat shock proteins (HSP27, HSP40, HSP60, HSP70, HSP90A, and HSP110), matrix metalloproteinases (MMP-1, -2, -3, -7, -9, -12 and -13), complement components C3 and C9, macrophage mannose receptor 1 (MRC1), S100A9, paraoxonase 1, human neutrophil peptides, uromodulin, gelsolin, meprin 1α (MEP1A), α-enolase, and transthyretin can serve as predictive markers for CRC.[37−48] Mutations of *KRAS*, *BRAF*, *NRAS*, *PIK3CA*, *APC*, *TP53*, *SMAD4*, *NF1*, *ARID1A*, *SOX9*, and *FAM123B* remain significant as genomic markers.[49−56]

Fecal microbes including Bacteroides such as *Alistipes*, *Prevotella*, Firmicutes such as Clostridium, *Parvimonas*, *Peptostreptococcus*, *Roseburia*, and *Oscillibacter*, Proteobacteria such as *Campylobacter*, *Escherichia*, *Halomonas*, and *Shewanella*, Verrucomicrobia, Fusobacteria, and Actinobacteria constitute the dysbiosis-associated microbial signatures for CRC. It is interesting to note that *Fusobacterium*, *Parvimonas*, *Gemella*, and *Leptotrichia* are early bacterial markers for CRC, whereas, β-proteobacteria is a late-stage CRC marker.[57−59] Proteins such as human neutrophil peptides, S100 proteins, hemoglobin, haptoglobin, AAT, lactoferrin, CEA, exopeptidases (dipeptidyl peptidases I and IV), cadherin-17, selenium binding protein 1, tumor pyruvate kinase type M2, and MMP-9 are biomarkers of the stool.[60] The list of proteomic, genomic, and microbial biomarkers is shown in Table 1.

Targeted therapies involve PIGF, VEGFA/B, EGFR (HER1/ERBB1), KIT, PDGFRβ, RAF, RET, VEGFR1−3, and PD-1 as molecular targets of U.S. Food and Drug Administration (FDA)-approved drugs for CRC.[61]

3.5. Different Categories of CNTs. Single-walled carbon nanotubes (SWCNTs) are hollow seamless cylinders made up of single graphene sheets or layers folded to form a tube.[62] They are in general narrow and curved in comparison to the multiwalled carbon nanotubes (MWCNTs).[63] Double-walled carbon nanotubes (DWCNTs) are highly analogous to SWCNTs but contain a double layer made up of two graphene layers folded onto one another.[64] MWCNTs constitute multiple sequences of graphene layers, and the distance is maintained uniformly. They are larger in size compared to

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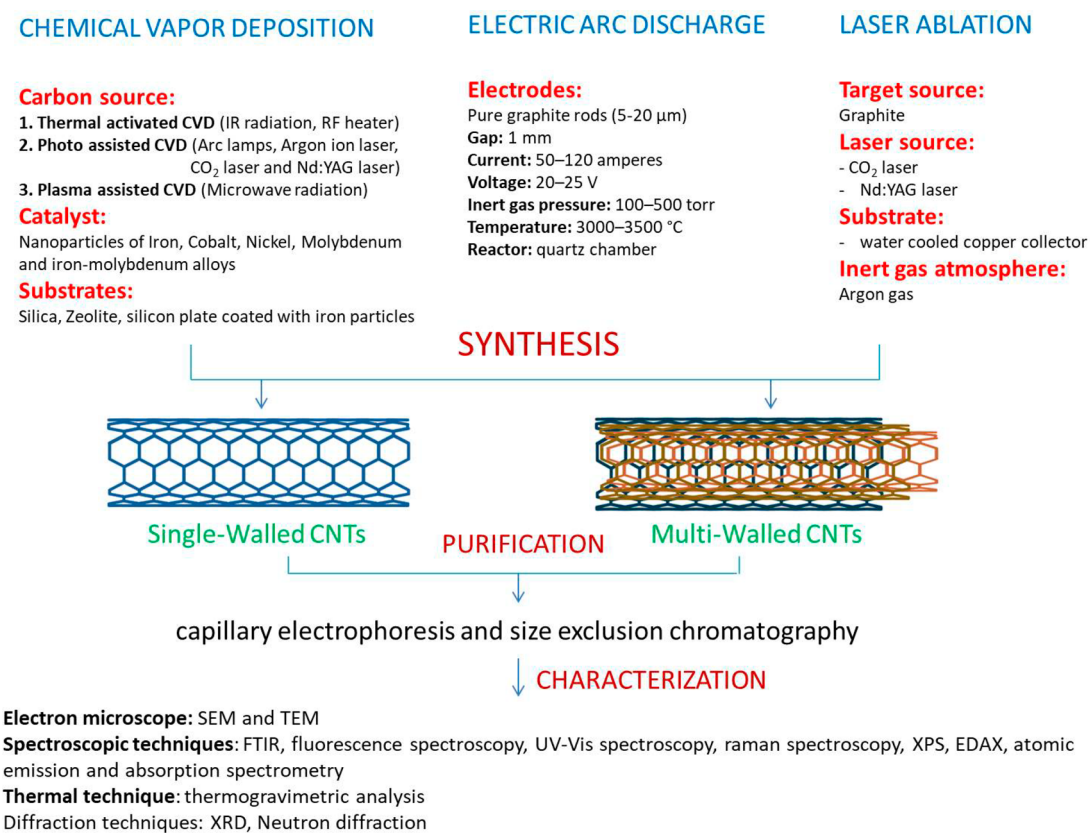

Figure 1. Different methods of synthesis, purification and characterization of CNTs.

SWCNTs.[65] Adding to the three types of CNTs such as MWCNTs, SWCNTs and DWCNTs, functionalized CNTs have been deemed to be the fourth category.[66] This describes the importance of functionalization in tumor targeting or other applications in nanomedicine. Besides these four types, they are further distinguished into solvent-dispersed, surfactant-assisted, and CNTs assisted with biological molecules.[67]

Among CNTs, the average diameters of SWCNTs range between 0.4 and 2 nm, whereas the diameters of DWCNTs and MWCNTs range between 2 and 100 nm, and 1 and 3.5 nm, respectively.[68,69] The size of MWCNTs and SWCNTs functionalized or loaded with anticancer drugs range between 5 and 700 nm for effects on *in vitro* cell lines. *In vivo*, the diameters may vary from 20 to 50 nm.[70]

3.6. Synthesis Methods and Characterization Techniques for CNTs. CNTs are synthesized by chemical vapor deposition, electric arc discharge, electrolysis, sonochemical methods, and laser ablation. The methods use a Nd:YAG or CO_2 laser and several hydrocarbon gases as the source, substrates such as water cooled copper collector, zeolite, and silica with iron, cobalt, nickel, and molybdenum as catalysts. The sources, substrates, and catalysts may be applicable or may vary based on the method applied for the synthesis. The synthesized materials can be purified from amorphous carbon, residual catalyst, and other impurities by gas phase, liquid phase, and intercalation methods of purification.[71,72] Although the production cost of CNTs has reduced from 45,000 USD to 100 USD per kg over the past decade, the application is still limited. If the prices decline further, the applications of CNTs may intensify.[73] Hence, cheaper alternative methods are being constantly studied that may utilize industrial waste such as fly ash from coal and bauxite residue along with waste from agricultural holdings, plastic pollutants, and tire waste as precursors for the fabrication of CNTs.[74,75]

After being synthesized, CNTs could be separated by capillary electrophoresis and size exclusion chromatography. Techniques include the following: as scanning and transmission electron microscopy, neutron diffraction, XRD, spectroscopic techniques that include FTIR, fluorescence spectroscopy, UV−vis spectroscopy, Raman spectroscopy, X-ray photoelectron spectroscopy (XPS), energy dispersive X-ray spectroscopy (EDAX), and atomic emission and absorption spectrometry. Besides, thermogravimetric analysis is a thermal technique used for such characterization purposes.[76] The methods currently adopted for fabricating, separating, and characterizing the CNTs have been presented in Figure 1.

3.7. Inherent Properties of CNTs. An interesting inherent property of MWCNTs is that they can mimic the microtubules which are considered as one of the principal components of the cytoskeleton of cancerous cells. Since microtubules can regulate migration, proliferation, and intracellular transport of cells, the drugs used for chemotherapy can bind to tubulins via protein−protein interactions. This can lead to cell cycle arrest and cell-killing. This behavior of CNTs can result in and effective response against tumor cells by promoting microtubule nucleation, creating changes in microtubule assembly or polymerization and leading to the development of abnormalities across the mitotic spindle.[77,78] This biomimetic property of CNTs is crucial for their cytotoxic effects as they can cause cytoskeletal instability (involving the centrosomes) and the disruption of microtubules, preventing the cancerous cells from migrating and proliferating. This is because the CNTs behave as submicrometric particles after drug loading, whereas the CNTs without any cargo behave as

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nanomaterials and can mimic the microtubules. The submicrometric CNTs are less cytotoxic toward normal cells, whereas the empty CNTs can target normal cells as they are highly nonspecific. Hence, drug-loaded CNTs are more specific with their functionalized constituents than the empty CNTs.[79]

The interactions of CNTs with tumor cells are dependent on their size as MWCNTs interact with microtubules, whereas SWCNTs communicate with the DNA for their cytotoxic effects.[80,81] This size-influence can improve the drug transport ability of large molecules by SWCNTs.[82,83] After crossing the lipid bilayer and being internalized into the cancerous cells, SWCNTs accumulate into the nucleus, bind to DNA, and alleviate the telomeric i-motif DNA. This can significantly impair the function of the telomeres, resulting in genotoxic effects and enhanced expression of tumor-suppressing genes, thereby preventing cell cycle arrest, apoptosis, and deteriorating effects that can happen as an outcome of the cells becoming senescent.[84,85]

