



# Current Advances in Chitosan Nanoparticles Based Oral Drug Delivery for Colorectal Cancer Treatment

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**Abstract:** As per the WHO, colorectal cancer (CRC) caused around 935,173 deaths worldwide in 2020 in both sexes and at all ages. The available anticancer therapies including chemotherapy, radiotherapy and anticancer drugs are all associated with limited therapeutic efficacy, adverse effects and low chances. This has urged to emerge several novel therapeutic agents as potential therapies for CRC including synthetic and natural materials. Orally administrable and targeted drug delivery systems are attractive strategies for CRC therapy as they minimize the side effects, enhance the efficacy of anticancer drugs. Nevertheless, oral drug delivery till today faces several challenges like poor drug solubility, stability, and permeability. Various oral nano-based approaches and targeted drug delivery systems have been developed recently, as a result of the ability of nanoparticles to control the release of the encapsulant, drug targeting and reduce the number of dosages administered. The unique physicochemical properties of chitosan polymer assist to overcome oral drug delivery barriers and target the colon tumour cells. Chitosan-based nanocarriers offered additional improvements by enhancing the stability, targeting and bioavailability of several anti-colorectal cancer agents. Modified chitosan derivatives also facilitated CRC targeting through strengthening the protection of encapsulant against acidic and enzyme degradation of gastrointestinal track (GIT). This review aims to provide an overview of CRC pathology, therapy and the barriers against oral drug delivery. It also emphasizes the role of nanotechnology in oral drug targeted delivery system and the growing interest towards chitosan and its derivatives. The present review summarizes the relevant works to date that have studied the potential applications of chitosan-based nanocarrier towards CRC treatment.

**Keywords:** chitosan, colorectal cancer, nanocarriers, oral delivery, drug targeting, nanotechnology

## Introduction

Colorectal cancer (CRC) is the third most prevailing cancer worldwide. In 2020, CRC caused 506,449 deaths in Asia, 244,824 in Europe, 63,987 in North America and 69,435 in Latin America and the Caribbean.<sup>1</sup> The morbidity is expected to increase up to 1.1 million worldwide by 2030.<sup>2</sup> CRC is usually seen in patients under the age of 50 and it is associated with dietary characteristics and eating habits that contribute to bacterial dysbiosis in the gastrointestinal tract (GIT) and colon cancer.<sup>3</sup>

CRC is a type of malignancy that affects the large intestine and rectum regions.<sup>4</sup> It is caused by the deactivation of the p53 pathway or adenomatous polyposis coli gene, the accumulation of mutations such as in K-ras, the transforming growth factor-beta pathway and the genesis of small polyps. The American Joint Committee on Cancer divided CRC into five phases with different treatment strategies for each stage.<sup>5</sup> Stage 0, the presence of abnormal colon cells or polyps on the mucosa, is completely curable with a surgical resection when found early enough. Similarly, surgical excision is the conventional treatment for stages I–II in most cases but with a 5-year survival rate of 37–74%. However, the survival rate

drops to 6% in advanced stages of CRC<sup>6</sup> and adjuvant pharmaceutical therapies such as chemotherapy are usually recommended following surgical resection.<sup>7</sup> In stages III–IV, adjuvant chemotherapy like oxaliplatin, 5-fluorouracil, cisplatin, doxorubicin and other emerging medicines are used. However, the administration of these medicines, has been linked to adverse effects, such as vomiting, hair loss and nausea and not demonstrated the expected efficacy.<sup>8</sup> Variety of drawbacks also associated with anticancer drugs, such as hydrophobicity, low water solubility, inadequate biodistribution, and susceptibility to multiple drug resistance.<sup>9</sup> This has urged the need for innovative approaches to enhance the physicochemical and pharmacodynamic properties of standard chemotherapeutics, or achieve target-specific delivery to optimise treatment effectiveness while reducing off-target side effects.<sup>10</sup>

Nanoparticles are among the most promising treatment modalities for cancers including CRC. Nanoparticles taken intravenously can circulate for extended period of time with reduced clearance rates from the renal system due to their ultra-small size and superior drug loading and encapsulation efficacy.<sup>11</sup> Furthermore, since nanoparticles allow for continuous or controlled release, the physical encapsulation of anticancer drugs improves their pharmacokinetic properties while reducing the number of dosages administered. This controlled release can circumvent multiple drug resistance since the cells actively ingest bigger amounts of the drug intracellularly.<sup>12–14</sup> Nanoparticles surface can be further modified with targeting ligands to achieve drug targeting by increasing the selectivity towards the desired cells/tissues while reducing the availability of the anticancer drug at the normal tissues.<sup>15</sup> In addition, amphiphilic lipid-based nanoparticles, such as micelles, possess a hydrophilic surface and a hydrophobic core and able to increase the solubility of hydrophobic drugs.<sup>16</sup>

Oral drug delivery is the most preferred and convenient route for drug administration and can be utilised for both systemic or local drug delivery.<sup>17</sup> The high surface area ( $>300\text{ m}^2$ ) of gastrointestinal track coated with a viscous mucosal layer facilitates drug adhesion and absorption.<sup>18</sup> Nevertheless, oral drug delivery can be challenging due to the complexity of GIT and obstacles that impact drug delivery like poor drug solubility, stability, and permeability.<sup>17</sup> Moreover, oral drugs may also have an unpleasant taste, induce gastric irritation, and be subjected to intestinal and hepatic barriers metabolism.<sup>19,20</sup> Oral route can be utilised to target a specific location in the GIT following proper design of the drug or the delivery carrier.<sup>21</sup> Several studies utilised oral route to target CRC throughout the rational design of cancer target discovery and development (CTDD).<sup>22</sup>

Numerous studies demonstrated the suitability of colon as a site for drugs absorption as it has less degradation and thus contributing to a greater drug bioavailability.<sup>23</sup> Special considerations are involved while designing CTDD as the drug need to be protected from gastric pH and enzymes. The colon's physiology differs greatly from that of the rest of the GIT. CTDD is affected by the inconsistency of colonic pH, transit time, and fluid volume, which are highly variable depending on the colon part, food, and metabolic enzymes.<sup>24</sup> Other important factors impacting CTDD efficacy include colonic fluid viscosity and the presence of microbial enzymes.<sup>25</sup>

Nanoparticles for drug delivery are prepared using numerous polymers, which range between natural, synthetic and semisynthetic.<sup>26–28</sup> Amongst these polymers, chitosan and its derivatives attracted much attention for oral route of administration owing to their suitable properties. Owing to the presence of amino groups, chitosan carries a cationic charge that is responsible for its permeation enhancing and mucoadhesion effect. It is also biodegradable, biocompatible and non-toxic.<sup>29</sup> Chitosan is a linear polysaccharide, comprises of randomly repeated units of N-acetyl-D-glucosamine and D-glucosamine linked by  $\beta(1\rightarrow4)$  glycosidic bonds.<sup>30</sup> Chitosan is a natural polymer obtained from chitin, the second most abundant naturally occurring polymer following cellulose. Both chitin and chitosan are extensively used in several applications such as food technology<sup>31</sup> and medical and pharmaceutical applications including wound healing,<sup>32</sup> tissue engineering<sup>33</sup> and gene delivery.<sup>34</sup> Chitosan has been formulated into chitosan nanoparticles (CSNPs) with attracted features especially in ocular and oral delivery. CSNPs are considered a very promising strategy to overcome the low stability and bioavailability of many active ingredients. Chitosan can efficiently bind to the negatively charged mucus membrane, increasing the retention time and enhancing the probability of their cellular uptake. Moreover, chitosan was also used as a coating agent due to its unique properties that enhances the permeation of anti-cancer drugs by transiently opening the tight junction between epithelial cells. Owing to its biodegradability via glycosidic linkage lysis by specified enzymes from the colonic microflora,<sup>35</sup> chitosan is very valuable for CRC targeting. Recently, Patil and Killedar developed water-insoluble gallic acid and quercetin into chitosan nanoparticles to assemble a stable and targeted colorectal cancer formulation.<sup>36</sup>

Kankala et al successfully prepared a versatile drug delivery platform based on nanocarriers by coating the ultrasmall Pt nanoparticles by a composite layer of chitosan over the Zn-doped MSNs to combat MDR in cancer efficaciously.<sup>37</sup> In 2017, 5-fluorouracil loaded PLGA-nanoparticles modified with chitosan were developed for target treatment of colorectal cancer. Coated NPs showed significant inhibition of colon cancer cells (HT-29) compared to uncoated NPs or free drug solution.<sup>38</sup>

Despite the availability of different types and grades of chitosan that differ mainly in the molecular weight and degree of deacetylation, original (un-modified) chitosan may lack the optimal properties for specific applications. Therefore, chemical modifications have been performed on chitosan structure mainly on the functional groups (-NH<sub>2</sub> and -OH). Modified chitosan derivatives have shown enhanced properties like bioactivity, biocompatibility and non-toxicity.<sup>39,40</sup> These chitosan modifications also facilitated CRC targeting where the drug need to be protected from acidic and enzyme degradation with minimal loss until it reaches the colon.<sup>41,42</sup> In 2021, Wang and his team combined of oxaliplatin and resveratrol successfully into N,O-carboxymethyl chitosan nanoparticles as a promise strategy for colorectal cancer therapy. The inhibition efficiency and anti-colon cancer activity of the combined treatment were more stronger than either type of nanoparticle alone or the free drugs.<sup>43</sup> In the same year, colon site-specific mucoadhesive system was developed by Samprasita et al composed of a-Mangostin-loaded thiolated chitosan NPs following by genipin (GP) crosslinking and the surface modification by EudragitVR L100.<sup>44</sup>

This review emphasized on chitosan nanoparticle applications in CRC. We first reviewed CRC pathology and therapy and explained the oral route of drug administration. Then, we explored nanotechnology in drug delivery and targeting before moving to detailed explanation of chitosan and CSNPs and their applications especially in CRC.

## Colorectal Cancer

Colorectal cancer is a type of malignancy that affects the large intestine and rectum regions.<sup>4</sup> In 2020, the number of death caused by colorectal was 506,449 in Asia, 244,824 in Europe, 63,987 in North America and 69,435 in Latin America and the Caribbean.<sup>1</sup> Colorectal cancer statistics are expected to grow with a morbidity estimation of 1.1 million worldwide by 2030.<sup>2</sup> Despite continual diagnosis and therapy advancements for both primary and metastatic type of CRC, long-term survival and cure rates are remained low. Males have a greater death rate from CRC than females, and this rate may rise as a result of several stimuli factors including age, unhealthy eating habits, obesity, smoking, alcohol use, high body mass index, sedentary lifestyle, and poor physical activities.<sup>45</sup> Adopting a healthy lifestyle appears to be the most important factor in preventing colorectal cancer.

## Pathophysiology

Colorectal cancer is the third most prevailing cancer worldwide, usually seen in patients under the age of 50 and it is associated with dietary characteristics and eating habits that contribute to bacterial dysbiosis in the GIT and colon cancer.<sup>3</sup> Colorectal cancer is now common in China, India, Singapore, Japan, and South Korea, despite the fact that it was long thought to be a disease only seen in Western nations. It is a multistage disease that can take 10–40 years to evolve from adenoma to carcinoma. Colorectal cancer is caused by the deactivation of the p53 pathway or adenomatous polyposis coli gene, the accumulation of mutations such as in K-ras, the transforming growth factor-beta pathway and the genesis of small polyps. These small polyps are prone to be transformed to large polyps, leading to tumour initiation. A preneoplastic indication of colon tissue is aberrant crypt foci where chemoprevention can be achieved by blocking or delaying the carcinogenesis process.<sup>46</sup>

T-lymphokine-activated killer cell-originated protein kinase signalling abnormalities promote cancer growth and is abundantly expressed and active in colorectal cancer, where it also stimulates cancer cell survival, inflammation and proliferation. Moreover, T-LAK-derived protein kinase, which interacts with the binding domain of DNA of the tumour suppressor p53 protein and controls the expression of transcription for target p21, has been suggested as a potential therapeutic target to fight colorectal cancer. Also, 3-Deoxysappanchalcone, a compound originated from *Caesalpiniasappan* L. is a natural component that capable of apoptosis induction via a p53-dependent pathway and hence, inhibiting colon cancer cell proliferation through direct targeting of signaling pathway of T-lymphokine-activated killer cell-originated protein kinase.<sup>47</sup> The Tumor, Node, Metastasis (TNM) classification of cancer and the International Union for Cancer Control as well as the Classification of Malignant Tumors provide the foundation for therapeutic

decisions. Colorectal cancer is categorised with reference to lymph node involvement (N stage), local invasion depth (T stage), and distant metastases (M stage).<sup>48</sup> The laterality of initial tumours is critical for a patient with synchronous metastatic colon cancer. Individuals with right-sided colon cancer had a considerably poorer overall survival rate than patients with left-sided colon cancer.<sup>49</sup>

## Biomarkers of Colorectal Cancer

Despite the accelerated progress of medicine, the prognosis of CRC patients is largely determined by the disease's stage at the time of diagnosis.<sup>50</sup> Considering the fact that early discovery of CRC reduces related morbidity and mortality and that detection of its precursor lesion can even reduce the occurrence, current CRC screening systems still have a number of flaws.<sup>51</sup> As a result, it is critical to develop novel non-invasive biomarkers that can detect tumour in the asymptomatic CRC early stages, when the disease is still treatable.

