Folic acid decorated chitosan nanoparticles and its derivatives for the delivery of drugs and genes to cancer cells

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Nanotechnology offers a number of nanoscale implements for medicine. Among these, nanoparticles are revolutionizing the field of drug and gene delivery. Chitosan is a natural polymer which provides a profitable tool to an innovative delivery system due to its inherent physicochemical and biological characteristics. Chitosan nanoparticles are promising drug and gene delivery carriers because of small size, better stability, low toxicity, inexpensiveness, simplicity, easy fabrication and versatile means of administration. Chitosan can also be easily modified chemically due to the presence of reactive functional hydroxide and amine groups. Folic acid is commonly engaged as a ligand, for targeting cancer cells, as its receptor, that transports folic acid into the cells through endocytosis and is over-expressed on the surface of several human epithelial cancer cells. Integrating folic acid into chitosan-based drug delivery inventions directs the systems with a well-organized targeting ability. The present review outlines several illustrations of this versatile system based on folate decorated chitosan, which have shown potential as auspicious delivery systems published over the past few years. In addition, it is probable to formulate chitosan nanocarriers that exhibit manifold usage beyond targeted delivery, such as nanotheranostics and cancer stem cell therapy.

Keywords: Cancer, chitosan, doxorubicin, drug delivery, folic acid, 5-fluorouracil, gene delivery.

CANCER is among the foremost causes of morbidity and mortality worldwide. According to reports, in 2017, the new cancer cases are estimated to about 1,688,780 with 600,920 cancer deaths in the US[1]. The therapy utilized for treating cancer is chemotherapy. In this technique, the fast growing cells are killed using single or a combination of drugs. The chemotherapeutic drugs utilized in this method are cytotoxic by way of interfering the mitosis or cell division of cancer cells. Moreover, chemotherapeutic drugs induce stress and initiate apoptosis which results in damaging of cells. However, normal cells are also found to be susceptible to these effects, in particular cells of bone morrow, digestive tract and hair follicles. Recently, nanotechnology provided several exciting possibilities in destroying cancer cells with minimum damage to the surrounding health tissues. Further, it also helped in the detection and elimination of cancer cells before it was prone to tumour[2,3]. A broad variety of substances like polymeric micelles, ligands, lipids and surfactants have been used as smart carriers to deliver drugs for cancer treatment. To enhance the delivery of drug to the therapeutic location by decreasing the delivery of the drug to the other sites, polymeric micelles are conjugated with ligands for active targeting of tumour cells[4].

Chitosan is a polysaccharide produced by deacetylation of chitin using sodium hydroxide. Chitosan is stable in neutral environments and solubilizes in acidic environments. It can transport a drug to an acidic environment and helps in discharging the drug to the preferred site[5]. Chitosan alone exhibits comparatively elongated blood circulation time and low uptake by the reticulo endothelial system (RES). Chitosan nanoparticles can target tumour sites via the unsystematic and malfunctioning vascular architecture in tumour tissue, called the enhanced permeability and retention (EPR) effect referred to as passive targeting[6]. EPR is a phenomenon in which the molecules of certain sizes are retained in tumour tissue more abundantly compared to normal tissues. Researchers reported this phenomenon whereby tumour cells need to induce the formation of blood vessels for their nutritional and oxygen supply in order to

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grow faster. This neovasculature usually is not normal and is also found to be defective vasculature with poorly arranged endothelial cells with wider fenestrations. Moreover, the lymphatic drainage system is absent in tumour tissues which makes them putative sites for retention of molecules like nanoparticles and other macromolecules[7].

Folic acid, a ligand, shows more affinity towards its receptors and hence folic acid and its conjugates exhibit advanced drug delivery to cancer cells[8]. The folated chitosan nanoparticles possess much more cell uptaking ability than unmodified chitosan, because of folate-mediated receptor endocytosis[9]. Hence, to attain an efficient drug delivery through active targeting, chitosan is combined with folic acid. The folic acid–chitosan conjugate comes together with folate receptors to reach the intercellular compartments. The folate receptor and conjugate get detached in an acidic medium and then the receptors revisit the cell surface while the folic acid conjugated chitosan are degraded by lysosome[10].