3.8. Roles of Functionalization and Other Parameters in Improved Tumor-Targeting by CNTs. CNTs are widely used as agents that carry polynucleotide molecules that specifically bind high-affinity molecules such as antisense agents and aptamers with the intention of blocking cellular division. They are also known to carry RNA silencing molecules such as siRNAs and plasmids. Thus, they can carry agents that possess cytotoxic and immunotherapeutic value which can be of immense value in phototherapy in addition to being used as diagnostic agents for malignancies of various origins.[86−88] The therapeutic benefits of CNTs improve significantly as they become more soluble and biocompatible after being functionalized. Functionalization can improve the intracellular targeting of the CNTs with higher affinity toward cytoplasmic organelles. This renders the CNTs with enhanced targeting of cancerous cells at lower doses and limited off-target toxicity on normal cells.[89] Therefore, functionalization is a key parameter for targeted therapy using CNTs. With regard to functionalization, CNTs have a large surface area and are extremely hydrophobic. The surface area and morphology of CNTs enable them to cross the plasma membrane efficiently. Also, amino functionalization is more toxic or potent on normal cells in comparison to functionalization with carboxylic acids, which is considered safe compared to amino-functionalization.[90,91]

Adding to this, the pH of the cells presents an enormous advantage in strategic tumor targeting as normal healthy cells demonstrate a pH of approximately 7.4, whereas the pH of cancerous cells is extremely acidic at the lysosomal compartment of the tumor cells (~5.5).[92] In this regard, the pH of the gastrointestinal tract is as follows: stomach, 2.0; duodenum, 6; small intestine, 6; terminal ileum, 7.4; cecum, 5.7; rectum, 6.7.[93,94] To neutralize this hindrance, nanomaterials can respond well to intestinal pH and can assist in the gastric survival of the drugs they carry, resulting in enhanced uptake, bioavailability, and rapid controlled release of such drugs.[95] At the physiological pH of 7.4, the nanomaterials remain stable, whereas at the lysosomal pH of 5.5, the drugs can be released from such materials via the enhanced permeability and retention (EPR) effect into the tumor microenvironment (TME).[96] Supportive reports for such effects leading to pH-dependent release of drugs from CNTs at the lysosomal pH have been reported previously.[97−100]

Adding to the effects of functionalization, the dose and dimensions of the CNTs play vital roles in unintended toxic effects. This was evident as MWCNTs are more toxic in comparison to SWCNTs by virtue of their length. The diameter is another parameter that contributes to the toxicity of CNTs.[101−103] The route of administration is another significant factor as each route has its own merits and demerits. Subsequently, the intravenous route exhibits higher biocompatibility, whereas the subcutaneous route is less expensive and can be self-administered. The oral route is noninvasive and easy for intake of the drugs. The intratumoral route is highly effective for tumor targeting precisely into the tumor environment, thereby preventing nonspecific adverse effects.[104−109] Biopersistence and biodistribution are other key factors to be considered for the safe use of CNTs as functionalization can improve the biocompatibility and biodegradation of CNTs.[110,111]

Therefore, functionalization, designation of proper dose and dimension, route of administration, along with biodegradation and biodistribution relate to the effective usage of CNTs in cancer therapy.

3.9. Evidences for the Use of Functionalized CNTs as Drug Delivery and Cytotoxic Agents. *3.9.1. SWCNTs.* SWCNTs (1.4 ± 0.1 nm) functionalized with carboxylic acid (*f*SWCNT-COOH) and polyethylene glycol (*f*SWCNT-PEG) did elicit limited cytotoxic effects on human colorectal adenocarcinoma Caco-2 cells after 24 h of treatment (10%). But only after 72 h of treatment, the cytotoxicity of *f*SWCNT-PEG improved to 35% at the dose range of 15.6−1000 μg/mL. Yet, the CNTs were efficient drug-delivery agents across the cancerous monolayer as evidenced by Lucifer Yellow permeability assay and rhodamine-123 accumulation.[112] The inhibitory activities of *f*SWCNT-COOH (1.4 ± 0.1 nm) in yet another study took place after 24 h in cells treated with doses higher than 100 μg/mL (5 to 1000 μg/mL range).[113]

Owing to their relatively larger size or length and higher cytotoxic effects, pristine SWCNTs are less used in drug delivery in comparison to SWCNTs, with the latter being preferred for better use. Since SWCNTs have limited length with superior biocompatibility, they can enter cancer cells by direct permeation or endocytosis. The CNTs enter the cells easily as a result of enhanced water solubility and dispersity since they possess carboxyl groups on their surfaces. The *f*SWCNT-COOH and *f*SWCNT-PEG with lengths of 253−328 nm did not have cytotoxic effects at the tested dose of 160 μg/mL, and the percentage of viability did not change after 24 h. Hence, they are better agents for loading and delivery of antioxidants like Coenzyme Q10 for uptake through the gastrointestinal tract.[114] Similarly, SWCNTs with a diameter range of 1.4−1.6 nm and length range of 400−800 nm did not induce toxic effects on enterocyte-like model Caco-2/TC cells up to 90 ng/mL. The cytotoxicity observed at doses higher than 100 ng/mL was due to the active poly-L-lysine in the medium in which the CNTs were dispersed, and not because of the CNTs. The study indicated that CNTs were inert and acted as carriers for poly-L-lysine.[115]

With respect to cytotoxicity on colorectal cancer cells *in vitro*, *f*SWCNT-COOH (0.4 μm−1.2 μm with an outer diameter of 15 ± 5 μm) was tested at the dose range of 5−1000 μg/mL. Improved production of reactive oxygen species (ROS) as the underlying mechanism of cytotoxicity was observed only after 100 μg/mL. The ROS levels later increased 5.2-fold to the control in 1000 μg/mL treated cells.[116] In a

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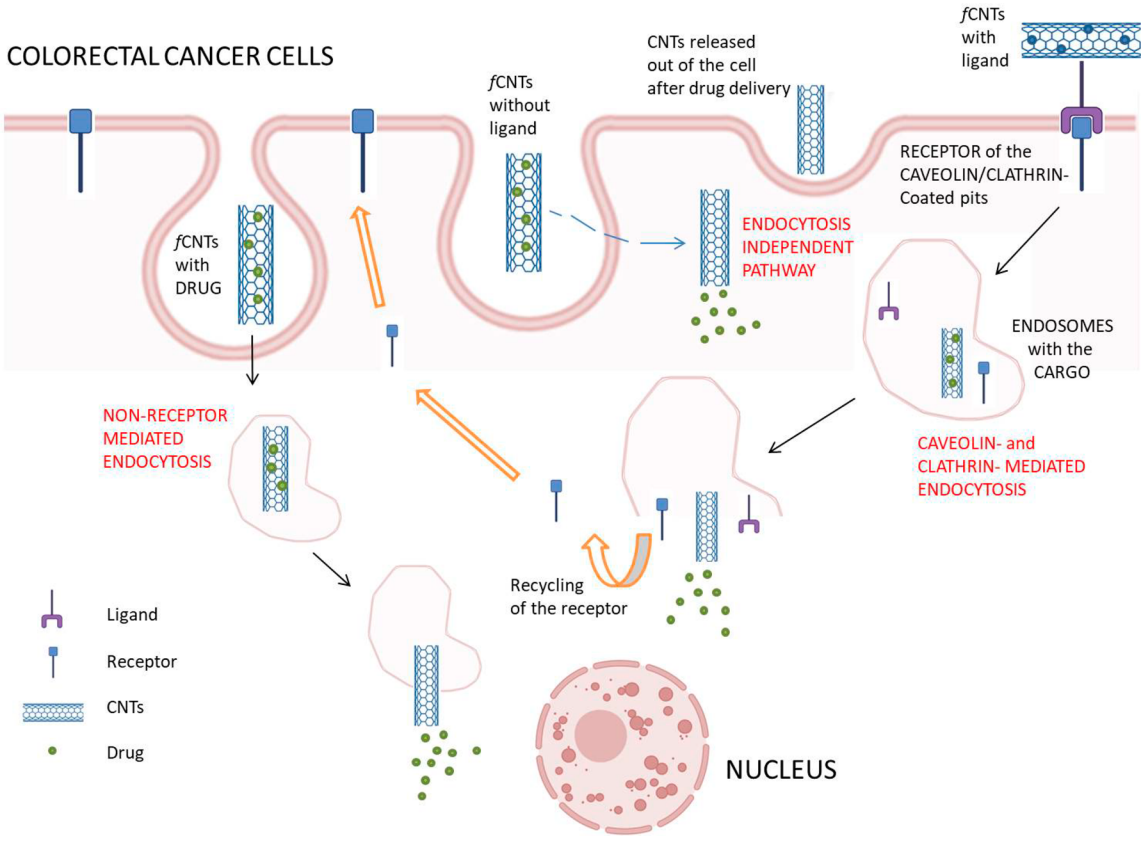

Figure 2. Different mechanisms for cellular uptake and drug delivery using *f*CNTs.

Table 2. SWCNTs (on Caco-2 Cells) and MWCNTs (on HT-29 Cells) for Drug Delivery Applications

type of CNTs	functionalization agent-cargo	particle dimensions	nontoxic dose	cytotoxic dose	ref
SWCNTs	COOH and PEG	1.4 ± 0.1 nm	1000 μg/mL after 24 h	35% cytotoxicity after 72 h	112
	COOH	1.4 ± 0.1 nm	5−100 μg/mL	>100 μg/mL after 24 h	113
	COOH and PEG-CoQ10	253.2 and 328.7 nm	160 μg/mL after 24 h		114
	Dispersed in poly-L-lysine	1.4−1.6 nm (diameter), 400−800 nm (length)	90 ng/mL	improved toxicity of poly-L-lysine at SWCNPs > 100 ng/mL	115
	COOH	0.4 μm−1.2 μm, 15 ± 5 μm (diameter)	<50 μg/mL after 24 h	Induction of ROS, LPO, alteration in antioxidant defenses after doses > 50 μg/mL after 24 h	116
MWCNTs	Fluorescein	20−500 nm (length), 5−60 nm (diameter)	<25 μg/mL	>25 μg/mL	120
	PEG-Oxaliplatin	40−50 nm (diameter), 15 nm (thickness)	5 μM, <48 h	5 μM, >48 h	121

similar study, nanobiocomposites (203 ± 6.6 nm) composed of SWCNTs functionalized with hyaluronic acid and loaded with a photosensitizer chlorin e6 (SWCNTs-HA-Ce6) were prepared. The analysis of toxic effects on Caco-2 cells (IC_{50} value of 2.56 μg/mL) by means of PDT at a confluence of 10 J/cm^2 showed higher cytotoxicity (84.6%) in comparison to the cells irradiated with a fluence of 5 J/cm^2 (77%).[117]

These studies indicate that *f*CNTs with lengths of ∼250−1200 nm and a diameter of ∼1.4 nm are toxic toward Caco-2 cells only after 24 h at doses higher than 100 μg/mL. The mechanisms for cellular uptake and drug delivery using *f*CNTs are presented in Figure 2.