CRC patients, on the other hand, have been treated for decades with relatively reliable diagnostic methods like as colonoscopy and histological study of tumour tissue, as well as imaging modalities such as x-rays, computed tomography, and magnetic resonance imaging. Nevertheless, based on these investigations, clinical illness assessment and tumour stage classification are unable to suggest clinically predictive information and relevant prognostic at the patient level.<sup>52</sup> As a result, both clinical and basic scientists are continually searching for more precise individual biomarkers to reduce the severe side effects of chemotherapy, disease relapse, and treatment resistance, which would ultimately improve patients' longevity and quality of life.

The presence of tumour traces in the blood and other bodily fluids has been recognised as a possible individual biomarker with a diagnostic, prognostic, and predictive value. The ability of detection of so-called circulating tumour cells (CTCs) in the blood samples of patients has been proven in multiple investigations to have a deleterious impact on their survival.<sup>53–55</sup> The detection of CTCs from blood is comparable to that of conventional tumour tissue biopsy, hence, the term “liquid biopsy” has been introduced. Liquid biopsy, in contrast to tissue biopsy, has various advantages, including easy and quick procedure, reduced cost, and minimum risk and pain for patients due to its limited invasiveness.

It has recently been discovered that the existence of circulating tumour DNA (ctDNA) is just as important as the presence of CTCs.<sup>56</sup> Furthermore, the biological function of microRNAs (miRNAs) in CRC pathogenesis is becoming clearer, and their existence in peripheral blood has been proposed as a major predictive/prognostic biomarker.<sup>57</sup> As a result, the concept “liquid biopsy” has been widely known and is now utilised to detect all circulating tumour features, primarily ctDNA, CTCs, circulating miRNAs, as well as others such mRNAs, exosomes, proteins, and others) in peripheral blood or other biological fluids.<sup>58</sup>

In the context of multiple clinical investigations, the liquid biopsy/circulating biomarker concept is steadily nearing clinical practice. Colorectal cancer is typically sporadic, although having a family history of the disease increases your chances of getting it. Prevalent mutations in colorectal cancer includes KRAS, TP53, BRAF, transforming-growth factor-beta receptor 2 (TGFB2), catenin-beta 1 (CTNNB1), APC, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit-alpha (PIK3CA), ERBB2, SMAD4, AT-rich interactive domain 1 A (ARID1A), SRY (sex-determining region Y) box 9 (SOX9) and family with sequence resemblance 123B (FAM123B).<sup>59</sup> By interfering with essential signalling pathways, mutations in these genes cause tumorigenesis. The mutations of WNT signalling pathway are seen in the early stages of colorectal cancer, with TP53 mutations appearing later.<sup>60</sup> Current colorectal cancer biomarker research aims to target these molecules, as well as a few others, in order to improve cancer management. The gold standard procedure for colon inspection is colonoscopy, which is used for a successful screening programme. However, the discomfort during the examination, high cost and the examination recommended interval of 10 years are the main limitations of this technique. Non-invasive screening fecal tests including fecal immunochemical test (FIT), guaiac fecal occult blood test (gFOBT) and fecal DNA tests are able to detect blood in stool in high sensitivity manner.<sup>61</sup> The FIT method uses antibodies, whereas the gFOBT method uses chemicals; however, the faecal DNA test examines colorectal cancer-related DNA biomarkers in faeces from cells released from the colon and rectal lining.

## Need for Novel Oral Drug Delivery Systems

Colorectal polyps, an abnormal noncancerous growth in the inner lining of the intestine, are the most common cause of CRC. However, only about 10% of polyps have been seen to progress to aggressive malignancy. This change is very slow, taking more than 10 to 20 years to complete, and it is more likely as polyps become larger.<sup>62</sup> Later, malignant cells infiltrate muscle and lymph nodes prior migrating to other tissues, such as the lungs and liver. CRC is divided into five phases by the American Joint Committee on Cancer, where the treatment strategy is highly related to the corresponding stage.<sup>5</sup> Stage 0 is characterised by the presence of abnormal colon cells or polyps on the mucosa when found early enough, and it is completely curable following a surgical resection. In most cases, surgical excision is the conventional treatment for stages I–II, with a 5-year survival rate of 37–74%. Unfortunately, the survival rate in advanced stages of CRC drops to 6% due to the considerable risk of recurrence and metastasis to distant organs.<sup>6</sup> As a result, adjuvant pharmaceutical therapies such as chemotherapy are usually recommended in the advanced stages of CRC to lower the risk of recurrence and metastasis following surgical resection.<sup>7</sup>

In stages III–IV, adjuvant chemotherapy like oxaliplatin, 5-fluorouracil, cisplatin, doxorubicin and other medicines are used to suppress tumour growth and extend survival. Mere administration of these medicines, however, has not demonstrated the expected efficacy at these stages and had been linked to a number of dose-dependent adverse effects, such as vomiting, hair loss and nausea.<sup>8</sup> Chemotherapeutics are given in multi-cycles to reduce their adverse effects, while varying the doses, frequency, and duration of each cycle based on the individual's condition. Anticancer drugs also have a variety of drawbacks, including the hydrophobicity, low water solubility, inadequate biodistribution, and susceptibility to multiple drug resistance.<sup>9</sup> This has urged researchers to conduct innovative approaches such as using nanotechnology-based drug carrier system to enhance the physicochemical and pharmacodynamic properties of standard chemotherapeutics, or achieving target-specific delivery of cytotoxic agents to various types of tumour tissues to optimise treatment effectiveness while reducing off-target effects.<sup>10</sup>

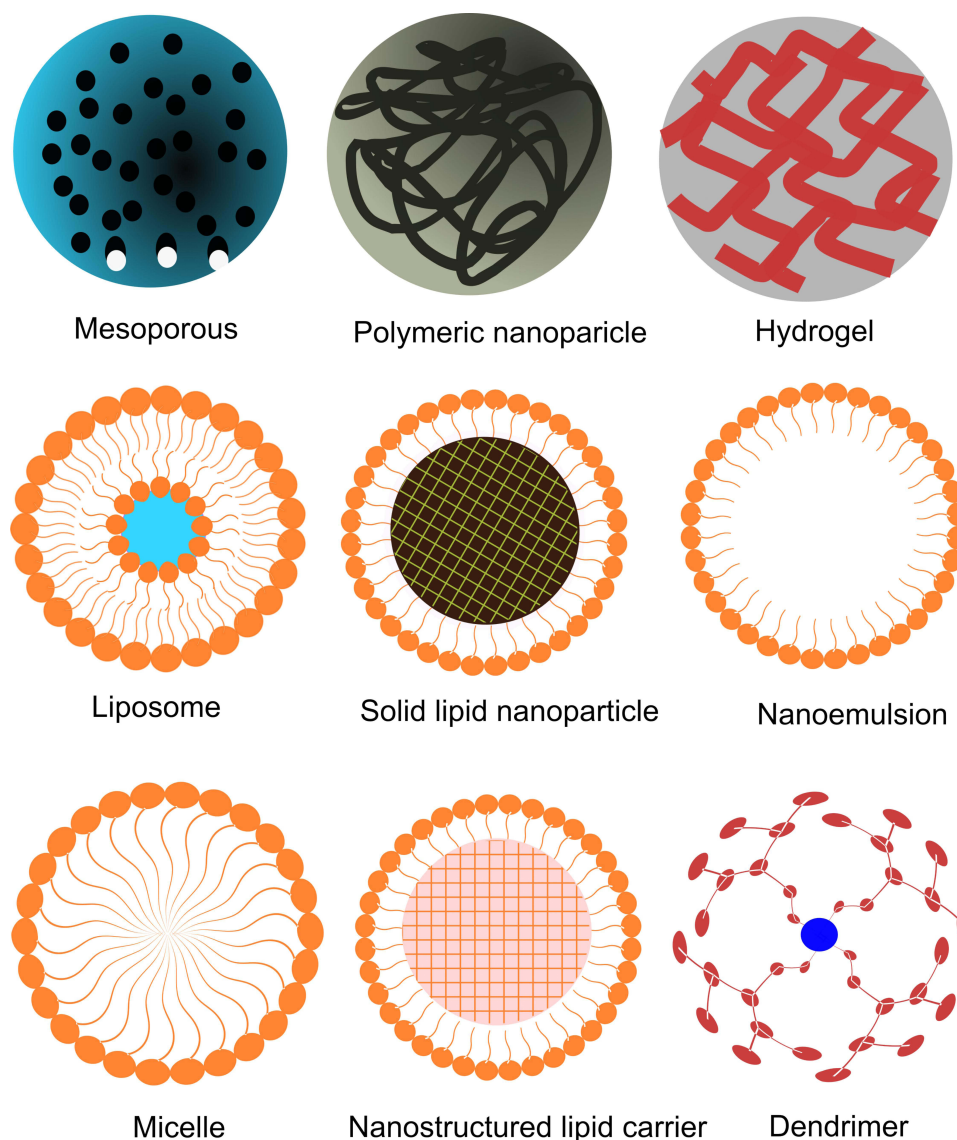
Nanoparticles are among the most promising treatment modalities for CRC and other cancers, as their properties are considered as a benefit in addressing the systemic drug delivery issues that diagnostics and anticancer drugs encounter. The surface area-to-volume ratio of nanoparticles varies between 1 and 100 nm, allowing for greater drug loading through encapsulation inside the core and adsorption on the surfaces of the nanocarrier. Nanoparticles can circulate for extended periods of time and have reduced clearance rates from the renal system due to their ultra-small size and superior drug loading and encapsulation efficacy.<sup>11</sup> Furthermore, since nanoparticles allow for continuous or controlled release, the physical confinement of diagnostics agents and anticancer drugs improves their pharmacokinetic properties while reducing the number of dosages administered. This controlled release can circumvent multiple drug resistance since the cells actively ingest bigger amounts of the drug intracellularly. Furthermore, the nanoparticles facilitates the anticancer mobility in the systemic circulation, increases cellular uptake and offers benefits over the bulk equivalent.<sup>63</sup>

Another advantage of using nanoparticles is the ability to customise their surface with targeting ligands (peptides or small-molecule ligands) to increase their selectivity towards the desired cells/tissues while reducing the availability of the chemotherapeutics at the normal tissues. Furthermore, amphiphilic lipid-based nanoparticles, such as micelles, possess a hydrophilic surface and a hydrophobic core and able to increase the solubility of hydrophobic drugs. In addition, quantum dots, nanoshells, nanosomes, and paramagnetic nanoparticles have structural properties that can be exploited for imaging and diagnostic purposes. Figure 1 illustrates different types of nanoparticles employed for colorectal cancer targeting.

## Emerging Oral Therapeutic Agents for Treating Colorectal Cancer (Synthetic & Natural)

The main treatment for stage 0 (the early stage) and stages I to III of non-metastatic CRC is radiotherapy with/without chemotherapy whereas chemotherapy is the option for stage IV (metastatic CRC (mCRC)). Several therapeutic agents were emerged as potential therapy for CRC with some gained regulatory approvals. They include synthetic and natural materials as small molecules or macromolecules. In this regard, we discuss the orally administered emerging therapeutic agents for CRC and divided them into synthetic and natural.





**Figure 1** Examples of nanoparticles used in the therapeutic management of colorectal cancer.

## Emerging Synthetic Therapeutic Agents

*KRAS* (Kirsten rat sarcoma virus) gene is a member in tyrosine kinase signaling pathways. It is one of the important identified oncogenes whereby downregulating these pathways becomes a target for tyrosine kinase inhibitors (TKIs).<sup>64</sup> Targeting growth factor receptors is one of the important mechanisms for the emerging therapeutic agents against CRC. These growth factors include platelet-derived growth factor receptors (PDGFRs), fibroblast growth factor receptors (FGFRs), vascular endothelial growth factor receptors (VEGFRs) and others.