The technologies and systems for transporting a drug in the body to accomplish its therapeutic effect for a particular cancer is known as drug delivery system which can be successfully attained through the application of smart carriers as shown in Figure 1. The nanoparticles are prospective candidates as drug delivery vehicles for tumour treatment because of their exclusive physicochemical characteristics[11]. Hence, in recent days, researchers concentrate more on nanoparticle drug delivery, which enables controlled drug release to specific sites with increased bioavailability of drug and eliminates the side-effects of drugs delivered. This article provides an insight on tumour-targeted drug delivery using folate–chitosan nanoparticles (FA–CS NPs) for delivering anticancer drugs and genes. Information on the physiochemical properties of chitosan and folic acid is briefly presented below.

Chitosan

Chitosan (CS) is a natural, linear carbohydrate polymer composed of both acetylated and deacetylated units. This cationic polysaccharide is easily obtainable, biocompatible, biodegradable and non-toxic in nature. Chitosan has gained increasing attention in therapeutic and biomedical applications. It is a deacetylated form of chitin, which is a polysaccharide rich in the exoskeleton of shellfish like shrimps, lobsters or crabs and cell walls of fungi (Table 1). Commercially produced CS possesses molecular weights ranging between 3800 and 20,000 Da and the deacetylation is about 66% to 95%. It is insoluble at neutral pH, but soluble in an acidic surrounding. The solubility of chitosan in neutral and basic solutions can be increased by quaternization[12]. The molecular weight and degree of deacetylation of chitosan affect the physicochemical and biological characteristics of chitosan. In general, lower molecular weight chitosan with lower degree of deacetylation demonstrates superior solubility and more rapid degradation than their higher molecular weight counterparts[13,14]. Chitosan, as a type of nanomaterial, carrying anti-tumour drug with noteworthy control-release behaviour, has fascinated widespread consideration attracting exhaustive studies[15].

Folic acid

Folic acid is non-immunogenic and economical than monoclonal antibodies. It possesses low molecular weight with better storage stability and dissolves in water[16]. Folic acid or pteroyl glutamic acid (PGA) consists of *p*-aminobenzoic acid connected to the one end of pteridine ring and L-glutamic acid at the other end. The folates in nature forms can vary in the nature of substituents, reduction state of the pteroyl group and the number of glutamyl groups linked to the pteroyl group. The majority of naturally occurring folates is pteroylpolyglutamates, which is

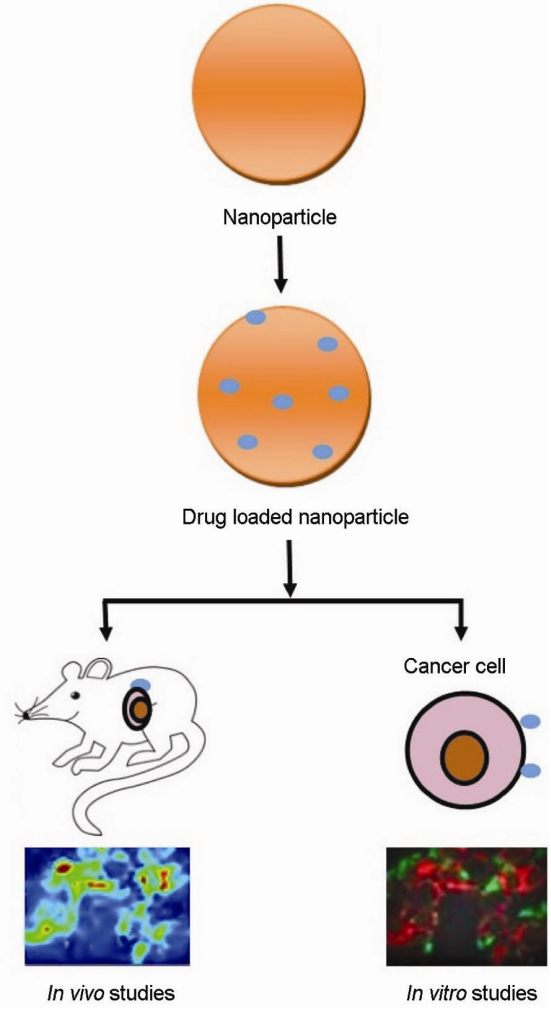
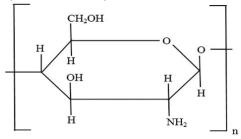
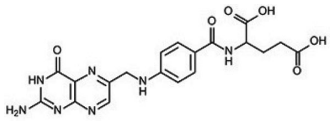


Figure 1. Schematic representation of nano-based drug delivery.