3.9.2. MWCNTs. MWCNTs (<1 μm) did improve the cytotoxic effects of doxorubicin and camptothecin as a complex in comparison (0.6 μg/mL) to the free drug on Caco-2 cells.[118] It is interesting to note that MWCNTs could not penetrate the Caco-2 cells at the concentration of 50 μg/mL although they were scattered around the cell surface.[119] MWCNTs with 10−30 layers, lengths of 20−500 nm, and diameters of 5−15 nm (interior) and 10−60 nm (external) were functionalized with fluorescein (CNT-FITC). CNT-FITCs were tested for cytotoxicity against HT-29 cells and induced 45% cell death at 25 μg/mL. The cell killing effects were further increased with increasing concentrations of the latter (23% and 7% viability for 100 and 200 μg/mL, respectively). The adhesive properties of 2D colonies were affected, a decrease in the area (89.8% at 200 μg/mL) of the model and a 2.4-fold decrease in volume of the 3D tumor spheroids were observed.[120] MWCNTs functionalized with PEG and loaded with Oxaliplatin (MWCNT-PEG-Oxaliplatin with ∼43.6% Oxaliplatin load) resulted in sustained release of the antineoplastic agent into HT-29 cells (34% after 6 h). The

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cytotoxicity of the MWCNTs (40−50 nm diameter and 15 nm thickness) increased gradually after incubation for 48 h with the formation of Pt-DNA adducts and γ-H2AX along with the induction of apoptosis.[121] The outcomes of research on drug delivery applications of SWCNTs and MWCNTs are enlisted in Table 2.

3.10. Internalization Mechanisms for CNTs. Owing to the limited penetrating abilities of small molecules and biological molecules like nucleic acid polymers and peptides, several carriers like CNTs have been utilized. CNTs can carry cargo of sizes more than 60 kDa based on hydrophobhic interactions over longer exposure durations and enter the cytoplasm of the mammalian cells by nonspecific endocytosis.[122,123] Among the common mechanisms for entry of CNTs into mammalian cells, phagocytosis, diffusion, and endocytosis have been widely credited.[124] Connecting to this behavior, clustered CNTs are internalized by endocytosis, whereas the distinct tubes enter by diffusion. Therefore, these mechanisms are necessary for transport across biological membranes. This is dependent both on tube properties and phagocytic behavior of the cancerous cells. The cellular fate may be attained in the cytoplasm, endosomes, perinuclear space, mitochondria and nucleus, while they are removed by exocytosis and biological degradation and elimination, making them safe candidates for drug delivery. In addition, they are also enzymatically degraded by myeloperoxidase.[125]

In this venture, the amino-groups functionalized on the CNTs bind those tubes to the cellular membrane and insert the CNTs by nanoneedle mechanism in case of loss in phagocytic behavior or failure in endocytosis. In short, the CNTs interact with the lipid bilayer of the cells and diffuse through the plasma membrane, whereas, CNTs loaded with biological macromolecules diffuse the cellular membrane by clathrin-mediated endocytosis. Similarly, RNA-coated CNTs can undergo exocytosis for entry into the cytoplasm or vesicles cancerous cells resembling endocytosis. The mechanism of cellular uptake and the CNTs being localized and distributed depends on the functionalization, dimensions (such as lengths and diameter), number of layers and the dose of such tubes. During endocytosis, the CNTs enter the endosomes and mature and accumulate into the lysosomes. This buildup can result in the vesicle to swell and rupture and release the *f*CNTs into the cytosol where the cargo is delivered.[126,127]

Overall, the studies on the use of CNTs highlight that they are internalized by cell membranes based on three mechanisms: 1. Caveolin- and Clathrin- mediated endocytosis (particles of sizes ≤100 nm); 2. Macropinocytosis (particles of sizes >300 nm); 3. Phagocytosis (particles of sizes ∼1000 nm). They are functionalized for better physical properties and performance intended for use in anticancer medications and photodynamic therapy (PDT).[127] The internalization of CNTs is usually energy-dependent and the uptake can become saturated at increased concentrations.[128]

3.11. Safety Testing of CNTs on Normal Cells. Functionalization of CNTs with drugs or complementary molecules via chemical bond formation or physioadsorption can improve the biocompatibility and reduce the toxic effects by showing an inert behavior. It increases the solubility in water with enhanced dispersion and limited agglomeration, leading to limited cytotoxic effects. The dose- and time-dependent cytotoxicity of CNTs is influenced by the structure, size (nontoxic length of ∼10 μm), and molecules used for functionalization.[129,130] The duration of exposure is another critical parameter for causing cell death.[131] The added advantage is that the CNTs can enter mammalian cells without the need for an additional transporter and cause systemic toxicity.[132,133]

Polycitric-acid-polymerized *f*MWCNTs with an average diameter of ∼89.2 nm and length of 400 μm at a concentration of 100 ng/mL were not as cytotoxic toward mesenchymal stem cells and showed very low apoptotic behavior. Hence, they could be used as scaffolding materials to study the patterns of cellular growth such as adhesion and proliferation.[134] The mechanism of cytotoxicity on rat MSCs *in vivo* was dependent on mitochondrial and DNA damage, elevated oxidative stress, and damage to cell membranes.[135] The acid-functionalized SWCNTs can disrupt the tight junction barriers and cause cytotoxicity in Madin-Darby canine kidney II epithelial cells.[136] The IC_{50} value of MWCNTs against the bronchial epithelial cell line BEAS-2B was 12 μg/mL, and the uptake was 17−18% at 10 μg/mL. The genomic and proteomic levels of migration inhibitory factor, interleukins IL-6 and IL-8 increased after treatment along with the phosphorylation of p38, ERK1 and HSP27.[137] In addition, the SWCNTs can induce genotoxicity in lung fibroblast V79 cells.[138,139]

In mouse models, *f*SWCNTs-COOH did not show any behavioral changes or mortality. The body weight and the weight of the majority of organs were not altered, whereas the increase in liver, lung, and spleen weights was insignificant. Functionalization with COOH was less toxic in comparison to amino-functionalization. The toxicity profile of MWCNTs was prominent in comparison to SWCNTs.[91] These studies conducted *in vitro* and *in vivo* indicate the toxicity of CNTs on normal cells is dependent on multiple factors.

3.12. Advantages of Using CNTs for Drug Delivery in Comparison to Other Nanomaterials. The initial advantage of using CNTs for drug delivery is that the entrapment efficacy of CNTs is significantly higher in comparison to liposomes, dendrimers, and other nanoparticles. Functionalization improves the solubility, biocompatibility, and enhances the specific targeting of a tumor at its site. In general, CNTs release the encapsulated drugs with higher efficiency at the physiological pH compared to liposomes, dendrimers, or other formulations. Also, the zeta potential of CNTs is neutral at acidic and neutral pH, whereas they are partially negative at alkaline pH in comparison to other types of nanomaterials.[140] The advantage of using a neutral or partially negative charged material like CNTs is that they aggregate at a slower rate in comparison to positively charged nanomaterials. Hence, CNTs can diffuse through the cell membrane more easily than positively charged nanoparticles which have a higher tendency of agglomeration. Thus, the negatively charged CNTs with the drug load are internalized through receptor-mediated endocytosis. Hence, the alteration of CNTs making them SMART at the membrane level based on the type of target cell is highly appreciated for efficient cellular uptake.[141] Therefore, functionalization may play a critical role in CNTs attaining an external charge and subsequent delivery into cancerous cells.

In comparison to other materials at the nanoscale, CNTs are known to possess greater tensile strength and enhanced biocompatibility in comparison to other nanomaterials and are chemically inert. Additional properties like high surface to volume ratio, superior conductivity, and easy access to be functionalized make them ideal candidates as drug vectors. Also, CNTs are excellent nonviral vectors as they can deliver

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the functionalized nucleic acids without being degraded. Adding to this, the CNTs are safe toward normal fibroblasts and elicit no inflammatory response on such cells *in vitro*. Carbon coatings are corrosion-resistant and can prevent inflammatory responses in biomedical devices like cardiovascular stents.[68,142,143]

3.13. CNPs in Preoperative Localization of Colorectal Tumors at the Clinical Level. Elevated lymphatic metastasis along the inferior mesenteric artery is the widely accepted route for spread of tumors to distant sites. Therefore, lymphadenectomy is critical for the therapy of CRC as disease-free survival improves in clinical patients.[144] CNPs can better assist the inspections accomplished after colonic polypectomy performed for the removal of precancerous lesions or early CRC tissues.[145] Pathology after the involvement of CNPs was enhanced, and this can contribute to identification of the metastatic state of the dissected lymph nodes effectively as it decreases the perils of postoperative comorbidities associated with excessive dissection of lymph nodes.[146−149] In addition, the nanomaterials are effective in the identification of sentinel lymph nodes.[150,151]