### Tyrosine Kinase Inhibitors Targeting VEGF or KIT

Nintedanib is a TKI that inhibits some of Src tyrosine kinases and targets several growth factor receptors including PDGFRs  $\alpha$  and  $\beta$ , FGFRs 1–3 and VEGFRs 1–3.<sup>65</sup> Nintedanib is approved in Europe and the USA, with 150 mg twice daily as a standard adult dose.<sup>64</sup>

Regorafenib is a multi-TKI with activities against Ret, Kit, PDGFR, and Raf kinases. It also inhibits VEGFR 2 and VEGFR 3, which is responsible for its antiangiogenic activity.<sup>66</sup> Regorafenib gained FDA and EMA approval for the treatment of mCRC.<sup>64</sup>

Axitinib is an antiangiogenic agent inhibiting VEGF and PDGF. Axitinib is an effective agent against mCRC.<sup>66</sup>

Apatinib (rivoceranib) is a TKI and VEGFR 2 inhibitor showing antiangiogenic and antineoplastic effects. Apatinib decreases tumor microvessel density and inhibits VEGF-stimulated endothelial cell migration and proliferation.<sup>66</sup> In a study involved patients undergone at least two standard treatments, Apatinib 500 mg once daily was effective against refractory mCRC. However, the dose was then reduced to avoid adverse events.<sup>66</sup> In VEGFA-overexpressed tumors, Apatinib decreases the hyperangiogenesis and hypoxia inside the tumor microenvironment, converting it from an immunosuppressive into an immunostimulatory one.<sup>66</sup>

Famitinib is a TKI that inhibits VEGFRs 2 and 3. In a clinical trial, Famitinib increased the progression-free survival in patients with refractory mCRC compared to the placebo. It showed an acceptable safety profile but the overall survival did not increase.<sup>64</sup>

Surufatinib (Sulfatinib) is a TKI exhibiting activities on anti-angiogenesis and regulating cancer immunity. Surufatinib inhibits VEGFRs 1–3, FGFR1 and colony stimulating factor 1 receptor (CSF1R). Surufatinib safety and efficacy is being investigated in a clinical trial (NCT04653480) in second-line RAS/BRAF mutant and microsatellite stable CRC.<sup>64</sup>

Masitinib is a TKI with a specific KIT targeting, which is mutated or overexpressed in many cancers. Activated c-KIT receptor and tryptase have important role in the cancer tissue angiogenesis, which help the cancer cell in the invasion and metastasis.<sup>66</sup>

### BRAF Inhibitors

BRAF is an enzyme in the RAF/MEK/ERK signaling pathway. BRAF<sup>V600E</sup> mutation is upregulated in many human cancers. In CRC, around 15% of the patients have mutated BRAF. BRAF inhibition showed in success in BRAF-mutant metastatic melanoma, therefore it can be also employed in mCRC treatment.<sup>64</sup> Vemurafenib and Encorafenib are among the inhibitors of BRAF<sup>V600E</sup>-mutated kinase. Encorafenib showed modest antitumor activity in patients with refractory mCRC bearing BRAF<sup>V600E</sup> mutation.<sup>66</sup>

### MEK Inhibitors

MEK1 and MEK2 are dual-specificity Thr/Tyr kinases. Binimetinib is a mitogen-activated inhibitor of both enzymes while Cobimetinib is a specific inhibitor of MEK1. In vitro, Cobimetinib induces the apoptosis of HCT-116 CRC cells and inhibits cell proliferation.<sup>64</sup>

### STAT3 Inhibitors

In colon cancer and other cancers, a signal transducer and activator of transcription factor 3 (STAT3) is active and have a significant role in cancer progression. In a clinical trial Phase III, Napabucasin as an inhibitor of STAT3 increased the survival of patients with mCRC compared with placebo.<sup>66</sup>

### PDI/PDL-1 Inhibitors

Programmed cell death 1 (PD-1) receptor is found on the immune cell surface including T cells. PD-1 ligand (PDL-1) was detected in several cancers and when binds to PD-1, it inhibits the activation of T cells. Therefore, interfering with the PD-1/PDL1 pathway restores T cell function and this leads to an improvement of the immune response against cancer.<sup>66</sup> Combination therapy of capecitabine and oxaliplatin together with immune checkpoint blockers anti-PD-1 and anti-PDL1 was explored in murine syngeneic preclinical models that are similar to the tumor type in patients.<sup>66</sup>

### PDZ Domains Inhibitors

PDZ domains represents the most abundant protein–protein interaction domains of the human proteome. PDZ is composed of 266 different domains located in 150 proteins. PDZ aberrant expression was found in CRC and other cancers.<sup>64</sup>

By themselves, these domains do not have any enzymatic activity but they orchestrate several pathways. Therefore, inhibition of PDZ domains results in multiple enzyme inhibition simultaneously.

NHERF1 (Na<sup>+</sup>/H<sup>+</sup> exchanger 3 regulating factor 1) is a PDZ containing protein overexpressed in the cytoplasm and nuclei of CRC cells. Therefore, a new class of anticancer drugs can be utilized based on the inhibition of NHERF1.

Several small molecules were designed employing molecular and chemical approaches then evaluated as NHERF1 inhibitors.<sup>66</sup>

### Cancer Stem Cells

Cancer stem cells (CSCs) are resistant to the radiation and chemotherapeutic agents. They involve in the self-renewal of many cancers. Colorectal CSCs and other solid tumor stem cells have an insistent activation of several signal transduction pathways, whereby Wnt/ $\beta$ -catenin pathway plays an important role in stemness maintenance and drug resistance of colorectal CSCs.<sup>66</sup> CD44 is a well-documented surface marker involved in the proliferation of cancer cells. It has an important role in regulating CSC stemness properties. CD44 was found to regulate the in vitro and in vivo growth of colon cancer xenografts in animals.<sup>66</sup> Therefore, CD44 can be utilized in CRC treatment options.

The anti-diabetes drug Metformin downregulates CD44 in primary oral cancer cells.<sup>66</sup> Combined with doxorubicin, Metformin inhibits both cancer stem cells and non-stem cancer cells in culture. Therefore, a new use of Metformin may emerge in the anticancer treatment plans.<sup>64</sup>

The antibiotic Salinomycin inhibits CSCs in different human cancers. The mechanism is suggested to be by interfering with ABC drug transporters and CSC pathways including the Wnt/ $\beta$ -catenin signaling pathway. Salinomycin derivative with cyclopropylamine at position C20 inhibits HMLER CD24-low/CD44-high cells. With high selectivity against CSCs, Salinomycin and its derivative could have the potential to prevent cancer resistance.<sup>66</sup>

The COX2 inhibitor celecoxib is used as nonsteroidal anti-inflammatory drug. Synergistically, celecoxib and the Hsp90 inhibitor 17-allylamino-17-demethoxygeldanamycin, may eradicate CSCs with CD44 overexpression.<sup>66</sup>

$\gamma$ -secretase inhibitors like MK-0752 are being investigated in various CSCs as modulators of the Notch signaling pathway. MK-0752 and ridaforolimus (MK-8669) as a combination are being clinically investigated (phase 1 clinical trial) in patients suffering from advanced and refractory pancreatic and colorectal cancer.<sup>66</sup>

### Emerging Natural Therapeutic Agents

Natural products serve as a main source of new anticancer drugs. It is estimated that about half of the anticancer drugs currently in use are derived from natural products whether directly or not directly. Anticancer natural products fall into several categories based on their structure including alkaloids, polysaccharides, polyphenols, diterpenoid and unsaturated fatty acids.<sup>67</sup> The use of chemotherapy for the treatment of CRC is commonly associated with side effects and drug resistance. Therefore, there is a need to find new and more effective treatments for CRC. Several natural products exhibit anti-CRC effects.<sup>68</sup>

#### Alkaloids

Irinotecan (CPT-11) is a water-soluble camptothecin derivative. Its mechanism involves the inhibition of DNA topoisomerase I, blocking DNA replication and inhibiting RNA synthesis.<sup>67</sup> Irinotecan has a proven anti-cancer effect against mCRC. Irinotecan has been used for standard CRC treatment. In combination with 5-fluorouracil/formyltetrahydrofolate (CF), it has better anti-cancer efficacy against the advanced mCRC.<sup>68</sup>

SN38 is the active analogue of irinotecan. It inhibits the growth of several cancer cells.<sup>67</sup> Its mechanism involves anti-angiogenic activity due to the inhibition of VEGF and hypoxia-inducible factor 1  $\alpha$  expression. SN38 inhibits both the endothelial cell proliferation or lumen formation and the angiogenic activity of glioma cells.

Despite of the variable anti-tumor activities of CPT-11 and SN38, they both have significant toxicity including cholinergic syndrome, neutropenia and delayed diarrhea. More research is being performed to improve their efficacy and safety through chemical structure modification and combination chemotherapy.<sup>68</sup>

#### Polysaccharides

Fucoidan is a sulfated polysaccharide isolated from the seaweed *Sargassum hemiphyllum*. It has been shown to induce apoptosis in HT-29 and HCT-116 cells.<sup>69</sup> Double-blind, randomized clinical trials was conducted in Taiwan and demonstrated that low-molecular-weight fucoidan (0.8 KDa) has significant activity against CRC in humans in combination with chemotherapy, with relatively few side effects.<sup>67</sup>



## Polyphenols

Resveratrol is a polyphenolic compound with significant inhibitory effects against wide range of cancers. Resveratrol induces apoptosis in CRC by inducing Fas redistribution and inhibiting Wnt signaling.<sup>70</sup> The grape seed extract, a rich source of Resveratrol, was shown to inhibit PI3K pathway and induce apoptosis in CaCo-2 cells.<sup>71</sup>

Curcumin is a polyphenol isolated from *Curcuma longa* with multiple effects against malignancy. Curcumin exhibits anti-angiogenic properties, and increases the expression of the p53 and cip1/p21. It is able to induce the apoptosis of CRC.<sup>72</sup>

## Terpenoids

Andrographolide is the major bioactive component of *Andrographis paniculata*. This plant has been used as a traditional herbal medicine in Asia for thousands of years. Andrographolide exhibits anti-cancer effects due to its DNA-binding activity and inhibition of NF- $\kappa$ B activation.<sup>68</sup> The combination of andrographolide and 5-fluorouracil induces the apoptosis of HCT-116 cells as a result of andrographolide binding and upregulation of BAX protein expression. The safety and efficacy of the combination treatment of andrographolides and capecitabine was shown in elderly patients with locally advanced CRC.<sup>73</sup>

## Unsaturated Fatty Acids

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are polyunsaturated fatty acids (PUFAs) with significant inhibitory effect against angiogenic factors like platelet growth factor, VEGF, and endothelial cell growth factor. Therefore, they are effective against CRC, and other cancers.<sup>74</sup> DHA and EPA as free acids or in the form of phospholipids inhibits the growth of CRC cells, and the studies have shown the strong growth-inhibitory effects of PUFAs in the HT-29 cell line more than in the Caco-2 and DLD-1 cell lines.<sup>68,75</sup>

## Oral Route of Drug Delivery

Oral drug delivery has been recognised as the most attractive approach of drug administration among various others and can be utilised for systemic or local drug delivery as well as enhance the drug solubility and dissolution of low solubility drugs.<sup>17</sup> It is the most preferred way by patients, ease of administration, convenience for self-administration, noninvasive, and feasibility for the formulations.<sup>19,20</sup> Furthermore, a high surface area ( $>300\text{ m}^2$ ) of gastrointestinal track coated with a viscous mucosal layer facilitates drug adhesion and absorption.<sup>18</sup> Nevertheless, oral drug delivery can be challenging due to the complexity of GIT, even among healthy people, which poses some obstacles that impact delivery such as poor drug solubility, stability, and permeability.<sup>17</sup> Drugs that are administered through the oral route have a slower absorption as they must be solubilised in the gastric fluid to be absorbed.<sup>20</sup> The main factors affecting GIT absorption of the drug is drug physicochemical characteristics (polymorphism, solubility, partition coefficient, and pH-pKa), biological factors (blood flow at absorption site, mucus, absorption surface area, ionic concentration, enzymes, and microbiome) as well as maintenance of sync condition (drug concentration at the absorption site).<sup>76</sup> Moreover, oral drugs may also have an unpleasant taste, induce gastric irritation, and/or be subjected to intestinal and hepatic barriers.<sup>19,20</sup>

The rectal route of administration is an alternative route of drug delivery that has some disadvantages in comparison to the oral route. The low surface area and rectal fluids can lead to irregular or incomplete absorption of the drug. Table 1 shows a comparison between characteristics of different parts of GIT.<sup>18</sup> Different segments of GIT vary anatomically and physiologically and impact the extent of dissolution and absorption of the drugs. Hence, the basic characteristics of the GIT should be considered during the formulation design of oral drug delivery system.

## Factors Influencing Gastrointestinal Tract

Regardless of absorption mode, each digested substance will encounter three major biological environments along with the gastrointestinal tract including the lumen (internal space), mucus, and tissue.

### Lumen

#### pH

The pH is the first biological barrier to any orally delivered drug, which denature/depurination most of the administered drugs and substantially reduces their effectiveness. The pH of GIT varies based on GIT part and fed state to unfed state. The pH inside the gastric environment is extremely acidic (pH 1–2) in unfed state and it could rise to pH 5 in fed state.