Table 1. Chemical structure and properties of chitosan and folic acid

Properties	Chitosan	Folic acid
Molecular formula	$(C_6H_{11}NO_4)_n$	$C_{19}H_{19}N_7O_6$
Chemical structure		
Form	Coarse ground flakes and powder	Orange to yellow crystalline powder
Stability	Stable	Stable
Water solubility	Solubility depends on its molecular weight	1.6 mg/l (25°C)
Storage temperature	Room temperature	2–8°C

attached to the γ-carboxyl of glutamatein, a form of peptide linkage with two to seven glutamates. Most of the natural folate gets fond of the polyglutamate chain. The enzyme γ-glutamyl hydrolase present in the small intestine removes the polyglumate chain and then the folate is absorbed and transported as a monoglutamate into the portal vein[17]. The folic-acid targeted nanoparticles were shown to recognize cancer cells with high efficiency and show reduced toxicity to the non-targeted site. The chemical properties of chitosan and folic acid are given in Table 1.

Chitosan nanoparticle synthesis

Before venturing into the conjugation of folic acid with chitosan nanoparticles, succinct information about the synthesis of chitosan nanoparticle is highlighted. Chitosan NPs can be prepared using numerous methods, namely, ionic gelation, polyelectrolyte complex method, desolvation, spray-drying and covalent crosslinking, which are briefly outlined below.

Ionic gelation method

Ionic gelation is the commonly preferred method for preparing chitosan nanoparticles, in which particular concentration of chitosan is liquefied in acetic acid and sodium tripolyphosphate is normally used as a linker. Chitosan NP is formed by electrostatic interaction between amine group of chitosan and negatively charged group of polyanion (tripolyphosphate)[18]. Chitosan nanoparticles can be formed by stirring at room temperature. The size of nanoparticles can be varied by varying the proportion of chitosan and stabilizer. For maximum production of nanoparticles, the weight ratio of chitosan : tripolyphosphate should be controlled and it was found to be within the range of 3 : 1 to 6 : 1 (ref. 19).

Polyelectrolyte complex method

Polyelectrolyte complex otherwise, self-assembled polyelectrolyte, is a term used to define complexes formed by charge neutralization and self-assembly of the oppositely charged groups, results in hydrophilicity. The size of the complexes can be varied from 50 to 700 nm (ref. 20). This method deals with simple procedures, avoids the use of chemical linkers, thereby decreasing toxicity and other unwanted effects of reagents. In these systems, the release of drugs may be intensely affected due to charge–charge interactions. However, these undesirable interactions have been exploited beneficially because of their ability to entrap the drug at molecular level for controlled drug release[21].

Desolvation method

It is frequently used technique for chitosan NP synthesis. The nanoparticles are synthesized using desolvating agents and hence named as desolvation method. Sodium sulphate and acetone are the most commonly used precipitating agents. The desolvating agents can be added continuously or intermittently. Chitosan nanoparticles are formed by drop wise addition of sodium sulphate into chitosan solution. The greater affinity of salt to water results in precipitation, inducing desolvation of chitosan leading to formation of chitosan nanoparticles[22].

Spray drying method

Spray drying is the process of making a waterless precipitate from a liquid by rapidly drying with a hot gas. Air is used as the drying medium, if the liquid is a combustible solvent such as ethanol or an oxygen-sensitive product like nitrogen is also used. Spray drying method can be used as a one-step preparation of nanoparticle powder, and it becomes a noble practice to advance the colloidal nanoparticle stability[23].

Covalent cross-linking method

Chitosan and its derivatives can be synthesized as nanoparticle drug carriers using covalent cross-linking technique. This method involves the development of covalent

bonds between the reactive amino groups of chitosan and functional linkers like dicarboxylic acid and glutaraldehyde[24].