Over the recent years, CNPs have been used for locating tumors of the colon and rectum. CNPs have reportedly been used in combination with titanium clips for neoadjuvant chemoradiotherapy before radical resection for colon and rectal cancers. The titanium clips and CNPs help in localizing the tumor easily and removing it early. This can improve the chances of identifying the best surgical option and reduction of the duration of laparoscopy. To support this claim, the CNPs were introduced into the submucosal layer of the normal noncancerous gastrointestinal wall of clinical CRC patients. The site of injection remained 1 cm distant from the tumor edge.[152,153] The use of endoscopic tattooing using CNPs seemed advantageous over conventional methods. This type of localization did not result in any adverse effects such as abdominal pain, fever, diarrhea, or any other symptoms related to any possible infections. Consistent with the previous reports, the duration for identifying the tumor and the time schedule of the operation decreased considerably between patients of the experimental and control group. The operative bleeding was significantly lower and the dissection rate of lymph nodes was higher in experimental group in comparison to the control group. The postoperative difficulties were not dissimilar among both the groups.[154] These clinical trials indicate that CNP-based localization of CRC and the subsequent neoadjuvant therapy is both beneficial and safe, as supported by previous studies.[155,156]

4. CONCLUSION

The current review highlighted the different roles of CNTs with respect to drug delivery and therapy at preclinical and CNPs as tumor localizers at clinical levels. The prospects of targeted therapy and tattooing for selective identification of tumor cells or tissues were discussed. The advantages of using CNTs as inert materials with improved properties enable them to act as attractive candidates for targeted therapy of CRC. The prerequisites with regard to a structure that supports proficient loading, surface area and stability, enhanced biocompatibility, and targeted release of drugs make CNTs smart options for drug delivery. The toxicity profile of CNTs on normal cells was elucidated further. It is important to observe that there are no reports describing the usage of CNTs at clinical levels. Yet, carbon-based nanoparticles have been used to localize tumors in clinical patients. This is supportive for the prospective use of CNTs in the clinic. With these derivations, the present review concludes that CNTs are efficient in drug delivery for CRC with limited toxicity and further preclinical studies are warranted in the near future for their use in the clinic.

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Notes

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REFERENCES

(1) Pandey, G.; Jain, P. Assessing the nanotechnology on the grounds of costs, benefits, and risks. *Beni-Suef University Journal of Basic and Applied Sciences* **2020**, *9*, 63.

(2) Bayda, S.; Hadla, M.; Palazzolo, S.; Kumar, V.; Caligiuri, I.; Ambrosi, E.; Pontoglio, E.; Agostini, M.; Tuccinardi, T.; Benedetti, A.; et al. Bottom-up synthesis of carbon nanoparticles with higher doxorubicin efficacy. *J. Controlled Release* **2017**, *248*, 144−152.

(3) Debnath, S. K.; Srivastava, R. Drug delivery with carbon-based nanomaterials as versatile nanocarriers: Progress and prospects. *Frontiers in Nanotechnology* **2021**, *3*, 15.

(4) Alzahrani, S. M.; Al Doghaither, H. A.; Al-Ghafari, A. B. General insight into cancer: An overview of colorectal cancer. *Molecular and Clinical Oncology* **2021**, *15*, 1−8.

(5) Gogoi, P.; Kaur, G.; Singh, N. K. Nanotechnology for colorectal cancer detection and treatment. *World J. Gastroenterol* **2022**, *28*, 6497−6511.

(6) Krasteva, N.; Georgieva, M. Promising Therapeutic Strategies for Colorectal Cancer Treatment Based on Nanomaterials. *Pharmaceutics* **2022**, *14*, 1213.

(7) Engelis, N. G.; Anastasaki, A.; Nurumbetov, G.; Truong, N. P.; Nikolaou, V.; Shegiwal, A.; Whittaker, M. R.; Davis, T. P.; Haddleton, D. M. Sequence-controlled methacrylic multiblock copolymers via sulfur-free RAFT emulsion polymerization. *Nature Chem.* **2017**, *9*, 171−178.

(8) Naeimi, R.; Najafi, R.; Molaei, P.; Amini, R.; Pecic, S. Nanoparticles: The future of effective diagnosis and treatment of colorectal cancer? *Eur. J. Pharmacol.* **2022**, *936*, 175350.
(9) Peng, H.; Xu, Z.; Wang, Y.; Feng, N.; Yang, W.; Tang, J. Biomimetic mesoporous silica nanoparticles for enhanced blood circulation and cancer therapy. *ACS Applied Bio Materials* **2020**, *3*, 7849−7857.
(10) Peng, H.; Zhang, X.; Yang, P.; Zhao, J.; Zhang, W.; Feng, N.; Yang, W.; Tang, J. Defect self-assembly of metal-organic framework triggers ferroptosis to overcome resistance. *Bioactive Materials* **2023**, *19*, 1−11.
(11) Khan, I.; Saeed, K.; Khan, I. Nanoparticles: Properties, applications and toxicities. *Arabian Journal of Chemistry* **2019**, *12*, 908−931.
(12) Guo, S.; Yao, X.; Jiang, Q.; Wang, K.; Zhang, Y.; Peng, H.; Tang, J.; Yang, W. Dihydroartemisinin-loaded magnetic nanoparticles for enhanced chemodynamic therapy. *Frontiers in pharmacology* **2020**, *11*, 226.
(13) Hong, G.; Diao, S.; Antaris, A. L.; Dai, H. Carbon nanomaterials for biological imaging and nanomedicinal therapy. *Chem. Rev.* **2015**, *115*, 10816−10906.
(14) Singh, R.; Torti, S. V. Carbon nanotubes in hyperthermia therapy. *Advanced drug delivery reviews* **2013**, *65*, 2045−2060.
(15) Harrison, B. S.; Atala, A. Carbon nanotube applications for tissue engineering. *Biomaterials* **2007**, *28*, 344−353.
(16) Bianco, A.; Kostarelos, K.; Prato, M. Applications of carbon nanotubes in drug delivery. *Curr. Opin. Chem. Biol.* **2005**, *9*, 674−679.
(17) Karimi, M.; Sahandi Zangabad, P.; Ghasemi, A.; Amiri, M.; Bahrami, M.; Malekzad, H.; Ghahramanzadeh Asl, H.; Mahdieh, Z.; Bozorgomid, M.; Ghasemi, A.; et al. Temperature-responsive smart nanocarriers for delivery of therapeutic agents: applications and recent advances. *ACS Appl. Mater. Interfaces* **2016**, *8*, 21107−21133.