**Table 1** Characteristics of Different Parts of the GIT

Segment	Luminal pH	Length (cm)	Mean Diameter (cm)	Transit Time (Hour)	Mucus Average Thickness ( $\mu\text{m}$ )	Mucus Turnover (Hour)
Stomach	0.8–5	20	NA	0–6 (0–2 fast) (2–6 fed)	245 $\pm$ 200	24–48
Duodenum	~7	17–56	4	2–6	15.5	
Jejunum	7.4	280–1000	2–2.5			24–48
Ileum			3			
Colon	7–8	80–313	4–4.8	6–70	135 $\pm$ 25	~48

The pH increases rapidly in duodenum (pH 6) and rises along the small intestine to reach pH 7.4 at the terminal ileum.<sup>18</sup> Cecum pH decreases just below pH 6 and again increases in the colon and reaches pH 6.7 at the rectum.<sup>77</sup> Nevertheless, the pH ranges can vary with factors like dietary intake, microbial metabolism, and diurnal cycle.<sup>78</sup> gastrointestinal pH changes affect the extent of drug molecules ionisation, subsequently impact drug absorption.<sup>79,80</sup>

### Enzyme

Enzymatic degradation of dosage forms and drugs can occur throughout the GIT and hence can affect their stability. The major site of the enzymes that are involved in food digestion is mouth (salivary amylase), stomach (pepsin).<sup>81</sup> Besides that, pancreatic enzymes secreted into the intestinal lumen including trypsin, amylase, lipase, and peptidases. Those pancreatic enzymes are not considered as the main challenge, although they are considered as a biological barrier. These enzymes are especially abundant in duodenum, and their concentrations are reduced significantly in the later intestinal parts. duodenum transit time is relatively short (10% of the whole small intestine residence time) and is not enough for the enzymatic degradation of the drugs. Duodenal pH is lower compared to the later small intestine parts, and thence the unwanted drug release inside the duodenum can be prevented via increasing delivery carriers pKa.

### Microbiome

The intestinal microbiome has over 500 bacterial species,<sup>82</sup> and they are important for digestion and metabolism of the ingested food as well as for intestinal health.<sup>83</sup> The colon contains the bulk of the intestinal microbiome and they ferment carbohydrates as the main source of nutrition.<sup>83</sup> The composition of the microbiome varies between individuals based on genetic and environmental factors.<sup>84</sup> Nevertheless, the dominant species appear to be consistent and represent the majority of the colonic flora.<sup>82</sup> These bacteria secrete many enzymes such as azoreductase,  $\alpha$ -arabinosidase,  $\beta$ -galactosidase,  $\beta$ -glucuronidase,  $\beta$ -xylosidase, deaminase, nitroreductase, urea hydroxylase.<sup>85</sup> These enzymes are exploited for CTDD.<sup>86</sup>

The density of the microbiome inside the small intestinal is lower compared to that of the large intestine. This is due to the intestinal fluid volume, rapid luminal flow, and bactericidal compounds secretion in this part of the GIT.<sup>87</sup> Small intestine microbiome composition can highly fluctuate over a short period as it is affected by dietary intake variations.<sup>88</sup> The colonic microbiome is claimed to play a crucial role in the regulation of metabolic function.<sup>87</sup> Small intestinal microbiota influences oral drug absorption have not yet been elucidated.

### Mechanical Degradation

Mechanical degradation inside the lumen including the peristalsis of the GIT muscles, the shear stresses by gastric juice flow rate within the lumen as well as the osmolarity along the GIT are factors that reduce the drug efficiency. Flowing gastric juice could reduce the contact time between the epithelial layer and drugs, hence impeding their absorption. Biologics and microencapsulation are the major elements sensitive to mechanical destruction through mechanical stresses.

### Transit Time

Gastrointestinal transit time is the key factor for drugs that have region-specific targeting or absorption characteristics. Gastric transit time varies from several minutes to hours, depending on several factors, such as age, gender, body posture, osmotic pressure, dietary intake, physical activity, etc.<sup>89</sup> The transit time in gastric can vary from 0 to 6 h based on fast/fed state,<sup>81</sup> while small intestine transit time is considered constant at approximately 4 h.<sup>90</sup> On the other hand, colon transit time is extremely variable from 6 to 70 h.<sup>91</sup> Gender is highly affecting the colonic transit time and females have significantly longer colonic transit times compared to males.<sup>92</sup>

### Mucus

Mucus is the second barrier for effective oral drug delivery. Mucus facilitates the digested moiety passage and protects the underlying epithelium from mechanical stress and pathogens by capturing any foreign moieties (especially hydrophobic molecules). Although mouth and oesophagus lack a distinct mucus layer, they are washed by salivary gland mucus.<sup>93</sup> The small intestine has a loose and unattached mucosal layer, while the stomach and colon have a two-layered mucus system composed of an outer loosely adherent layer and an inner firmly adherent layer.<sup>93</sup> It is claimed that mucus is the primary barrier against oral drug bioavailability due to the difficulty to diffuse into the mucus layer. While the inner mucus layer facilitates the absorption and improves the drug uptake efficiency, justifying the dual role of mucus in the absorption/desorption of oral administered drugs. Goblet cells are continuously secreted mucus; consequently, it is cleared in each part of the GIT (50–270 min).<sup>20</sup> This defines the final mucus layer thickness (Table 1).

### Fluid Volume

Controlling fluidity is crucial for gastrointestinal function.<sup>81</sup> The fluid environment allows digestive enzymes to contact with food, and this aids in transit the intestinal contents along the GIT without damaging the epithelial lining and it facilitates the nutrients and drugs solubility and absorption.<sup>81</sup> The daily water balance in a healthy adult human GIT is determined by the secretion from saliva (1.5 L), gastric juice (2.5 L), pancreatic, and other intestinal components (~2.5 L), and absorption from the small and large intestine (7 L and 1.9 L, respectively).<sup>81</sup> The pH is influenced by fluid-to-matter ratios, which can potentially alter drug absorption and delivery, especially in lower GIT. For instance, diet can have a big impact on the GIT free fluid volume, bile salts, and digestive enzyme levels.<sup>81</sup> Moreover, intestinal fluid secretion affects the viscosity of the mucous-gel layer,<sup>81,93</sup> which can alter the drug's cellular uptake at the site of action. Increased fluid secretion and reduced fluid reabsorption dilute the digestive enzymes and affect the intestinal microbiota which in turn alter the digestion of carbohydrates<sup>94</sup> and contribute to changes in intestinal transit time.<sup>95</sup> Variations in intestinal fluid volume can impact the way drugs are processed in the GIT.

### Tissue

The drug molecule properties determine where it will be absorbed and how it will cross the intestinal epithelial cells. Drugs may be absorbed in four ways: transcellular (transcytosis of the drug by crossing epithelial cells membrane), paracellular (permits only small hydrophilic molecules diffusion through epithelial cell spaces), facilitated transport, and carrier-mediated transcellular.<sup>96</sup> Tight junctions, that affect the paracellular absorption pathway for orally delivered drugs, are the primary extracellular biological barrier against oral administration.<sup>97</sup>

The size and chemistry of the drug affect their transport through tissue. Generally, hydrophilic small drugs prefer paracellular routes, while hydrophobic drug molecules prefer to be absorbed via transcellular routes.<sup>98,99</sup> Passive drug diffusion via intercellular pathways is only conceivable for substances as small as 0.5–3 nm, which is too small for most drug molecules to be delivered.<sup>100,101</sup>

## Approaches for Oral Colon Delivery in Colorectal Cancer

Numerous studies demonstrated that the colon is a suitable site for drugs absorption as it has less reactive enzymes against drugs, CTDD protects the drug from hydrolysis by enzymes and decreases their degradation in the duodenum and jejunum, subsequently greater drug systemic bioavailability<sup>23</sup> and prolonged colonic residence time of the drug is accompanied by the synergistic effect of absorption enhancers on the drug absorption.<sup>102</sup> The goal of developing oral CTDD systems is to avoid or limit the release of drugs in the upper GIT while allowing explicit drug release in the colorectal area for local and/or systemic

delivery. To accomplish this, the physiological parameters that affect colonic drug delivery must be taken into consideration. CTDD can be characterised as either prodrug, pH-dependent systems, time-dependent systems, or ligand/receptor-mediated systems. Each of these formulation methods has its own set of benefits and drawbacks. A combination of two or more formulation systems has been examined to overcome the limits of a single system and to maximise CTDD.

Nanotechnology has significantly advanced current drug formulations for improved absorption and site-specificity. The use of nanosystems facilitates the delivery of active compounds to cancer tissues in the colon. The usage of nanoparticles in drug formulations has a lower dose requirement compared to the amount needed for conventional dosing. Nanoparticles can be tailored with various charges to treat colonic diseases. Some examples of known polycation nanoparticles can interact with inflamed tissues of the intestine, while polyanion nanoparticles interact with cationic components (cytokines) in colorectal cancer. Nanoparticles are also prone to be uptaken by macrophages at the inflamed areas of the colon, thus allowing the drug to remain in the colon for a longer time.

Moreover, nanometre-scale sizes of nanoparticles contribute to their efficacy as drug delivery systems. Nanosized particles possess increased epithelial permeability and retention, that promotes selective delivery into inflamed tissues,<sup>103</sup> prevents removal through diarrhoea,<sup>104</sup> and enhances uptake by immune cells accumulated in the inflamed colon portions. Bioadhesion is the attachment of anionic nanoparticles to inflamed tissues by electrostatic interactions with positively charged proteins like eosinophil and transferrin.<sup>105</sup> Cationic nanocarriers are used in the nanoparticles designed as a mucoadhesive system, which is attached to the anionic intestinal mucosa.<sup>106</sup> This cationic delivery system can be used in GIT targeting as it enhances the drug cellular uptake into the mucosal surface. pH-dependent nanocarriers have been also used for CTDD. The ideal polymer should withstand gastric acidic pH and proximal small intestine while being dissolved in pH of the terminal ileum and colon. Thus, pH-dependent drug delivery systems with dissolution threshold of pH 6.0–7.0 are expected to delay drug dissolution and prevent unwanted drug release in the upper GIT before reaching the colon.<sup>107</sup> This makes the delayed-release profile of the drug formulation.

Nanoparticles with different polymers that are capable of entrapping drugs into their matrix until further release in the colon, have been designed. Polymeric nanoparticles selectively release drugs through the enzymatic activity of colonic bacteria or by manipulating colon transit time for time-dependent release nanoparticles.

Oral drug administration in the form of a prodrug can enhance its bioavailability and prevent early degradation in the upper GIT. Colon-targeted prodrug is the inactive derivative of a drug molecule that becomes an active ingredient upon enzymatic hydrolysis in the colon. One strategy is disulfide bond conjugates formation between the prodrug and the carrier. The disulfide bond is stable in the upper GIT and can be disintegrated via extracellular bacterial reduction process or intracellular enzymes. This results in the carrier breakage and prodrug release into the colon.

For a more successful local therapy of colorectal cancer with less toxic side effects, ligand/receptor-mediated drug delivery systems can improve target specificity through the interaction of specific receptors expressed at disease locations and the targeted ligands on the carrier surface have been investigated. It can be also combined with other delivery strategies to improve its GIT stability and site-specificity. Folic acid is a receptor-selective targeting ligand that targets folic acid receptors overexpressed in many types of tumours including colorectal cancer.<sup>108</sup> Folate receptor functionalised nanoparticles can enhance selective drug uptake into cancer cells. Folic acid functionalised liposomes enhanced the cytotoxicity of daunorubicin via facilitating folate receptor-mediated drug uptake.<sup>109</sup> 5-fluorouracil loaded Folic acid conjugated liposomes enhance the selective drug delivery to cancer cells as it showed higher anti-cancer activity and significantly decrease the volume of the tumour when compared to free 5-fluorouracil.<sup>110</sup> Hyaluronic acid is a polysaccharide that has a high affinity for CD44 receptors, which is overexpressed in many tumours including colorectal cancer.<sup>111</sup> Previous studies have examined the effectiveness of hyaluronic acid nanoparticles as CTDD for colorectal cancer.<sup>112,113</sup> Hyaluronic acid-functionalised camptothecin/curcumin-loaded polymeric nanoparticles (HA-CPT/CUR-NPs) showed significant targeting capability towards Colon-26 cancer cells.<sup>113</sup> Peptide is gaining popularity as a possible ligand for targeted drug delivery. Peptides have several pros, such as simplicity, low cost, chemical variety, biocompatibility, and stimulus responsiveness.<sup>114,115</sup> Moreover, peptide ligands show higher binding affinity and specificity with receptors compared to small molecule ligands.<sup>108,116</sup> In addition, the metabolic instability caused by proteases can be addressed by peptide sequence alteration, encouraging the use of peptide ligands in targeted drug delivery systems. Peptide functionalised nanoparticles are mainly being investigated as a viable strategy for tumour-