Chitosan–folic acid conjugation and active targeting

Better therapeutic effects cannot be attained if the therapeutic drug is directly conjugated to the targeting ligand (FA) because of the reduction in biological activity of drug due to conjugation and also because the ligand-receptor recognition system was disturbed. This limitation can be overcome by polymeric micelles and/or peptide sequences[25]. The biological activity of the drug can be preserved by physically entrapping the drug molecule within the nanocarrier (CS). The two carboxyl groups of folic acid are named α and γ. Between these two carboxyl groups, the γ-group shows more reactivity than the α-group in conjugating with the reactive amino group of chitosan. The solubility of folic acid in water is improved by increasing the temperature or changing the pH, but this may disturb the stability of folic acid. Hence dimethylsulphoxide (DMSO) is used as a solvent for folic acid and chosen as a conjugation medium. On the contrary, chitosan dissolves in acidic solutions and is insoluble in organic solvents. Hence aqueous buffers or in particular combinations with organic solvents miscible with water are chosen as a medium for conjugation reaction[26]. The coupling agents may be used for direct conjugation of folic acid to the γ-COOH group of chitosan. The grafting of folic acid to chitosan is also achieved with the help of linkers. 2,2′-(ethylenedioxy)-bis-(ethylamine) (EDBE) has been utilized as a linker between folic acid and carboxymethylated[27] or succinylated chitosan[28]. In addition to chitosan and chitosan derivatives, it is also grafted with copolymers for the delivery of chemotherapeutic agents and drugs[29].

A targeted drug delivery system consists of ligand or an antibody and can effectively deliver the drug only to the targeted site as shown in Figure 2. The effectiveness of the drug delivery system depends on physiochemical characteristics of the therapeutic agent, ligand and the time duration for which the delivery system requests to be presented for action[30]. In some cases the drug is directly attached to the nano-carrier in which case the pharmacological property of therapeutic agent will be lost. To avoid this, a ligand is used for active targeting of drug delivery. Folic acid, an attractive ligand to folate receptors, is known to be a potential targeting substance to deliver the therapeutic agents through receptor-mediated endocytosis because of the overexpression of folate receptors in the number of human cancer cells that serve as a symbol of tumour, and provide a distinguishable marker from normal cells[31,32]. The effective delivery of drugs to the target site can be attained through biological interactions like antigen–antibody binding called targeted drug delivery. Active targeting utilizes the characteristics exhibited by the tumour tissue such as over expression of tumour-associated antigens on the surface of the tumour tissue[33]. The interaction between the over-expressing antigen on the tumour site and the targeting components causes accumulation of drug in the target tissue. As the drugs are accumulated only in tumour sites, it decreases the side-effects and allows the cellular uptake through receptor-mediated endocytosis[34] (Figure 3).

Folic acid conjugated chitosan for the delivery of drugs

There have been many studies on delivery of anti-cancer drugs by conjugating highly biocompatible chitosan nano-carriers with folic acid. The utilization of folic acid as a targeting component can accomplish the targeted delivery and enhance the competency of carrier internalization[35]. Chemotherapeutic drugs can cause side-effects, including hair loss, birth defects, ulcer, fatigue and liver disease and affect bone marrow function. These side-effects can be prevented by conjugating the drugs with chitosan nanoparticles[36]. The most commonly used anti-cancer drugs such as doxorubicin (DOX), 5-fluorouracil (5-FU), paclitaxel (PTX) and mitomycin C (MMC) have a common problem of solubility, which can be overcome by linking with folated chitosan for the targeted delivery to cancer cells and these are discussed below. The chemical structure of the above mentioned poorly soluble anti-cancer drugs is given in Figure 4.