(18) Ghasemi, A.; Amiri, H.; Zare, H.; Masroor, M.; Hasanzadeh, A.; Beyzavi, A.; Aref, A. R.; Karimi, M.; Hamblin, M. R. Carbon nanotubes in microfluidic lab-on-a-chip technology: current trends and future perspectives. *Microfluid. Nanofluid.* **2017**, *21*, 1−19.
(19) Tang, J.; Kong, B.; Wu, H.; Xu, M.; Wang, Y.; Wang, Y.; Zhao, D.; Zheng, G. Carbon Nanodots Featuring Efficient FRET for Real-Time Monitoring of Drug Delivery and Two-Photon Imaging. *Adv. Mater.* **2013**, *25*, 6569−6574.
(20) Rawla, P.; Sunkara, T.; Barsouk, A. Epidemiology of colorectal cancer: incidence, mortality, survival, and risk factors. *Prz Gastroenterol* **2019**, *14*, 89−103.
(21) Kuipers, E. J.; Grady, W. M.; Lieberman, D.; Seufferlein, T.; Sung, J. J.; Boelens, P. G.; van de Velde, C. J. H.; Watanabe, T. Colorectal cancer. *Nat. Rev. Dis Primers* **2015**, *1*, 15065−15065.
(22) Siegel, R. L.; Miller, K. D.; Jemal, A. Cancer statistics, 2020. *CA: A Cancer Journal for Clinicians* **2020**, *70*, 7−30.
(23) Sung, H.; Ferlay, J.; Siegel, R. L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians* **2021**, *71*, 209.
(24) Siegel, R. L.; Miller, K. D.; Fuchs, H. E.; Jemal, A. Cancer statistics, 2021. *CA: a cancer journal for clinicians* **2021**, *71*, 7−33.
(25) Siegel, R. L.; Miller, K. D.; Fuchs, H. E.; Jemal, A. Cancer statistics, 2022. *CA: A Cancer Journal for Clinicians* **2022**, *72*, 7−33.
(26) Yang, Y.; Han, Z.; Li, X.; Huang, A.; Shi, J.; Gu, J. Epidemiology and risk factors of colorectal cancer in China. *Chin J. Cancer Res.* **2020**, *32*, 729−741.
(27) Alyabsi, M.; Algarni, M.; Alshammari, K. Trends in Colorectal Cancer Incidence Rates in Saudi Arabia (2001−2016) Using Saudi National Registry: Early-Versus Late-Onset Disease. *Frontiers in oncology* **2021**, *11*, 730689.
(28) Cai, J.; Yuan, Z.; Zhang, S. Abdominal pain, diarrhea, constipation–which symptom is more indispensable to have a colonoscopy? *Int. J. Clin Exp Pathol* **2015**, *8*, 938−942.
(29) Hamilton, W.; Round, A.; Sharp, D.; Peters, T. J. Clinical features of colorectal cancer before diagnosis: a population-based case−control study. *Br. J. Cancer* **2005**, *93*, 399−405.
(30) Winner, M.; Mooney, S. J.; Hershman, D. L.; Feingold, D. L.; Allendorf, J. D.; Wright, J. D.; Neugut, A. I. Management and outcomes of bowel obstruction in patients with stage IV colon cancer: a population-based cohort study. *Dis Colon Rectum* **2013**, *56*, 834−843.
(31) Fukuchi, M.; Tsukagoshi, R.; Sakurai, S.; Suzuki, M.; Naitoh, H.; Yamauchi, H.; Tabe, Y.; Fukasawa, T.; Kiriyama, S.; Kuwano, H. Adult Intussusception Caused by Descending Colon Cancer during Chemotherapy of Stomach Cancer Recurrence. *Case Rep. Gastroenterol* **2012**, *6*, 88−93.
(32) Abdulamir, A. S.; Hafidh, R. R.; Abu Bakar, F. The association of Streptococcus bovis/gallolyticus with colorectal tumors: the nature and the underlying mechanisms of its etiological role. *J. Exp Clin Cancer Res.* **2011**, *30*, 11−11.
(33) Yang, J.; Du, X. L.; Li, S. T.; Wang, B. Y.; Wu, Y. Y.; Chen, Z. L.; Lv, M.; Shen, Y. W.; Wang, X.; Dong, D. F.; Li, D.; Wang, F.; Li, E. X.; Yi, M.; Yang, J. Characteristics of Differently Located Colorectal Cancers Support Proximal and Distal Classification: A Population-Based Study of 57,847 Patients. *PloS one* **2016**, *11*, e0167540−e0167540.
(34) Nakayama, G.; Tanaka, C.; Kodera, Y. Current Options for the Diagnosis, Staging and Therapeutic Management of Colorectal Cancer. *Gastrointest Tumors* **2013**, *1*, 25−32.
(35) Xie, Y.-H.; Chen, Y.-X.; Fang, J.-Y. Comprehensive review of targeted therapy for colorectal cancer. *Signal transduction and targeted therapy* **2020**, *5*, 1−30.
(36) Alrawi, S. J.; Schiff, M.; Carroll, R. E.; Dayton, M.; Gibbs, J. F.; Kulavlat, M.; Tan, D.; Berman, K.; Stoler, D. L.; Anderson, G. R. Aberrant crypt foci. *Anticancer Res.* **2006**, *26*, 107−119.
(37) Hauptman, N.; Glavač, D. Colorectal Cancer Blood-Based Biomarkers. *Gastroenterol Res. Pract* **2017**, *2017*, 2195361.
(38) Ren, L.; Yi, J.; Li, W.; Zheng, X.; Liu, J.; Wang, J.; Du, G. Apolipoproteins and cancer. *Cancer Med.* **2019**, *8*, 7032−7043.
(39) Rudzińska, M.; Parodi, A.; Soond, S. M.; Vinarov, A. Z.; Korolev, D. O.; Morozov, A. O.; Daglioglu, C.; Tutar, Y.; Zamyatnin, A. A. The role of cysteine cathepsins in cancer progression and drug resistance. *Int. J. Mol. Sci.* **2019**, *20*, 3602.
(40) Janciauskiene, S.; Wrenger, S.; Günzel, S.; Gründing, A. R.; Golpon, H.; Welte, T. Potential Roles of Acute Phase Proteins in Cancer: Why Do Cancer Cells Produce or Take Up Exogenous Acute Phase Protein Alpha1-Antitrypsin? *Frontiers in oncology* **2021**, *11*, 622076−622076.
(41) Sawayama, H.; Miyamoto, Y.; Hiyoshi, Y.; Shimokawa, M.; Kato, R.; Akiyama, T.; Sakamoto, Y.; Daitoku, N.; Yoshida, N.; Baba, H. Preoperative transferrin level is a novel prognostic marker for colorectal cancer. *Ann. Gastroenterol Surg* **2021**, *5*, 243−251.
(42) Gould, T.; Jamaluddin, M. F. B.; Petit, J.; King, S. J.; Nixon, B.; Scott, R.; Pockney, P.; Dun, M. D. Finding Needles in Haystacks: The Use of Quantitative Proteomics for the Early Detection of Colorectal Cancer. In *Advances in the Molecular Understanding of Colorectal Cancer*; IntechOpen, 2018.
(43) Albrethsen, J.; Møller, C. H.; Olsen, J.; Raskov, H.; Gammeltoft, S. Human neutrophil peptides 1, 2 and 3 are biochemical markers for metastatic colorectal cancer. *European journal of cancer* **2006**, *42*, 3057−3064.
(44) Mozdiak, E.; Metzger, J.; Pejchinovski, M.; Gopalakrishnan, K.; James, S.; Arasaradnam, R. P. P308 Exploratory study of urinary peptide markers in colorectal cancer and linkage to histopathology. *Gut* **2021**, *70*, A200.
(45) Zhan, P.; Zhao, S.; Yan, H.; Yin, C.; Xiao, Y.; Wang, Y.; Ni, R.; Chen, W.; Wei, G.; Zhang, P. α-enolase promotes tumorigenesis and metastasis via regulating AMPK/mTOR pathway in colorectal cancer. *Molecular carcinogenesis* **2017**, *56*, 1427−1437.
(46) Wang, B.; Hu, H.; Zhang, H.; Zhong, D. Immunohistochemical Assessment of Transthyretin Association with Colorectal Adenocarci-