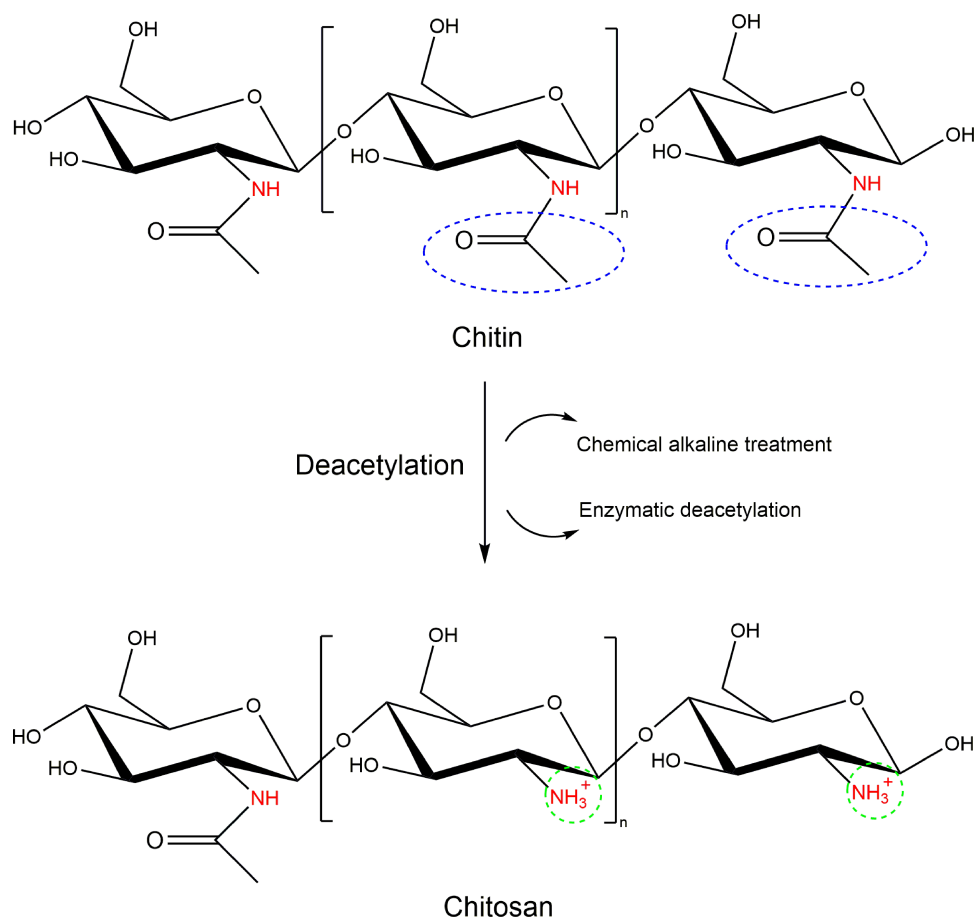
targeted drug delivery. For instance, TWYKIAFQRNRK, TK peptide, has been investigated for CTDD of anticancer drugs. TK peptide has a high affinity to  $\alpha\beta1$  integrin, that is upregulated in colorectal cancer. Hence, TK peptide-functionalised PEG-PLA micelles loaded with doxorubicin showed significantly stronger toxicity and more effectively penetrated the tumour spheroids, indicating TK peptide as a potential targeting ligand for CTDD.<sup>117</sup>

### Polysaccharide as CTDD

Natural polysaccharides like pectin, chitosan, xylan, and guar gum have been used in CTDD systems, as they can remain intact in the upper GIT, but are degraded by microflora to release the drug.<sup>118</sup> Moreover, polysaccharide structural modifications or derivatives can enhance drug release behaviour, site-specificity, and stability.<sup>119</sup> Polysaccharide mucoadhesiveness may be beneficial for drug absorption through extended contact between drug delivery carriers and the mucosal surface. Polysaccharide-based drug delivery systems provide additional advantages such as low toxicity, low cost, immunogenicity, biodegradability, and biocompatibility.<sup>120</sup> As a result, the microbiota-triggered polysaccharide-based system offers a viable approach for CTDD. Nevertheless, polysaccharide-based delivery systems have certain potential limitations, such as variable polysaccharide chemistry and a wide range of molecular weights.<sup>120</sup> Moreover, the rate of drug release is affected by the type and concentration of polysaccharides.

### Chitosan and Its Physicochemical Properties

Chitosan is a linear polysaccharide, comprises of randomly repeated units of N-acetyl-D-glucosamine and D-glucosamine linked by  $\beta(1\rightarrow4)$  glycosidic bonds.<sup>121</sup> Chitosan is the most valuable derivative of chitin, the second most abundant naturally occurring polymer following cellulose.<sup>122</sup> Chitin can be found in the exoskeleton of insects and crustaceans, in addition to the cell walls of certain fungi.<sup>123</sup> Chitosan, the partially deacetylated form of chitin, can be obtained by chitin deacetylase enzyme or chemical alkaline treatment using concentrating NaOH as shown in (Figure 2).<sup>124,125</sup> Chitin polymer can be called chitosan when the degree



**Figure 2** Deacetylation of chitin into chitosan.



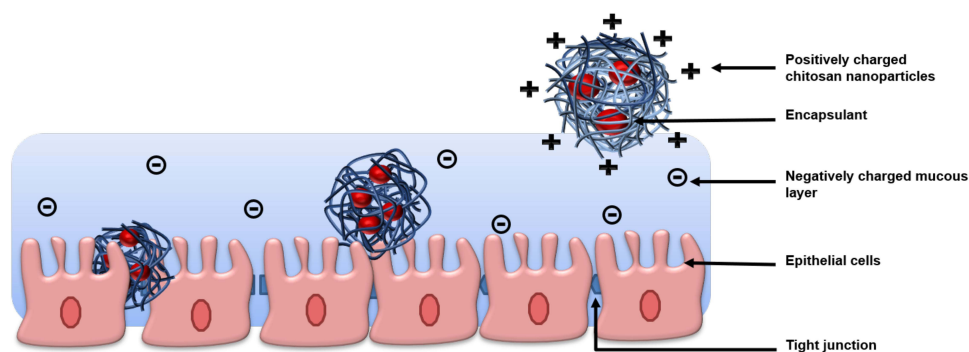
of deacetylation (DD) exceeds 50%.<sup>126</sup> Both chitin and chitosan have attracted tremendous attention in various fields, such as food technology,<sup>31</sup> textile industry,<sup>127</sup> and diverse medical and pharmaceutical applications including wound healing,<sup>32</sup> tissue engineering<sup>33</sup> and gene delivery.<sup>34</sup>

Unlike chitin, chitosan is readily soluble at pH below its pKa ( $pK_a \approx 6.5$ ) ascribing to the protonation of its amino group ( $-NH_2$ ) at C-2 in each repeating D-glucosamine unit turning the chitosan into a cationic polymer.<sup>30</sup> Besides this cationic nature that is responsible for chitosan permeation enhancing and mucoadhesion effects, being also biodegradable, biocompatible and non-toxic enable chitosan to be a polymer of choice for various drug delivery applications.<sup>29</sup>

Chitosan has become of great interest to be formulated into CSNPs. CSNPs are considered a very promising strategy to overcome the low stability and bioavailability of many active ingredients by combining the favourable natural characteristics of both the polymer itself and nanotechnology. Chitosan can efficiently bind to the negatively charged mucus membrane, increase the retention time and enhance the probability of their cellular uptake. Moreover, chitosan is known for transiently opening the tight junction between epithelial cells, which allows the permeation of the hydrophilic loaded drug.<sup>128</sup>

## Interaction Between Chitosan and Colon Mucosa

Mucus sticks to most particles, it is as a tenacious barrier prevents their penetration to the epithelial surface. Mucus layer is continuously secreted and degraded or digested, thus rightly called dynamic.<sup>129</sup> Mucins are consisted of highly glycosylated proteins ( $>106$  Da) that are the main components of mucus covering epithelia. They are involved with several functions such as forming a viscous matrix that controls the adhesion of the mucosal layer to the cells as well as drug targeting and pathogens penetration.<sup>129,130</sup> Thus, the development of novel systems for transmucosal drug delivery requires the conceptualization of targeting strategy. Chitosan interacts with mucins as well as it absorbs and changes the rheology of mucus. The interaction between mucins and chitosan depends mainly on electrostatic attraction, hydrophobic interactions and hydrogen bonding that determines the fate and behaviour of chitosan-based drug delivery systems. Thus, CSNPs could go through the mucus and reach the cell surface or be shed with the mucus. Several intrinsic factors have effects on the interaction between chitosan nanocarriers and mucins specially that related to the nature of the polymer such as the molecular weight and surface charge of CSNPs.<sup>131</sup> The value of pH of the media is one of the environment factors, the  $pK_0$  of chitosan is around 6.5, thus the amine group will be protonated at pH lower than the  $pK_0$ .<sup>132</sup> On the other hand, mucins are glycoproteins with pI between 2 and 3, therefore they will be charged negatively in medium pH  $>$  pI, promoting electrostatic interaction between mucins and chitosan especially at pH between 2.4 and 6.3.<sup>131</sup> Moreover, the negative charge of the sialic acid, sulfate groups on the oligosaccharide chains of mucins and surrounding anions aid gel formation as well as they enhance the association in the mucus layer and subsequent internalization via endocytosis of CSNPs.<sup>133</sup> The positive surface charge of CSNPs may improve also the drug penetration by opening tight junctions of epithelial cell layers as shown in (Figure 3).<sup>134</sup> In fact, the interaction between mucins and chitosan is still difficult to understand and predict. It's worth noting that some studies have found that interacting chitosan with mucins in vivo changes the biopolymer attachment to mucosal surfaces.<sup>135,136</sup>



**Figure 3** Schematic illustration of the interaction between chitosan loaded nanoparticles with the mucus layer.

## Nanotechnology in Drug Targeted Delivery System

Any drug delivery system can carry therapeutic agents, only if it was able to reach the site of action from the site of administration crossing all the biological barriers. Besides, most of the active pharmaceutical ingredients (API) are often likely to exhibit limited bioavailability, poor water solubility and inadvertent serious side effects.<sup>137</sup> Therefore, it is a major challenge to deliver therapeutic agents to specific organ or tissue for the treatment of certain disease. In order to overcome those drawbacks, nanotechnology has been widely embraced to develop effective targeting drug loading systems.<sup>138</sup> Table 2 summarizes general advantages and potential limitation of nanotechnology in drug delivery.<sup>139</sup>

Equipping nanotechnology with existing cancer therapies to enhance drug efficiency and limit its systematic toxicity, known as cancer nanotechnology.<sup>140</sup> Nanocarriers for cancer treatment are aimed to deliver the loaded-molecules to and around the targeted pathologic tissue, killing cancer cells without harming the normal ones.<sup>141</sup> Compared to micro-particles, nanoparticles have larger surface area with tremendous modifiable options enabling the delivery of the anti-tumour drug to the targeted location with less probability of opsonisation. Additionally, nanoparticles facilitate the drug diffusion and accumulation in tumour cell, through enhanced permeability and retention (EPR) effect.<sup>142</sup> Various types of materials have been used to fabricate nanoparticles for colorectal cancer treatment including liposomal,<sup>143</sup> polymeric, inorganic,<sup>144</sup> mesoporous silica nanoparticles.<sup>145</sup> Among the forenamed nanoparticles, polymeric nanoparticles have numerous advantages due to their ease of synthesis and modification, ability of loading and protecting the loaded-molecules from degradation by surrounding environment, high in vivo and in vitro stability profiles and their potential use for targeted controlled releases.<sup>146</sup> Chitosan based nanoparticles stand out among polymeric nanoparticles by being efficient, readily available, environment friendly, non-immunogenic, biocompatible and biodegradable nanoparticles.<sup>147</sup> It has been reported that chitosan demonstrates an anticancer activity through antioxidant defence mechanism, cellular enzymatic, immunoenhancing, cellular enzymatic, antiangiogenic and apoptotic pathways.<sup>40</sup> Furthermore, CSNPs permeate through cancer cell membranes selectively, enhance the drug potency and bioavailability in the cancer cells while being non-toxic to normal cells.<sup>40</sup> Nanoparticles can target the cancer cells by two distinguished approaches, namely, passive and active targeting approaches (Table 3). CSNPs can combine both approaches, the passive targeting through EPR effect, and the active targeting through ligand conjugations.<sup>148</sup>

**Table 2** Advantages and Potential Limitations of Nanotechnology in Drug Delivery

Advantages	Limitations
Encapsulating of hydrophilic or hydrophobic drugs	Poor drug loading
Drug release control	Burst or rapid release before reaching site of action
Protecting the loaded-drugs from degradation	Probability of clearance through kidney filtration due to its nanosize
Enhancing drug absorption and permeation	Formulation designing difficulty

**Table 3** Main Differences Between Passive and Active Targeting

Passive Targeting	Active Targeting
Targeting is possible because of the unique changes in the cancer environment of incomplete vasculature	Targeting depend on the overexpressed species in the tumour, and the ligand conjugated on nanocarrier.
Less selective	Highly selective
Restricted in use	Very versatile
More likely to cause side effects	Less likely to cause side effects