Doxorubicin

DOX is the most universally recommended antitumour drug for various types of cancers. It is a weakly soluble

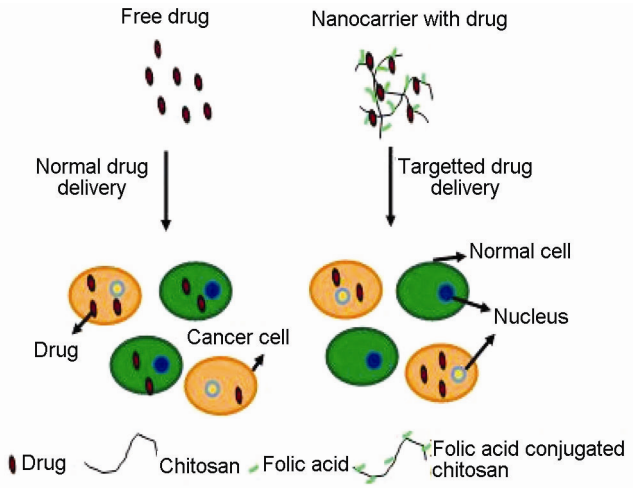


Figure 2. Difference between targeted and normal delivery of drugs.

chemotherapeutic agent that produces anticancer mechanisms via suppressing the synthesis of nucleic acid by cancer cells. The chitosan nanoparticle was loaded with DOX and then conjugated to folic acid for efficient drug delivery. The cytotoxic effects were studied through MTT assay of doxorubicin loaded folated chitosan nanoparticles (DOX-CNPs) using retinoblastoma cells (Y-79). The results showed that folated chitosan nanoparticles loaded with DOX possess better cytotoxic effect than the unconjugated DOX-CNPs and DOX alone. This result was clarified by an improved intracellular uptake of DOX-CNPs-FA (30%) compared to DOX-CNPs (13.24%) and DOX (5.01%), resulting from the elevated attraction of folic acid for folate receptors[37]. The chitosan nanoparticles were loaded with both doxorubicin and pyrrolidinedithiocarbamate (PDTC) with more significant scientific inference against liver cancers. The encapsulating efficiency of DOX and PDTC was about 77.64% and 86.54% respectively. DOX-loaded nanoparticles demonstrated a lower IC_{50} signifying that FA-CS nanocarriers enhanced cell uptake efficiency, which was also demonstrated by fluorescence microscopy[38]. The anticancer activity of folate-linked carboxymethyl chitosan (CMCS)-iron (II, III) oxide (Fe_3O_4)-doped cadmium telluride (CdTe) quantum dot (QDs) nanoparticles (CFLMNPs) was studied in normal L02 and HepG2 cancer human hepatocytes. The outcome of this research indicates that the carrier possesses high drug loading capacity and the drug was taken up by the cell through the mechanism of folate receptor mediated endocytosis[39]. Hu *et al.*[40] industrialized a drug delivery system via conjugating the folate with trimethylchitosan (FTMC)/graphene oxide (GO) nanocomplexes (FGNCs) for the targeted delivery of DOX to the tumour cells. An insignificant cytotoxicity of FGNCs was observed in HeLa and A549 cells. The higher uptake level in HeLa cells with folate–receptor demonstrated the targeting capability of FGNCs. The efficiency of targeting delivery might decline the toxicity of the loaded anticancer therapeutic agent[40]. DOX was incorporated into the biodegradable succinyl chitosan nanoparticles functionalized with folic acid which were explained to be non-toxic *in vitro*. The nanoparticles showed excellent loading capacity for doxorubicin and reported a pH-dependent drug release[28]. The folic acid linked cholesterol-modified glycol chitosan (FCHGC) micelles were produced and employed for the targeted drug delivery of DOX to the cancer cells. The results of the study demonstrated that cytotoxicity was significantly increased against FR-positive HeLa cells than the free DOX[41]. The pH-dependent drug releasing mechanism was studied by Chen *et al.*[42]. To attain targeted drug delivery of DOX, smart pH-dependent polymeric micelles were developed. The pH-responsive micelles depended on deoxycholic acid and folic acid co-modified hydroxypropyl chitosan of various substitutions. The results specified that pH-responsiveness of DOX-release behaviour was heavily reliant on the attached proportions of two hydrophobic components. The DOX-release behaviour was due to the gradual hydrolysis of amide bond and electrostatic repulsion between the protonated DOX and the amino residue of the Chitosan backbone under a tumourous environment[42]. Folate-conjugated Chitosan was used as a shell material to coat the pluronic F127 for delivery of DOX to cancer cells. The drug release pattern of shell structured system was slow and sustained than the uncoated polymeric micelle delivery system. It was suggested that this controlled drug release was succeeded by dissemination of drug via polymer wall and enzymatic degradation of chitosan. Cell viability was not disturbed by nanoparticles at concentrations lesser than 1 mg/ml (ref. 43). Folate-conjugated chitosan was loaded with other polymers or inorganic materials like graphene oxide