https://doi.org/10.1021/acsomega.2c06242
ACS Omega 2023, 8, 10656−10668

noma. *Clinical Laboratory* **2021**, DOI: 10.7754/Clin.Lab.2020.200641.
(47) Ahn, S. B.; Sharma, S.; Mohamedali, A.; Mahboob, S.; Redmond, W. J.; Pascovici, D.; Wu, J. X.; Zaw, T.; Adhikari, S.; Vaibhav, V.; Nice, E. C.; Baker, M. S. Potential early clinical stage colorectal cancer diagnosis using a proteomics blood test panel. *Clinical Proteomics* **2019**, *16*, 34.
(48) Prizment, A. E.; Linabery, A. M.; Lutsey, P. L.; Selvin, E.; Nelson, H. H.; Folsom, A. R.; Church, T. R.; Drake, C. G.; Platz, E. A.; Joshu, C. Circulating Beta-2 Microglobulin and Risk of Cancer: The Atherosclerosis Risk in Communities Study (ARIC). *Cancer Epidemiol Biomarkers Prev* **2016**, *25*, 657−664.
(49) Garcia-Carbonero, N.; Martinez-Useros, J.; Li, W.; Orta, A.; Perez, N.; Carames, C.; Hernandez, T.; Moreno, I.; Serrano, G.; Garcia-Foncillas, J. KRAS and BRAF Mutations as Prognostic and Predictive Biomarkers for Standard Chemotherapy Response in Metastatic Colorectal Cancer: A Single Institutional Study. *Cells* **2020**, *9*, 219.
(50) Alharbi, A.; Bin Dokhi, H.; Almuhaini, G.; Alomran, F.; Masuadi, E.; Alomran, N. Prevalence of colorectal cancer biomarkers and their impact on clinical outcomes in Riyadh, Saudi Arabia. *PLoS One* **2021**, *16*, e0249590.
(51) Hamada, T.; Nowak, J. A.; Ogino, S. PIK3CA mutation and colorectal cancer precision medicine. *Oncotarget* **2017**, *8*, 22305−22306.
(52) Zhang, L.; Shay, J. W. Multiple Roles of APC and its Therapeutic Implications in Colorectal Cancer. *J. Natl. Cancer Inst* **2017**, *109*, djw332.
(53) Kandioler, D.; Mittlböck, M.; Kappel, S.; Puhalla, H.; Herbst, F.; Langner, C.; Wolf, B.; Tschmelitsch, J.; Schippinger, W.; Steger, G.; et al. TP53 mutational status and prediction of benefit from adjuvant 5-fluorouracil in stage III colon cancer patients. *EBioMedicine* **2015**, *2*, 825−830.
(54) Fang, T.; Liang, T.; Wang, Y.; Wu, H.; Liu, S.; Xie, L.; Liang, J.; Wang, C.; Tan, Y. Prognostic role and clinicopathological features of SMAD4 gene mutation in colorectal cancer: a systematic review and meta-analysis. *BMC Gastroenterology* **2021**, *21*, 297.
(55) Mei, Z.; Shao, Y. W.; Lin, P.; Cai, X.; Wang, B.; Ding, Y.; Ma, X.; Wu, X.; Xia, Y.; Zhu, D.; Shu, Y.; Fu, Z.; Gu, Y. SMAD4 and NF1 mutations as potential biomarkers for poor prognosis to cetuximab-based therapy in Chinese metastatic colorectal cancer patients. *BMC Cancer* **2018**, *18*, 479.
(56) Muzny, D. M.; Bainbridge, M. N.; Chang, K.; Dinh, H. H.; Drummond, J. A.; Fowler, G.; Kovar, C. L.; Lewis, L. R.; Morgan, M. B.; Newsham, I. F.; Reid, J. G.; Santibanez, J.; Shinbrot, E.; Trevino, L. R.; Wu, Y.-Q.; Wang, M.; Gunaratne, P.; Donehower, L. A.; Creighton, C. J.; Wheeler, D. A.; Gibbs, R. A.; Lawrence, M. S.; Voet, D.; Jing, R.; Cibulskis, K.; Sivachenko, A.; Stojanov, P.; McKenna, A.; Lander, E. S.; Gabriel, S.; Getz, G.; Ding, L.; Fulton, R. S.; Koboldt, D. C.; Wylie, T.; Walker, J.; Dooling, D. J.; Fulton, L.; Delehaunty, K. D.; Fronick, C. C.; Demeter, R.; Mardis, E. R.; Wilson, R. K.; Chu, A.; Chun, H.-J. E.; Mungall, A. J.; Pleasance, E.; Gordon Robertson, A.; Stoll, D.; Balasundaram, M.; Birol, I.; Butterfield, Y. S. N.; Chuah, E.; Coope, R. J. N.; Dhalla, N.; Guin, R.; Hirst, C.; Hirst, M.; Holt, R. A.; Lee, D.; Li, H. I.; Mayo, M.; Moore, R. A.; Schein, J. E.; Slobodan, J. R.; Tam, A.; Thiessen, N.; Varhol, R.; Zeng, T.; Zhao, Y.; Jones, S. J. M.; Marra, M. A.; Bass, A. J.; Ramos, A. H.; Saksena, G.; Cherniack, A. D.; Schumacher, S. E.; Tabak, B.; Carter, S. L.; Pho, N. H.; Nguyen, H.; Onofrio, R. C.; Crenshaw, A.; Ardlie, K.; Beroukhim, R.; Winckler, W.; Getz, G.; Meyerson, M.; Protopopov, A.; Zhang, J.; Hadjipanayis, A.; Lee, E.; Xi, R.; Yang, L.; Ren, X.; Zhang, H.; Sathiamoorthy, N.; Shukla, S.; Chen, P.-C.; Haseley, P. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* **2012**, *487*, 330−337.
(57) Jiang, Z.; Jacob, J. A.; Li, J.; Wu, X.; Wei, G.; Vimalanathan, A.; Mani, R.; Nainangu, P.; Rajadurai, U. M.; Chen, B. Influence of diet and dietary nanoparticles on gut dysbiosis. *Microbial pathogenesis* **2018**, *118*, 61−65.
(58) Jiang, Z.; Li, L.; Chen, J.; Wei, G.; Ji, Y.; Chen, X.; Liu, J.; Huo, J. Human gut-microbiome interplay: Analysis of clinical studies for the emerging roles of diagnostic microbiology in inflammation, oncogenesis and cancer management. *Infection, Genetics and Evolution* **2021**, *93*, 104946.
(59) Olovo, C. V.; Huang, X.; Zheng, X.; Xu, M. Faecal microbial biomarkers in early diagnosis of colorectal cancer. *J. Cell Mol. Med.* **2021**, *25*, 10783.
(60) Alvarez-Chaver, P.; Otero-Estévez, O.; Páez de la Cadena, M.; Rodríguez-Berrocal, F. J.; Martínez-Zorzano, V. S. Proteomics for discovery of candidate colorectal cancer biomarkers. *World journal of gastroenterology* **2014**, *20*, 3804−3824.
(61) Shin, M. H.; Kim, J.; Lim, S. A.; Kim, J.; Lee, K.-M. Current Insights into Combination Therapies with MAPK Inhibitors and Immune Checkpoint Blockade. *Int. J. Mol. Sci.* **2020**, *21*, 2531.
(62) Tomada, J.; Dienel, T.; Hampel, F.; Fasel, R.; Amsharov, K. Combinatorial design of molecular seeds for chirality-controlled synthesis of single-walled carbon nanotubes. *Nat. Commun.* **2019**, *10*, 3278.
(63) Saifuddin, N.; Raziah, A.; Junizah, A. Carbon nanotubes: a review on structure and their interaction with proteins. *Journal of Chemistry* **2013**, *2013*, 1.
(64) Beg, S.; Rahman, M.; Jain, A.; Saini, S.; Hasnain, M. S.; Swain, S.; Imam, S.; Kazmi, I.; Akhter, S. Emergence in the functionalized carbon nanotubes as smart nanocarriers for drug delivery applications. In *Fullerenes, Graphenes and Nanotubes*; Grumezescu, A. M., Ed.; William Andrew Publishing, 2018; Ch. 4, pp 105−133.
(65) Sakharova, N. A.; Pereira, A. F.; Antunes, J. M.; Fernandes, J. V. Mechanical Characterization of Multiwalled Carbon Nanotubes: Numerical Simulation Study. *Materials* **2020**, *13*, 4283.
(66) Beg, S.; Rizwan, M.; Sheikh, A. M.; Hasnain, M. S.; Anwer, K.; Kohli, K. Advancement in carbon nanotubes: basics, biomedical applications and toxicity. *J. Pharm. Pharmacol.* **2011**, *63*, 141−163.
(67) Brian, M. Practical applications of carbon nanotubes in medicine. *Basic Biotechnology ejournal* **2009**, 445.
(68) Anzar, N.; Hasan, R.; Tyagi, M.; Yadav, N.; Narang, J. Carbon nanotube - A review on Synthesis, Properties and plethora of applications in the field of biomedical science. *Sensors International* **2020**, *1*, 100003.
(69) Caruso, G.; Merlo, L.; Tot, E.; Pignataro, C.; Caffo, M. Nanotechnology and the New Frontiers of Drug Delivery in Cerebral Gliomas. In *Nano- and Microscale Drug Delivery Systems*; Grumezescu, A. M., Ed.; Elsevier, 2017; Ch. 6, pp 95−112.
(70) Pu, Z.; Wei, Y.; Sun, Y.; Wang, Y.; Zhu, S. Carbon Nanotubes as Carriers in Drug Delivery for Non-Small Cell Lung Cancer, Mechanistic Analysis of Their Carcinogenic Potential, Safety Profiling and Identification of Biomarkers. *Int. J. Nanomed.* **2022**, 6157−6180.
(71) Jagadeesan, A. K.; Thangavelu, K.; Dhananjeyan, V. Carbon Nanotubes: Synthesis, Properties and Applications. In *21st Century Surface Science*; Pham, P., Goel, P., Kumar, S., Yadav, K., Eds.; IntechOpen, 2020; p 27.
(72) Gacem, A.; Modi, S.; Yadav, V. K.; Islam, S.; Patel, A.; Dawane, V.; Jameel, M.; Inwati, G. K.; Piplode, S.; Solanki, V. S. Recent Advances in Methods for Synthesis of Carbon Nanotubes and Carbon Nanocomposite and their Emerging Applications: A Descriptive Review. *J. Nanomater.* **2022**, *2022*, 7238602.
(73) Zhang, Q.; Huang, J.-Q.; Qian, W.-Z.; Zhang, Y.-Y.; Wei, F. The Road for Nanomaterials Industry: A Review of Carbon Nanotube Production, Post-Treatment, and Bulk Applications for Composites and Energy Storage. *Small* **2013**, *9*, 1237−1265.
(74) ABOUL-ENEIN, A. A.; AWADALLAH, A. E.; EL-DESOUKI, D. S.; ABOUL-GHEIT, N. A. Catalytic pyrolysis of sugarcane bagasse by zeolite catalyst for the production of multi-walled carbon nanotubes. *Journal of Fuel Chemistry and Technology* **2021**, *49*, 1421−1434.
(75) Alam, J.; Yadav, V. K.; Yadav, K. K.; Cabral-Pinto, M. M.; Tavker, N.; Choudhary, N.; Shukla, A. K.; Ali, F. A. A.; Alhoshan, M.; Hamid, A. A. Recent advances in methods for the recovery of carbon