## Passive Targeting

Passive targeting is mainly associated with EPR effect, where the substrates get accumulated at the target site due to the incomplete lymphatic system and newly formed leaky vasculature surrounding tumour stroma.<sup>149</sup> Passive targeting approach relies on the physicochemical characteristics of the formulated nanocarrier including particle size (PS), surface charge, morphology, circulating time, and surface chemistry as well as the tumour biology characteristic such as leakiness and vascularity.<sup>148</sup> Without any specific ligand targeting, nanoparticles can extravasate from leaky tumour vessels, reaching the cancer cells and can increase the concentration of the drugs acting on these cells for days or even weeks as a result of the sluggish lymphatic drainage.<sup>150</sup> The PS has a notable influence on the drug extravasation, since the vascular cut-off pore sizes in cancer and healthy cells are different. While only particles smaller than 2 nm are permeable through normal cells, the pores size of most tumours are from 10 to 1000 nm.<sup>151</sup> For efficient permeation of nanoparticles through the fenestrations of the endothelial tissue, the PS should be less than 400 nm.<sup>152</sup> The physicochemical properties of the polymeric nanoparticles can be easily modified by altering the formulation composition or by the synthetic method. In case of CSNPs, they accumulate in the low-pH tumour microenvironment, which leads to protonating of chitosan, swelling of the nanoparticle and releasing of the drug in the targeted cancer cells.<sup>40</sup>

## Active Targeting

In order to improve nanoparticles selective accumulation in the tumour tissue, active targeting was developed as a complementary strategy to the passive EPR-based targeting approach.<sup>149</sup> In active targeting, high affinity targeting molecules with innate ability can be conjugated on the surface of nanodelivery systems in order to attach to specific receptor expressed at the target site.<sup>153</sup> Several types of ligand have been utilized including protein, monoclonal antibodies, folic acid, aptamers, small molecules, nucleic acid and polysaccharides.<sup>154</sup> Tumour targeting ligands on the nanocarriers can specifically bind to tumour cells that express unique biomarkers distinguishing them from the surrounding normal healthy cells.<sup>155</sup> The binding between targeting molecules and receptors takes place via endosome-depending mechanism, in which cancer cells can be directly killed.<sup>156</sup> Table 4 demonstrates both active targeting (targeting molecule and its targeted receptor), the effect of passive targeting (EPR effect) which can be seen from the physicochemical characteristics of the prepared nanoparticles, and their impact on the colon cancer cell lines.

## The Role of Chitosan and Its Derivatives for Colorectal Cancer Targeted Drug Delivery

Chitosan polymer has received immense attention in developing nanocarriers as drug delivery systems for anticancer agents.<sup>157</sup> In terms of colorectal cancer targeting, chitosan can form complexes with anionic drugs, reduce premature release of entrapped drugs at the upper GIT and release the encapsulant at colon part of body due to its biodegradability via glycosidic linkage lysis by means of the specified enzyme of the colonic microflora.<sup>35</sup> Drug release from chitosan matrix is related to matrix swelling behaviour and chitosan-drug interaction that are affected by the chitosan molecular weight and the pH of the medium. Also, drug release is still affected by its solubility and its molecular weight, as well as the release profile is influenced by polymer-drug ratio. Drug release from CSNPs can occur via different mechanisms: diffusion through the swollen matrix, release from the surface of nanoparticles and release due to polymer erosion. However, in most of the conditions, the release occurs as a result of the combination of than one mechanism.<sup>14,158</sup> Nevertheless, pure chitosan's utility and applications are limited due to its insolubility in water and also most organic solvents.<sup>159</sup> Different functional groups such as -NH<sub>2</sub> and -OH are reactive groups on the chitosan molecule that are involved in chemical modification. Modified chitosan derivatives have displayed superior inherent properties such as bioactivity, biocompatibility, bioactivity and non-toxicity as well as several bioactive effects like antibacterial, antiviral, anticancer effect.<sup>39,40</sup> The functional groups C2-NH<sub>2</sub> and C6-OH readily involve in chemical modifications in order to introduce new chemical moieties. These chitosan modifications also facilitate in drug delivery to the colon, where therapeutic agents need to be protected from acidic pH of the stomach and enzymes degradation with minimal drug lose until they reach the colon.<sup>41,42</sup> Chitosan has three different types of reactive functional groups: a primary hydroxyl group at the C-6, an amino group at the C-6 and a secondary hydroxyl group at the C-3. The most common method for

**Table 4** Chitosan-Based Nanoparticles Studies of Colon Targeted Drug Delivery System

Formulation	Active Compound	Nanoparticles Characteristics	Active Targeting Molecule	Targeted Receptor	Cell Line	Outcome	Ref
Chitosan modified citrus pectinate nanoparticles	Curcumin	PS= $178 \pm 0.896$ nm, ZP= $+35.7 \pm 1.41$ mV	Galactose moiety of pectin	Galectin 3 (Gal-3) protein	-	Displayed pH-responsive release, presented mucoadhesive property at pH representing the colon region	[199]
Cetuximab-conjugated chitosan modified citrus pectinate composite nanoparticles	Curcumin	PS= $249.33 \pm 5.15$ nm, ZP= $+39.1 \pm 0.87$ mV	Cetuximab, a monoclonal antibody	Epidermal growth factor receptor	Caco-2, HCT116	Enhanced internalization and cellular uptake, enhanced apoptosis by increasing cycle arrest in the G2/M phase	[200]
Imatinib-conjugated CSNPs (IMT-NPs)	Imatinib	PS= $208 \pm 0.01$ nm, ZP= $-32.56 \pm 0.03$ mV	Imatinib	-	CT-26	Wide spread of IMT-NPs within epithelial tissues of colon after 1 h, high accumulation of chitosan in tumour cells improving EPR, enhanced anti-cancer effect by increasing the demotion effect of p-glycoprotein leading to S phase arrest in cancer cells.	[201]
Mesoporous silica nanoparticle based galactosylated chitosans (5-FU@MSN-NH <sub>2</sub> /GC)	5-fluorouracil	PS= $511.57 \pm 9.69$ nm, PDI < 0.2, ZP= $29.9 \pm 0.8$ mV	Galactose-moieties of galactosylated chitosans	Galectin receptor	SW-620	Enhanced anti-cancer activity of (5-FU@MSN-NH <sub>2</sub> /GC) through cell apoptosis and higher cytotoxic activity against SW620 cell line. Specifically recognize and bind to galectin-receptor on cancer cells	[202]
Photothermal (IR780) and photodynamic 5-Aminolevulinic acid loaded CSNPs	5-Aminolevulinic acid (5-ALA), IR780	PS= 180 nm	-	-	CT-26	Improved nanoparticles local accumulation in colon tumour cells (CT-26), Elevated oxidative stress, including the increasing the production of ROS, superoxide and $^{1}O_2$ by the photothermally enhanced photodynamic effects against CT-26.	[203]
CSNPs	Gallic acid-quercetin	Size= 218.33 nm ZP= $+11.50$ mV	-	-	HCT-116	A higher change in aberrant crypt foci in CSNPs than polyherbal extract that were observed, with a significant decrease in the colonic catalase, glutathione and superoxide dismutase levels.	[36]
Chitosan mesoporous	Zinc nitrate hexahydrate	PS= 20–40 nm	-	-	DLD-1	The NPs stimulated the apoptotic process, moreover, they induced in vitro DNA fragmentation in DLD-1 cell line with significantly different affinity by 20% versus 52% reduction by Cisplatin. The NPs (IC <sub>50</sub> ) showed anti-invasive activity and dramatically inhibited the migration of DLD-1 cell line.	[204]

(Continued)

**Table 4** (Continued).

Formulation	Active Compound	Nanoparticles Characteristics	Active Targeting Molecule	Targeted Receptor	Cell Line	Outcome	Ref
Chitosan-coated solid lipid nanoparticles	5-Fluorouracil cinnamon oregano	Size= 254.77 nm PDI= 0.28 ZP= +15.26 mV EE (cinnamon)= 77.3% EE (Oregano)= 69.1%	-	-	HCT116	Both cinnamon and oregano inhibited HCT 116 cells. Combining both extracts with 5-Fluorouracil showed highest cell inhibition (62.8% and 71.1%), high apoptotic cells (28.9% and 39.7%), high caspase-3 activation (2.94 and 4.46) and high suppression of mitochondrial membrane potential (58.1 and 21.76) for encapsulated and unencapsulated formulation, respectively.	[205]
Chitosan-gum arabic nanoparticles	Curcumin	Size= 136 nm ZP= +48 mV EE= 95%	-	-	HCT116 HT-29	An in vitro release study showed that nanoparticles could reach the colon without degrading by the gastric of the stomach. In addition, the formulation led to better anticorectal cancer activities against HCT-116 and HT 29 cell lines due to it provided easier cellular uptake as well as more readily induced cell apoptosis.	[206]
Chitosan-cellulose nanoparticles	5-Fluorouracil	PS= 48.73 ± 1.52 nm EE= 86 ± 2.75%	-	-	HCT-116	The formulation triggered release 42.37 ± 0.43% of encapsulant in SGF pH 1.2 and 76.82 ± 1.29% in the colorectal fluid with pH 7.4. In in vitro cytotoxicity assays, at 250 µg/mL concentration of nanoparticles eliminated only 8.16 ± 2.11% of CCD112 normal cells and 56.42 ± 0.41% of HCT-116 cancer cells.	[207]
Chitosan collides nanoparticles	Curcumin 7-ethyl-10-hydroxycamptothecin	Curcumin nanoparticles PS= 81.2 ± 8.0 nm	-	-	DLD1 HCT116	The synergy of curcumin and 7-ethyl-10-hydroxycamptothecin was shown to provide a suitable strategy for the simultaneous treatment of inflammatory bowel diseases and related colorectal cancer. The combinatorial therapy also resulted in a remarkable tumor shrinkage (~6%) of the total tumors exceeding 4 mm.	[208]



Chitosan coated polylactic-co-glycolic acid/polycaprolactone nanoparticles	5-Fluorouracil	PLGA as coated material PS= $302.2 \pm 9.1$ nm PDI= $0.226 \pm 0.019$ ZP= $+15.21 \pm 1.72$ mV EE= $44.05 \pm 3.98\%$ Polycaprolactone as coated material PS= $271.3 \pm 2.8$ nm PDI= $0.296 \pm 0.032$ ZP= $+12.08 \pm 1.87$ mV EE= $51.16 \pm 2.77\%$	-	-	HT-29	Nanoparticles exhibited a sustained release behaviour as well as the cytotoxicity study showed a greater cytotoxic effect against HT-29 cells compared with 5-Fluorouracil solution and unloaded nanoparticles.	[38]
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**Abbreviations:** PS, particle size; ZP, zeta potential; PDI, polydispersity index; EE, encapsulation efficiency.

chemical modifications of chitosan is N-substitution, in which the amino group ( $-\text{NH}_2$ ) of chitosan serves as the functional group that reacts.<sup>30</sup>

## Chitosan Derivatives with Functional Modifications

### Trimethyl Chitosan

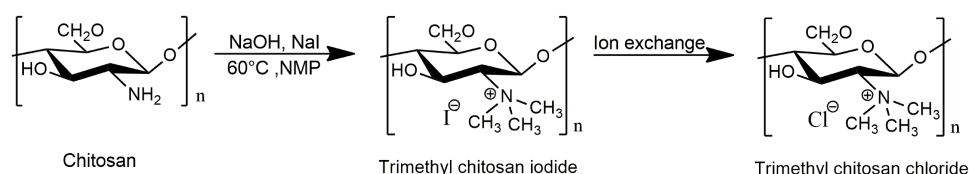
Trimethyl Chitosan (TMC) is among of the highly desirable quaternized chitosan derivatives that first synthesized by Muzzarelli and Tanfani.<sup>160</sup> Typically, TMC is synthesized through reaction of chitosan with methyl iodide in strong alkaline environment at elevated temperature. The iodide ion is then substituted by chloride by means of an ion exchange process as shown in (Figure 4).<sup>161,162</sup> N-Methylated chitosan with the hydrophobic groups  $\text{N}(\text{CH}_3)_2$  and the hydrophilic groups  $\text{N}^+(\text{CH}_3)_3$  is amphiphilic, thus it can be self-assembled to vesicles and also is considered as a suitable derivative for nanoparticles preparation.<sup>161</sup> TMC has been proven to be useful in enhancing the paracellular transport of targeted drug delivery systems through increasing the retention time of encapsulant on the mucosal surface as well as binding the protonated amino groups on the C-2 position with negatively-charged mucus and by opening the tight junctions of epithelial cells.<sup>163,164</sup> In addition, the degree of quaternization increases the permeation-enhancing effect. Several studies have applied TMC due to its solubility in a wide range of pH (1–9), thus the advantageous properties of the polymer are maintained regardless of the varying pH values along GIT.<sup>165</sup>