https://doi.org/10.1021/acsomega.2c06242
ACS Omega 2023, 8, 10656−10668

nanominerals and polyaromatic hydrocarbons from coal fly ash and their emerging applications. *Crystals* **2021**, *11*, 88.
(76) Herrero-Latorre, C.; Álvarez-Méndez, J.; Barciela-García, J.; García-Martín, S.; Peña-Crecente, R. Characterization of carbon nanotubes and analytical methods for their determination in environmental and biological samples: a review. *Analytica chimica acta* **2015**, *853*, 77−94.
(77) Pampaloni, F.; Florin, E.-L. Microtubule architecture: inspiration for novel carbon nanotube-based biomimetic materials. *Trends Biotechnol.* **2008**, *26*, 302−310.
(78) Rodriguez-Fernandez, L.; Valiente, R.; Gonzalez, J.; Villegas, J. C.; Fanarraga, M. L. Multiwalled carbon nanotubes display microtubule biomimetic properties in vivo, enhancing microtubule assembly and stabilization. *ACS Nano* **2012**, *6*, 6614−6625.
(79) González-Domínguez, E.; Iturrioz-Rodriguez, N.; Padin-Gonzalez, E.; Villegas, J.; García-Hevia, L.; Pérez-Lorenzo, M.; Parak, W. J.; Correa-Duarte, M. A.; Fanarraga, M. L. Carbon nanotubes gathered onto silica particles lose their biomimetic properties with the cytoskeleton becoming biocompatible. *Int. J. Nanomed.* **2017**, *12*, 6317.
(80) Umemura, K. Hybrids of Nucleic Acids and Carbon Nanotubes for Nanobiotechnology. *Nanomaterials (Basel)* **2015**, *5*, 321−350.
(81) Ma, J.; Wang, J.-N.; Tsai, C.-J.; Nussinov, R.; Ma, B. Diameters of single-walled carbon nanotubes (SWCNTs) and related nanochemistry and nanobiology. *Frontiers of Materials Science in China* **2010**, *4*, 17−28.
(82) Wu, Y.; Phillips, J. A.; Liu, H.; Yang, R.; Tan, W. Carbon nanotubes protect DNA strands during cellular delivery. *ACS Nano* **2008**, *2*, 2023−2028.
(83) Shi Kam, N. W.; Jessop, T. C.; Wender, P. A.; Dai, H. Nanotube molecular transporters: internalization of carbon nanotube-protein conjugates into Mammalian cells. *J. Am. Chem. Soc.* **2004**, *126*, 6850−6851.
(84) Chen, Y.; Qu, K.; Zhao, C.; Wu, L.; Ren, J.; Wang, J.; Qu, X. Insights into the biomedical effects of carboxylated single-wall carbon nanotubes on telomerase and telomeres. *Nat. Commun.* **2012**, *3*, 1074.
(85) Wolski, P.; Wojton, P.; Nieszporek, K.; Panczyk, T. Interaction of human telomeric i-motif DNA with single-walled carbon nanotubes: insights from molecular dynamics simulations. *J. Phys. Chem. B* **2019**, *123*, 10343−10353.
(86) Son, K. H.; Hong, J. H.; Lee, J. W. Carbon nanotubes as cancer therapeutic carriers and mediators. *International journal of nanomedicine* **2016**, *11*, 5163.
(87) Elhissi, A.; Ahmed, W.; Hassan, I. U.; Dhanak, V.; D'Emanuele, A. Carbon nanotubes in cancer therapy and drug delivery. *Journal of Drug Delivery* **2012**, *2012*, 837327.
(88) Bura, C.; Mocan, T.; Grapa, C.; Mocan, L. Carbon Nanotubes-Based Assays for Cancer Detection and Screening. *Pharmaceutics* **2022**, *14*, 781.
(89) Tang, L.; Xiao, Q.; Mei, Y.; He, S.; Zhang, Z.; Wang, R.; Wang, W. Insights on functionalized carbon nanotubes for cancer theranostics. *J. Nanobiotechnol.* **2021**, *19*, 423.
(90) Chenthamara, D.; Subramaniam, S.; Ramakrishnan, S. G.; Krishnaswamy, S.; Essa, M. M.; Lin, F.-H.; Qoronfleh, M. W. Therapeutic efficacy of nanoparticles and routes of administration. *Biomaterials Research* **2019**, *23*, 20.
(91) Mohammadi, E.; Zeinali, M.; Mohammadi-Sardoo, M.; Iranpour, M.; Behnam, B.; Mandegary, A. The effects of functionalization of carbon nanotubes on toxicological parameters in mice. *Human & Experimental Toxicology* **2020**, *39*, 1147−1167.
(92) Song, L.; Ho, V. H.; Chen, C.; Yang, Z.; Liu, D.; Chen, R.; Zhou, D. Efficient, pH-triggered drug delivery using a pH-responsive DNA-conjugated gold nanoparticle. *Adv. Healthcare Mater.* **2013**, *2*, 275−280.
(93) Pfeiffer, J. K. Innate Host Barriers to Viral Trafficking and Population Diversity: Lessons Learned from Poliovirus. In *Advances in Virus Research*; Maramorosch, K., Shatkin, A. J., Murphy, F. A., Eds.; Academic Press, 2010; Vol. *77*, Ch. 4, pp 85−118.
(94) Fallingborg, J. Intraluminal pH of the human gastrointestinal tract. *Danish medical bulletin* **1999**, *46*, 183−196.
(95) Yoshida, T.; Lai, T. C.; Kwon, G. S.; Sako, K. pH- and ion-sensitive polymers for drug delivery. *Expert opinion on drug delivery* **2013**, *10*, 1497−1513.
(96) Liu, Y.; Wang, W.; Yang, J.; Zhou, C.; Sun, J. pH-sensitive polymeric micelles triggered drug release for extracellular and intracellular drug targeting delivery. *Asian Journal of Pharmaceutical Sciences* **2013**, *8*, 159−167.
(97) Heger, Z.; Polanska, H.; Krizkova, S.; Balvan, J.; Raudenska, M.; Dostalova, S.; Moulick, A.; Masarik, M.; Adam, V. Co-delivery of VP-16 and Bcl-2-targeted antisense on PEG-grafted oMWCNTs for synergistic in vitro anti-cancer effects in non-small and small cell lung cancer. *Colloids Surf., B* **2017**, *150*, 131−140.
(98) Yu, B.; Tan, L.; Zheng, R.; Tan, H.; Zheng, L. Targeted delivery and controlled release of Paclitaxel for the treatment of lung cancer using single-walled carbon nanotubes. *Materials Science and Engineering: C* **2016**, *68*, 579−584.
(99) Cao, Y.; Huang, H.-Y.; Chen, L.-Q.; Du, H.-H.; Cui, J.-H.; Zhang, L. W.; Lee, B.-J.; Cao, Q.-R. Enhanced lysosomal escape of pH-responsive polyethylenimine−betaine functionalized carbon nanotube for the codelivery of survivin small interfering RNA and doxorubicin. *ACS Appl. Mater. Interfaces* **2019**, *11*, 9763−9776.
(100) Razzazan, A.; Atyabi, F.; Kazemi, B.; Dinarvand, R. In vivo drug delivery of gemcitabine with PEGylated single-walled carbon nanotubes. *Materials Science and Engineering: C* **2016**, *62*, 614−625.
(101) Villegas, J. C.; Álvarez-Montes, L.; Rodríguez-Fernández, L.; González, J.; Valiente, R.; Fanarraga, M. L. Multiwalled carbon nanotubes hinder microglia function interfering with cell migration and phagocytosis. *Adv. Healthcare Mater.* **2014**, *3*, 424−432.
(102) Chen, T.; Nie, H.; Gao, X.; Yang, J.; Pu, J.; Chen, Z.; Cui, X.; Wang, Y.; Wang, H.; Jia, G. Epithelial-mesenchymal transition involved in pulmonary fibrosis induced by multi-walled carbon nanotubes via TGF-beta/Smad signaling pathway. *Toxicology letters* **2014**, *226*, 150−162.
(103) Das, R.; Leo, B. F.; Murphy, F. The Toxic Truth About Carbon Nanotubes in Water Purification: a Perspective View. *Nanoscale Res. Lett.* **2018**, *13*, 183.
(104) Al Shoyaib, A.; Archie, S. R.; Karamyan, V. T. Intraperitoneal Route of Drug Administration: Should it Be Used in Experimental Animal Studies? *Pharm. Res.* **2020**, *37*, 12.
(105) Spahn, J. D.; Szefler, S. J. Pharmacology of the Lung and Drug Therapy. In *Pediatric Respiratory Medicine*, Second ed.; Taussig, L. M., Landau, L. I., Eds.; Mosby: Philadelphia, 2008; Ch. 16, pp 219−233.
(106) Jonaitis, L.; Marković, S.; Farkas, K.; Gheorghe, L.; Krznarić, Ž.; Salupere, R.; Mokricka, V.; Spassova, Z.; Gatev, D.; Grosu, I.; Lijović, A.; Mitrović, O.; Saje, M.; Schafer, E.; Uršič, V.; Roblek, T.; Drobne, D. Intravenous versus subcutaneous delivery of biotherapeutics in IBD: an expert's and patient's perspective. *BMC Proceedings* **2021**, *15*, 25.
(107) Bittner, B.; Richter, W.; Schmidt, J. Subcutaneous Administration of Biotherapeutics: An Overview of Current Challenges and Opportunities. *BioDrugs: clinical immunotherapeutics, biopharmaceuticals and gene therapy* **2018**, *32*, 425−440.
(108) Alqahtani, M. S.; Kazi, M.; Alsenaidy, M. A.; Ahmad, M. Z. Advances in Oral Drug Delivery. *Front Pharmacol* **2021**, *12*, 618411.
(109) Huppert, L. A.; Daud, A. I. Intratumoral therapies and in-situ vaccination for melanoma. *Human Vaccines & Immunotherapeutics* **2022**, *18*, 1890512.
(110) González-Lavado, E.; Iturrioz-Rodríguez, N.; Padín-González, E.; González, J.; García-Hevia, L.; Heuts, J.; Pesquera, C.; González, F.; Villegas, J.; Valiente, R.; et al. Biodegradable multi-walled carbon nanotubes trigger anti-tumoral effects. *Nanoscale* **2018**, *10*, 11013−11020.
(111) Kotchey, G. P.; Zhao, Y.; Kagan, V. E.; Star, A. Peroxidase-mediated biodegradation of carbon nanotubes in vitro and in vivo. *Advanced drug delivery reviews* **2013**, *65*, 1921−1932.
(112) Coyuco, J. C.; Liu, Y.; Tan, B.-J.; Chiu, G. N. C. Functionalized carbon nanomaterials: exploring the interactions