### Carboxymethyl Chitosan

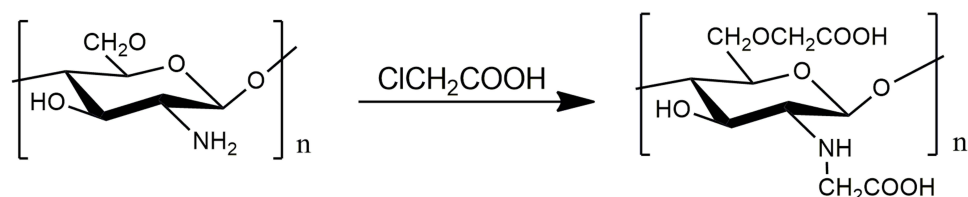
Besides TMC, chitosan solubility can be also enhanced through another common modification method; carboxymethylation. Carboxymethyl chitosan is developed by introducing a carboxymethyl group in the structure of chitosan as a hydrophilic modification. It is synthesised by carboxymethylation of the amine and hydroxyl moieties of chitosan. This modification increases chitosan solubility in neutral and basic solutions without negatively affecting other important characteristics.<sup>166</sup> Carboxymethylation reaction takes place specially either at C-6 hydroxyl groups or at the  $\text{NH}_2$  moiety that produces water-soluble N,O-carboxymethyl chitosan compounds and comprises either a primary amino group ( $-\text{NH}_2$ ) or as secondary amine ( $-\text{NH}-\text{CH}_2\text{COOH}$ ) as shown in (Figure 5).<sup>167,168</sup> Carboxymethyl chitosan have been synthesised with different degrees of carboxymethyl substitution and molecular weights to prepare nanoparticles by means of ionotropic gelation with calcium ions for targeted anticancer drug delivery.<sup>43,169,170</sup>

### N-Succinyl-Chitosan

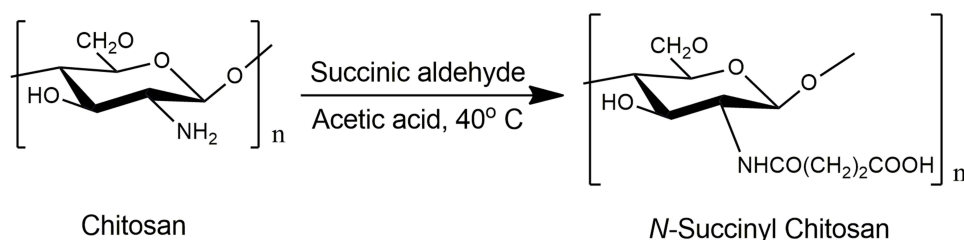
N-succinyl-chitosan, which is an acyl chitosan derivative obtained by introduction of succinyl groups into chitosan N-terminal of the glucosamine units. Polyion complexes can be generated between the  $\text{NH}_3^+$  and  $-\text{COOH}-$  groups in the succinyl chitosan as shown in (Figure 6).<sup>171,172</sup> The degree of succinylation can be easily modulated by adjusting reaction



**Figure 4** Synthesis of N,N,N-trimethyl chitosan.



**Figure 5** Synthesis of N,O-carboxymethyl chitosan.



**Figure 6** Chemical structure of N-succinyl-chitosan.

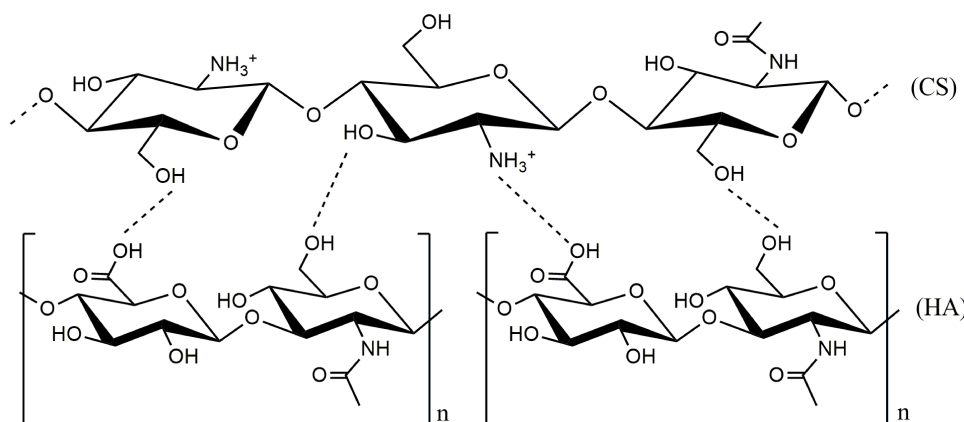
conditions through succinic anhydride.<sup>173,174</sup> N-succinyl-chitosan exhibits a good ability to react with several kinds of agents due to the reactive functional  $-\text{NH}_2$  and  $-\text{COOH}$  groups.<sup>175–177</sup> Furthermore, this derivative has shown a good water solubility in a wide pH-range with biocompatibility *in vitro* and *in vivo*, low toxicity, long-term retention in the systemic circulation and high accumulation in tumour tissue.<sup>173,178</sup>

### Hyaluronic Acid-Conjugated Chitosan

Hyaluronic acid, a linear glycosaminoglycan consists of alternating  $\beta$ -1,4 linked d-glucuronic acid (GlcUA) and  $\beta$ -1,3 linked N-acetyl-d-glucosamine (GlcN Ac) units. It has been experimentally shown its ability to be as a tumor-targeting moiety through virtue of its high affinity for the cell surface receptors CD44 and RHAMM which, in a cancer stage-specific manner, overexpressed in colorectal carcinoma.<sup>179–181</sup> These lead to enhance the antitumor efficiency of antitumor agents and to increase drug accumulation in tumor cells by facilitating targeted drug delivery via CD44 and RHAMM.<sup>182,183</sup> The deposition of hyaluronic acid preferentially on the chitosan-based nanoparticles' surface can improve cells targeting with overexpressed receptors, enhance *in vivo* stability and prolong circulation times of therapeutic agents.<sup>182,184</sup> Figure 7 represents the possible chemical structure of hyaluronic acid-conjugated chitosan.<sup>185</sup>

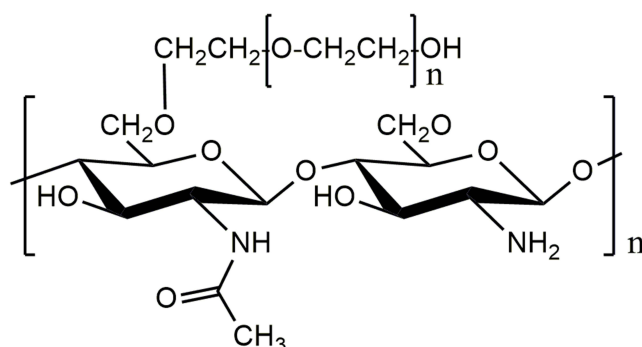
### PEG-Conjugated Chitosan

Polyethylene glycol (PEG) has gained wide recognition as an ideal graft-forming polymer because of its solubility in aqueous and organic solvents as well as having weak immunogenicity, low toxicity, good biodegradability, and biocompatibility. PEG is among of the few synthetic polymers that has been approved for using in biomedical field.<sup>186</sup> PEGylation of chitosan via hydroxyl groups was first synthesised by Gorochovceva and Makuska as shown in (Figure 8).<sup>168,187</sup> PEGylation is a suitable method for introducing new physicochemical properties to chitosan such as the solubility at wide range of pH values.<sup>188</sup> It is also a critical way in developing CSNPs as biomedical carriers and can improve the colloid stability of the particle system.<sup>189</sup>



**Figure 7** Possible chemical structure of hyaluronic acid (HA)-conjugated chitosan (CS).

**Notes:** Adapted from: Sionkowska A, Gadomska M, Musiał K, Piątek J. Hyaluronic Acid as a Component of Natural Polymer Blends for Biomedical Applications: A Review. *Mol.* 2020;25: 4035. doi.org/10.3390/molecules25184035.<sup>185</sup> © 2020 by the authors. Licensee MDPI, Basel, Switzerland. Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).



**Figure 8** Chemical structure of PEG-conjugated chitosan.

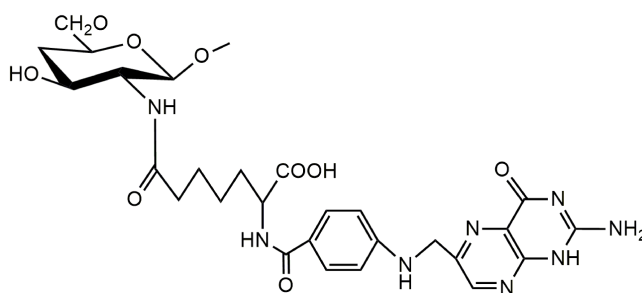
Several synthetic strategies were developed for chemical conjugation of chitosan with PEG in previous studies. Among of the desirable approaches, the substitution of amino group of chitosan through *n*-succinimidyl ester (NHS) derivative (known as NHS-amino coupling) of PEG is considered as a simple method and does not require using complex reaction pathways or equipments.<sup>188</sup> PEG chains prevent the adhesion of opsonins existing in the blood by generating a barrier layer surrounding the particles that can be “invisible” to phagocytic cells. Due to these benefits, chitosan-g-PEG was applied as a potential copolymer for colon cancer drug delivery.<sup>190</sup>

### Folic Acid-Conjugated Chitosan

Folate receptors are considered as one of the most expressed among receptors on many cancer cells types, besides, their expression levels are limited in normal cells. The cell lines HT-29 and HCT-116 have over levels of folate receptors that can be led to easier interaction with folate targeted nanoparticles; more release and more uptake of folic acid conjugates at colorectal cancer cells.<sup>145,191</sup> Folic acid is a stable, inexpensive and has a high affinity for the folate receptors.<sup>192</sup> Folic acid can conjugate covalently to chitosan molecules via folate's  $\gamma$ -carboxyl moiety.<sup>152</sup> In particular, the conjugates enter the cells through endocytosis via folate receptor and are then transported to cell cytoplasm via by vesicular trafficking. Later, the unligated folate receptor return to the cell surface once more to transport more folic acid conjugates.<sup>193</sup> Folic acid-conjugated CSNPs were used in several studies to improve drug uptake and drug accumulation into colorectal cancer cells. **Figure 9**<sup>194</sup> represents the chemical structure of folic acid-conjugated chitosan. **Table 5** represents the most recent examples of chitosan and chitosan derivatives as nanoparticles of anticancer drugs for colorectal cancer treatment.

### Chitosan Nanoparticles for siRNA Delivery

siRNA, sometimes known as short interfering RNA or silencing RNA, is a class of double-stranded RNA molecules, usually 20–25 nucleotides in length with 3' overhanging nucleotides on either end that play a variety of roles in biology.<sup>195</sup> SiRNA was used for several purposes including suppression proliferation of colorectal cancer, induction of cell apoptosis as well as prevention of colorectal cancer metastasis due to its ability to bind to the corresponding mRNA



**Figure 9** Chemical structure of folic acid-conjugated chitosan.

**Table 5** Summary of Recent Studies of Chitosan and Chitosan Derivatives as Anticorectal Cancer Drug Nanocarrier

Formulation	Active Compound	Targeted Receptor	Nanoparticles Characteristics	Cell Line	Outcome	Ref
Folic acid conjugated CSNPs	Protoporphyrin IX	Folate receptor	PS= 100 nm ZP= 20 mV LE= 35–40%	HT-29 Caco-2	Nanoparticles were taken up by HT-29 and Caco-2 cell lines after short-term of uptake period and the drug accumulated in cancer cells as a result of the folate receptor as well as the folic acid modification.	[209]
Hyaluronic acid-coupled CSNPs	Oxaliplatin	-	PS= 152 ± 5.2 nm, PDI= 0.155 ZP= +10.0 ± 0.5 mV	HT-29 in C57BL mice	Improved colon and tumour-targeting of the hyaluronic acid-coupled formulation compared to the uncoupled one	[210]
Folate-targeted chitosan/chondroitin nanoparticles (Bor/CS/Chs-FA)	Bortezomib	DSPE-PEG conjugated to folic acid	PS= 196.5 ± 1.2 nm, PDI= 0.21 ± 0.5	HCT116, HT-29	Overexpression of folate receptor leading to enhanced anti-tumour effect, improved sustained release and loading capacity	[211]
Mesoporous silica nanoparticles coated with folic acid chitosan-glycin complex (MSNsPCOL/CG-FA)	Colchicine (COL)	Folate receptor	PS= 330 ± 22.2 nm ZP >+ 40 mV	HCT116	MSNsPCOL/CG-FA showed low toxicity (4%) against normal cells compared to COL alone (60%).Enhanced anti-tumour effect mainly by primarily intrinsic apoptosis.	[145]
N,O-carboxymethyl CSNPs	Oxaliplatin/ resveratrol	-	Oxaliplatin as encapsulant PS= 190 nm EE= 60%	SW480 cells and CT26	Both nanoparticle formulations exhibited higher anticancer activity than the free drugs by enhancing the permeability and retention effect in solid tumors and improving the solubility and stability of drugs by the used drug delivery system. the cytotoxicity was confirmed against SW480 cells and CT26 cells was confirmed. In vivo study, resveratrol regulated the microenvironment of cancer cells.	[43]
			Resveratrol as encapsulant PS= 164.2 nm EE= 65%			
Hyaluronic acid-coupled CSNPs encapsulated in Eudragit S100-coated pellets	Oxaliplatin	CD44 and RHAMM	PS= 152 ± 5.2 nm ZP= +10.0 ± 0.5 mV EE= 40 ± 3.9%	HT-29	The distribution of coated pellets after oral administration was significantly different from that of uncoated. The oxaliplatin was protected by coated pellets from the whole upper GIT and delivered also to the colon and in the vicinity of tumors. Hyaluronic acid coupling on the surface of nanoparticles made the drug delivery more specific to tumors of the colon.	[210]

(Continued)



Table 5 (Continued).

Formulation	Active Compound	Targeted Receptor	Nanoparticles Characteristics	Cell Line	Outcome	Ref
N-succinyl CSNPs	Paclitaxel and gemcitabine	-	PS= ~200 nm PDI= 0.2 ZP= -10 mV	HT-29	The prepared nanoparticles showed a swelling behaviour at colonic pH that resulted in a sequential release profile for both the drugs. The drug combination also exhibited a cytotoxic effect against HT-29 cell line, besides of a remarkable G2/M phase arrest.	[212]
Thiolated chitosan-alginate nanoparticles coated Eudragit L100	$\alpha$ -Mangostin	-	PS= 767.4 $\pm$ 73.4 nm PDI= 0.680 $\pm$ 0.148 ZP= +50.8 $\pm$ 2.5 mV EE= 57.7 $\pm$ 6.5%	HT-29	Eudragit coating reduced the release of $\alpha$ -mangostin at gastric environment while it increased its release at the colon site. Moreover, the coated nanoparticles provided higher mucoadhesion that resulted in the drug remaining at the colon site via forming disulfide between thiolated chitosan and mucosa.	[44]
Folic acid-conjugated chitosan/ chondroitin sulfate self-assembled nanoparticles	Bortezomib	Folate receptors	Size= ~196.5 $\pm$ 1.2 nm PDI= ~0.21 $\pm$ 0.5 ZP= ~+18 mV EE= 21.4%	HCT116 HT-29 A549	Nanoparticles facilitated the drug release under an acidic tumor microenvironment. In vitro study showed higher cellular uptake and apoptosis in colorectal cancer cells (HCT-116 and HT-29), via binding the folate receptor, compared to that in lung cancer cells (A549).	[211]
N-trimethyl chitosan	Camptothecin	-	-	CT26	The final formulation remarkably showed cytotoxic activities and apoptotic effects against CT26 cell line. Tumor growth and prolonged the survival time were efficiently suppressed by N-trimethyl chitosan nanocolloids compared with free camptothecin in the CT26 colon carcinoma subcutaneous model.	[213]
N,N,N-trimethyl chitosan chloride/poly (acrylic acid)/silver (TMC/PAA/Ag) nanocomposites	-	-	PS= 30–65 nm	HCT116	TMC/PAA/Ag (3%) had the highest cytotoxicity effect (the less cell viability %) on colon cancer line compared with TMC/PAA/Ag (2%) and TMC/PAA nanocomposites.	[214]
PLGA nanoparticles coated with folic acid-chitosan ( $\alpha$ T-PCF-NPs)	Alpha terpineol	Folate receptor	PS= 263.95 nm PDI= 25% ZP= +38.20 mV EE= 88.1%	HT-29	$\alpha$ T-PCF-NPs had an inhibitory effect on HT-29 cells compared to HFF cells through pro-apoptotic effect and anti-apoptotic genes. In vivo study reported a decrease in the size of murine tumors in exposure of $\alpha$ T-PCF-NPs.	[215]

Folic acid conjugated hesperetin encapsulated chitosan nanoparticles (CFH)	Doublecortin like kinase I (DCLKI)	Folate receptor	PS= 182 nm ZP= +17 mV EE= 88.2%	HCT116	CFH-DCLKI nanoparticles enhance apoptosis and inhibited the migration and invasion of HCT116 cells. Real time PCR and Western blot results have demonstrated that the treatment with CFH-DCLKI nanoparticles The CSC markers STAT1, NOTCH1 and DCLK1 were reduced the expression significantly after treating the HCT116 cells by CFH-DCLKI nanoparticles compared to the CFH alone. Furthermore, the colonosphere growth in the 3D spheroid model was also inhibited significantly.	[216]
Chitosan folate nanoparticle	Hesperetin	Folate receptor	PS= 457 nm PDI= 60% ZP= +18 EE= 98.3%	HCT-15	Chitosan folate hesperetin nanoparticle caused a proliferation inhibition with a IC50 value of 28 $\mu$ M. Additionally, they induced apoptosis and inhibited colony formation via regulating the expression of proapoptotic genes expression.	[217]
Folic-acid-modified chitosan nanoparticles	5-Fluorouracil	Folate receptor	PS= 235 $\pm$ 12 PDI= 25% ZP= +20 $\pm$ 2 mV EE= 59 $\pm$ 2%	caco-2	The caco-2 cell death was enhanced in the presence of folic acid due to the overexpression of folate receptors on the caco-2 cancer cells.	[218]
Aminated mesoporous silica nanoparticles-alginate/folic acid conjugated O-Carboxymethyl chitosan-gelatin (AMSN-Alg/FA-CMCT-gel)	5-Fluorouracil (5-FU) Bisdemethoxycurcumin (BDC)	Folate receptor	PS= 203 nm ZP= +42.42 mV EE= 85.8% (BDC), 89.4% (5-FU)	HCT116	The combinatorial of 5-FU and BDC in HCT116 cells showed an anticancer effect as well as the intracellular uptake of the combination into nanocarriers was also confirmed by Confocal Microscopy.	[219]
solid lipid nanoparticles of irinotecan coupled with chitosan surface modification	5-fluorouracil and curcumin	Folate receptor	PS= 1201.50 $\pm$ 386.34 nm PDI= 50 $\pm$ 0.09% ZP= +48.70 $\pm$ 1.55 mV EE= 62.24 $\pm$ 2.90%	HCT116	The sulforhodamine B assay results showed the superior cytotoxicity of SLNs on HCT-116 cells over the standard drug. Biodistribution studies conducted on Wistar rats reported that higher concentration of prepared SLNs in the colon after surface modification.	[220]
Thiolated chitosan-based nanoparticles coated by Eudragit® S100	Alpha-mangostin resveratrol	-	(Without coating) PS= 540 nm ZP= +39 mV	-	The dual loaded of Alpha-mangostin and resveratrol into the NPs decreased the half-maximal inhibitory concentration levels of NPs dramatically, which indicated a synergistic efficiency of the system against colon cancer cells.	[221]

**Abbreviations:** PS, particle size; ZP, zeta potential; PDI, polydispersity index; EE, encapsulation efficiency; LE, loading efficiency.

**Table 6** Summary of Recent Studies of Chitosan and Chitosan Derivatives Nanoparticles for siRNA Delivery in Colorectal Cancer Therapy

Formulation	Active Compound	Nanoparticles Characteristics	Cell Line	Outcome	Ref
Carboxymethyl dextran-CSNPs	Snail siRNA doxorubicin	PS= $172 \pm 6$ nm PDI= 0.33 ZP= $+11.8$ mv $\pm$ 0.5 EC (siRNA)= 79% EC (doxorubicin) = 77%	HCT116	Delivering snail siRNA and doxorubicin via CSNPs led to significant changes of Epithelial mesenchymal transition genes as (Down regulation the expression of Vimentin and MMP-9 and upregulation of E-cadherin) well as inhibited growth, migration and induced apoptosis cell death of HCT-116 cells.	[197]
Carboxymethyl dextran-CSNPs	HMGA2 siRNA doxorubicin	PS= $174 \pm 6$ nm PDI= 0.3 ZP= $+11.8 \pm 0.5$ EC (siRNA)= 78% EC (doxorubicin) = 75%	HT-29	HMGA2 siRNA and doxorubicin nanoparticles had an effective to induce tumour cell death and also decreased the expressions of HMGA2, MMP-9 as well as vimentin and enhance E-cadherin expression.	[222]
Carboxymethyl chitosan and labeled fluorescein isothiocyanate-chitosan hydrochloride	Anti- $\beta$ -catenin siRNA	PS= $374 \pm 7.2$ nm ZP= $+21.8$ mV EE= $76.7 \pm 3.7\%$	HT-29	siRNA was protected against acidic degradation with controlled release through responding to external stimulus under pH 5.5. The $\beta$ -catenin protein that promotes colon cancer proliferation was reduced to around 40.10%.	[223]
Carboxymethyl dextran trimethyl CSNPs	NIK/STAT3-specific siRNA BV6	PS= $\sim 105$ nm PDI= $< 0.2$ ZP= $+17$ mV	CT26	The combination therapy reduced proliferation, improved proapoptotic effects, colony formation, cell migration, and angiogenesis, besides the expression of factors including HIF and IL-10 in tumor cells.	[224]

**Abbreviations:** PS, particle size; ZP, zeta potential; PDI, polydispersity index; EE, encapsulation efficiency; EC, encapsulation capacity.

and further induce the mRNAs cleavage.<sup>196</sup> However, the targeted delivery of siRNA has faced several challenges, including low intracellular uptake and rapid degradation. The positively charged of chitosan and its mucopermeable and mucoadhesive properties have led to the extensive use it for encapsulate the negatively charged siRNA by the electrostatic interaction, forming positively charged stable polyplexes.<sup>184</sup> In addition, in order to enhance the efficiency of suppressors of cellular resistance several studies combined therapeutic agents and siRNA within one nanocarrier-based delivery system.<sup>197</sup> Table 6 summarises the recent studies of chitosan and chitosan derivatives nanoparticles based-siRNA delivery.

## Future Perspective

Due to its physicochemical properties, chitosan and its derivatives nanoparticles confirmed their efficacy for enhancing drug stability and targeting treatments of colorectal cancer that are administrated orally. We anticipate more research in the next years aim to improve the chitosan characteristics by physical and chemical modifications in order to increase the specific drug accumulation within the colorectal cancer cells. From the aspect of the adverse effects of anticancer treatment, the toxicity and immunogenicity of the chitosan nanocarriers towards more normal cell lines need to be studied particularly to investigate their long-term effects.

## Conclusion

Over the last few decades, various efforts were devoted in nanomedicine science to develop novel strategies of smart targeted drug delivery systems. In this approach, targeting cancerous tissues of colorectal with minimum side effects and cellular toxicity is the ultimate goal. The delivery systems based on chitosan nanocarriers have shown high ability to carry therapeutic agents to specific region of the body according to the polymer characteristics. The above-discussed applications of chitosan and its derivatives-based oral nanocarrier systems for colorectal cancer demonstrated their biocompatibility and versatility. CSNPs control drug delivery by means of their pH-sensitivity, surface flexibility, mucoadhesive and penetration properties. Chitosan modification further enhances the efficiency of anticancer drugs by increasing their retention time, accumulation, cellular uptake and cytotoxicity to tumor cells. Studies in colon tumor HT-29, Caco-2, HCT-116, SW480, CT26 and MCF-7 cell lines have shown high anticancer activity as well as significant decrease in tumor volume. In summary, oral chitosan-based nanocarriers conjugated/or loaded with therapeutic agents or targeting ligands represent a great promise in developing colon targeted drug delivery systems with low toxicity level and less monitoring of treatment.

Further understanding of the factors influencing nanoparticle characterization and uptake will help researchers to identify the best approach for targeting oral drug to the colon cancer. While many studies have been done in recent years to develop a successful system for oral drug and gene delivery, the approaches have yet to progress beyond animal studies and establish relevant efficacy in humans. However, the major advantages of chitosan, including mucoadhesion effects, non-toxic, ease of modification, ease of protein or DNA complex formation, biodegradability, and low cost explain the continuing improving of these promising carriers for efficient drug delivery to colon cancer. Therefore, it is envisaged that the use of chitosan in cancer-targeted drug delivery systems will improve the prospects of biomedical and biotechnology applications in the future.

## Acknowledgment

The authors wish to thank Universiti Sultan Zainal Abidin for the support.

## Funding

This research was funded by Universiti Sultan Zainal Abidin through UniSZA/2021/DPU2.0/05 grant (grant number R0332).

## Disclosure

The authors report no conflicts of interest in this work.

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