https://doi.org/10.1021/acsomega.2c06242
ACS Omega 2023, 8, 10656−10668

with Caco-2 cells for potential oral drug delivery. *Int. J. Nanomedicine* **2011**, *6*, 2253−2263.
(113) Jos, A.; Pichardo, S.; Puerto, M.; Sánchez, E.; Grilo, A.; Cameán, A. M. Cytotoxicity of carboxylic acid functionalized single wall carbon nanotubes on the human intestinal cell line Caco-2. *Toxicology in Vitro* **2009**, *23*, 1491−1496.
(114) Yang, S.; Niu, Y.; Li, L.; Liu, J.; Ma, M.; Duan, X.; Zhang, L.; Wang, L.; Qu, L. PEG-conjugated single-walled carbon nanotubes enhance the cellular uptake of Coenzyme Q10: in vitro evaluation and mechanism study. *J. Nanopart. Res.* **2021**, *23*, 1−14.
(115) Ansón-Casaos, A.; Grasa, L.; Pereboom, D.; Mesonero, J. E.; Casanova, A.; Murillo, M. D.; Martínez, M. T. In-vitro toxicity of carbon nanotube/polylysine colloids to colon cancer cells. *IET nanobiotechnology* **2016**, *10*, 374−381.
(116) Pichardo, S.; Gutiérrez-Praena, D.; Puerto, M.; Sánchez, E.; Grilo, A.; Cameán, A. M.; Jos, A. Oxidative stress responses to carboxylic acid functionalized single wall carbon nanotubes on the human intestinal cell line Caco-2. *Toxicology in Vitro* **2012**, *26*, 672−677.
(117) Sundaram, P.; Abrahamse, H. Effective photodynamic therapy for colon cancer cells using chlorin e6 coated hyaluronic acid-based carbon nanotubes. *International journal of molecular sciences* **2020**, *21*, 4745.
(118) Garriga, R.; Herrero-Continente, T.; Palos, M.; Cebolla, V. L.; Osada, J.; Muñoz, E.; Rodríguez-Yoldi, M. J. Toxicity of Carbon Nanomaterials and Their Potential Application as Drug Delivery Systems: In Vitro Studies in Caco-2 and MCF-7 Cell Lines. *Nanomaterials (Basel)* **2020**, *10*, 1617.
(119) Clark, K. A.; O'Driscoll, C.; Cooke, C. A.; Smith, B. A.; Wepasnick, K.; Fairbrother, D. H.; Lees, P. S.; Bressler, J. P. Evaluation of the interactions between multiwalled carbon nanotubes and Caco-2 cells. *Journal of Toxicology and Environmental Health, Part A* **2012**, *75*, 25−35.
(120) Perepelytsina, O. M.; Ugnivenko, A. P.; Dobrydnev, A. V.; Bakalinska, O. N.; Marynin, A. I.; Sydorenko, M. V. Influence of carbon nanotubes and its derivatives on tumor cells in vitro and biochemical parameters, cellular blood composition in vivo. *Nanoscale Res. Lett.* **2018**, *13*, 1−16.
(121) Wu, L.; Man, C.; Wang, H.; Lu, X.; Ma, Q.; Cai, Y.; Ma, W. PEGylated multi-walled carbon nanotubes for encapsulation and sustained release of oxaliplatin. *Pharm. Res.* **2013**, *30*, 412−423.
(122) Shi Kam, N. W.; Jessop, T. C.; Wender, P. A.; Dai, H. Nanotube molecular transporters: internalization of carbon nanotube− protein conjugates into mammalian cells. *J. Am. Chem. Soc.* **2004**, *126*, 6850−6851.
(123) Yaron, P. N.; Holt, B. D.; Short, P. A.; Lösche, M.; Islam, M. F.; Dahl, K. N. Single wall carbon nanotubes enter cells by endocytosis and not membrane penetration. *J. Nanobiotechnol.* **2011**, *9*, 45.
(124) Raffa, V.; Ciofani, G.; Vittorio, O.; Riggio, C.; Cuschieri, A. Physicochemical properties affecting cellular uptake of carbon nanotubes. *Nanomedicine (London, England)* **2010**, *5*, 89−97.
(125) Costa, P. M.; Bourgognon, M.; Wang, J. T. W.; Al-Jamal, K. T. Functionalised carbon nanotubes: From intracellular uptake and cell-related toxicity to systemic brain delivery. *J. Controlled Release* **2016**, *241*, 200−219.
(126) Tîlmaciu, C.-M.; Morris, M. C. Carbon nanotube biosensors. *Frontiers in chemistry* **2015**, *3*, 59.
(127) Zare, H.; Ahmadi, S.; Ghasemi, A.; Ghanbari, M.; Rabiee, N.; Bagherzadeh, M.; Karimi, M.; Webster, T. J.; Hamblin, M. R.; Mostafavi, E. Carbon nanotubes: Smart drug/gene delivery carriers. *International journal of nanomedicine* **2021**, *16*, 1681.
(128) Cui, X.; Wan, B.; Yang, Y.; Ren, X.; Guo, L. H. Length effects on the dynamic process of cellular uptake and exocytosis of single-walled carbon nanotubes in murine macrophage cells. *Sci. Rep* **2017**, *7*, 1518.
(129) He, H.; Pham-Huy, L. A.; Dramou, P.; Xiao, D.; Zuo, P.; Pham-Huy, C. Carbon nanotubes: applications in pharmacy and medicine. *BioMed Res. Int.* **2013**, *2013*, 1.
(130) Vardharajula, S.; Ali, S. Z.; Tiwari, P. M.; Eroğlu, E.; Vig, K.; Dennis, V. A.; Singh, S. R. Functionalized carbon nanotubes: biomedical applications. *Int. J. Nanomedicine* **2012**, *7*, 5361−5374.
(131) Czarnecka, J.; Wiśniewski, M.; Forbot, N.; Bolibok, P.; Terzyk, A. P.; Roszek, K. Cytotoxic or Not? Disclosing the Toxic Nature of Carbonaceous Nanomaterials through Nano−Bio Interactions. *Materials* **2020**, *13*, 2060.
(132) Cui, H.-F.; Vashist, S. K.; Al-Rubeaan, K.; Luong, J. H.; Sheu, F.-S. Interfacing carbon nanotubes with living mammalian cells and cytotoxicity issues. *Chemical research in toxicology* **2010**, *23*, 1131−1147.
(133) Lamberti, M.; Pedata, P.; Sannolo, N.; Porto, S.; De Rosa, A.; Caraglia, M. Carbon nanotubes: Properties, biomedical applications, advantages and risks in patients and occupationally-exposed workers. *International journal of immunopathology and pharmacology* **2015**, *28*, 4−13.
(134) Garnica-Gutiérrez, R. L.; Lara-Martínez, L. A.; Palacios, E.; Masso, F.; Contreras, A.; Hernández-Gutiérrez, S.; Cervantes-Sodi, F. Effect of functionalized carbon nanotubes and their citric acid polymerization on mesenchymal stem cells in vitro. *J. Nanomater.* **2018**, *2018*, 1.
(135) Song, G.; Guo, X.; Zong, X.; Du, L.; Zhao, J.; Lai, C.; Jin, X. Toxicity of functionalized multi-walled carbon nanotubes on bone mesenchymal stem cell in rats. *Dental materials journal* **2019**, *38*, 127−135.
(136) Singh, A. P.; Mia, M. B.; Saxena, R. K. Acid-functionalized single-walled carbon nanotubes alter epithelial tight junctions and enhance paracellular permeability. *Journal of biosciences* **2020**, *45*, 1−12.
(137) Hirano, S.; Fujitani, Y.; Furuyama, A.; Kanno, S. Uptake and cytotoxic effects of multi-walled carbon nanotubes in human bronchial epithelial cells. *Toxicology and applied pharmacology* **2010**, *249*, 8−15.
(138) Kisin, E. R.; Murray, A. R.; Keane, M. J.; Shi, X.-C.; Schwegler-Berry, D.; Gorelik, O.; Arepalli, S.; Castranova, V.; Wallace, W. E.; Kagan, V. E.; et al. Single-walled carbon nanotubes: geno-and cytotoxic effects in lung fibroblast V79 cells. *Journal of Toxicology and Environmental Health, Part A* **2007**, *70*, 2071−2079.
(139) Mrakovcic, M.; Meindl, C.; Leitinger, G.; Roblegg, E.; Fröhlich, E. Carboxylated short single-walled carbon nanotubes but not plain and multi-walled short carbon nanotubes show in vitro genotoxicity. *Toxicological sciences* **2015**, *144*, 114−127.
(140) Mody, N.; Tekade, R. K.; Mehra, N. K.; Chopdey, P.; Jain, N. K. Dendrimer, liposomes, carbon nanotubes and PLGA nanoparticles: one platform assessment of drug delivery potential. *AAPS PharmSciTech* **2014**, *15*, 388−399.
(141) Forest, V.; Pourchez, J. Preferential binding of positive nanoparticles on cell membranes is due to electrostatic interactions: A too simplistic explanation that does not take into account the nanoparticle protein corona. *Materials Science and Engineering: C* **2017**, *70*, 889−896.
(142) Smart, S.; Cassady, A.; Lu, G.; Martin, D. The biocompatibility of carbon nanotubes. *Carbon* **2006**, *44*, 1034−1047.
(143) Madani, S. Y.; Naderi, N.; Dissanayake, O.; Tan, A.; Seifalian, A. M. A new era of cancer treatment: carbon nanotubes as drug delivery tools. *Int. J. Nanomedicine* **2011**, *6*, 2963−2979.
(144) Sadahiro, S.; Ishikawa, K.; Suzuki, T.; Mukoyama, S.; Tanaka, Y.; Yasuda, S.; Tajima, T.; Makuuchi, H. [Optimal lymph node dissection for colorectal cancer]. *Nihon Geka Gakkai zasshi* **2001**, *102*, 497−500.
(145) Wang, R.; Wang, Y.; Li, D.; Yu, L.; Liu, G.; Ma, J.; Wang, W. Application of carbon nanoparticles to mark locations for re-inspection after colonic polypectomy. *Surgical Endoscopy* **2016**, *30*, 1530−1533.
(146) Yan, J.; Xue, F.; Chen, H.; Wu, X.; Zhang, H.; Chen, G.; Lu, J.; Cai, L.; Xiang, G.; Deng, Z.; Zheng, Y.; Zheng, X.; Li, G. A multi-center study of using carbon nanoparticles to track lymph node metastasis in T1−2 colorectal cancer. *Surgical Endoscopy* **2014**, *28*, 3315−3321.

https://doi.org/10.1021/acsomega.2c06242
ACS Omega 2023, 8, 10656−10668

(147) Chen, Y.-J.; Yeh, S.-T.; Kao, P.-S.; Ou, L.-H.; Lin, C.-S. A reappraisal of lymph node dissection in colorectal cancer during primary surgical resection. *World J. Surg Oncol* **2020**, *18*, 97.
(148) Tang, L.; Sun, L.; Zhao, P.; Kong, D. *Effect of activated carbon nanoparticles on lymph node harvest in patients with colorectal cancer* **2019**, *21*, 427−431.
(149) Cai, H. K.; He, H. F.; Tian, W.; Zhou, M. Q.; Hu, Y.; Deng, Y. C. Colorectal cancer lymph node staining by activated carbon nanoparticles suspension in vivo or methylene blue in vitro. *World journal of gastroenterology* **2012**, *18*, 6148−6154.
(150) Yan, J.; Xue, F.; Chen, H.; Wu, X.; Zhang, H.; Chen, G.; Lu, J.; Cai, L.; Xiang, G.; Deng, Z.; Zheng, Y.; Zheng, X.; Li, G. A multi-center study of using carbon nanoparticles to track lymph node metastasis in T1−2 colorectal cancer. *Surg Endosc* **2014**, *28*, 3315−3321.
(151) Sun, J.; Zhang, J. Assessment of lymph node metastasis in elderly patients with colorectal cancer by sentinel lymph node identification using carbon nanoparticles. *Journal of B.U.ON.: official journal of the Balkan Union of Oncology* **2018**, *23*, 68−72.
(152) Lin, N.; Qiu, J.; Wu, W.; Yang, W.; Wang, Y. Preoperative carbon nanoparticles and titanium clip combined labeling method for transverse colon tumor surgery. *Asian journal of surgery* **2019**, *42*, 844−845.
(153) Lin, N.; Yu, C.; Yang, J.; Wang, Y. Nano-carbon and titanium clip combined labeling assisted surgery before neoadjuvant radiotherapy and chemotherapy for rectal cancer. *Asian journal of surgery* **2020**, *43*, 383−384.
(154) Wang, Q.; Chen, E.; Cai, Y.; Chen, C.; Jin, W.; Zheng, Z.; Jin, Y.; Chen, Y.; Zhang, X.; Li, Q. Preoperative endoscopic localization of colorectal cancer and tracing lymph nodes by using carbon nanoparticles in laparoscopy. *World J. Surg Oncol* **2016**, *14*, 231−231.
(155) Wang, R.; Mo, S.; Liu, Q.; Zhang, W.; Zhang, Z.; He, Y.; Cai, G.; Li, X. The safety and effectiveness of carbon nanoparticles suspension in tracking lymph node metastases of colorectal cancer: a prospective randomized controlled trial. *Japanese journal of clinical oncology* **2020**, *50*, 535−542.
(156) Li, J.; Deng, X.; Wang, L.; Liu, J.; Xu, K. Clinical application of carbon nanoparticles in lymphatic mapping during colorectal cancer surgeries: A systematic review and meta-analysis. *Digestive and Liver Disease* **2020**, *52*, 1445−1454.

https://doi.org/10.1021/acsomega.2c06242
ACS Omega 2023, 8, 10656−10668