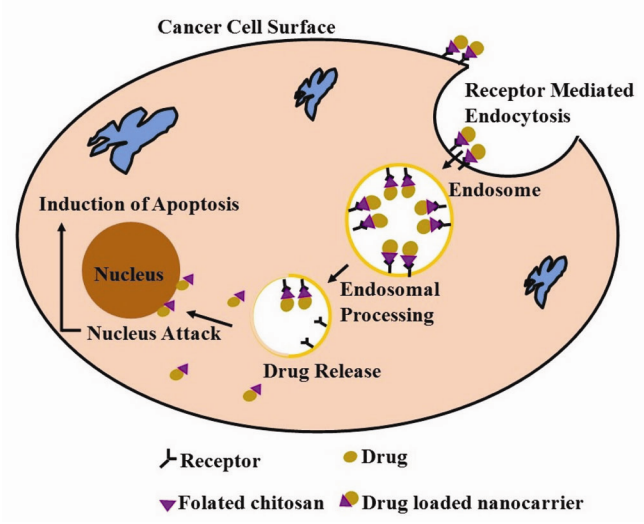


Figure 3. Folic acid–chitosan mediated endocytosis of drugs in cancer cells.

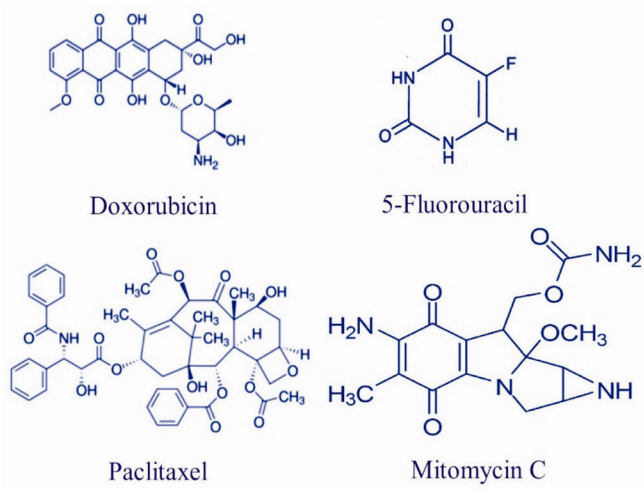


Figure 4. Chemical structure of anticancer drugs.

(GO), carbon nanotube (CNT) with the anticancer drug doxorubicin (DOX) through π–π staking. These inorganic nanosheets are soluble in water and polar solvents and it potentially enables the drug delivery system with great drug loading capability and controlled drug release. The research demonstrated a slow drug release at physiological pH (~11% after 72 h) and an increased release at low pH, particularly in the first 24 h (~35%)[44,45]. Folate–receptor targeted nanoparticles using chitosan–folic acid and dextran succinate-doxorubicin (ChitoFA and DexSU-DOX) conjugates were fabricated and studied using KB cells. The results revealed that folic acid-dextran (FA-Dex) nanoparticles effectively suppressed tumour growth by folate receptor targeting. These results suggested that DOX-loaded FADex nanoparticles are potential carriers for anticancer drug delivery[46]. The folate-grafted chitosan nanoparticles showed greater efficiency in cell uptake, approximately twice that of the non-folate grafted chitosan nanoparticles. The IC_{50} of DOX-loaded NPs were almost 10-fold lesser than that of free DOX. The results on the other hand, found no noteworthy change between cytotoxicity profiles of DOX-loaded CS and FA-CSNPs, as recommended by IC_{50} of DOX-loaded CSNPs and FA-CSNPs containing DOX of 34 and 26 μg/ml respectively. Folated chitosan is also used to coat single-walled carbon nanotubes for the targeting system. This targeting system demonstrated no significant toxicity compared to free DOX and showed more stability under physiological conditions along with effective release of DOX. The targeting system exhibited cytotoxicity to the hepatocellular carcinoma cell line (SMMC-7721) and suppressed the growth of liver cancer in nude mice[47].

5-Fluorouracil

Fluorouracil (5-FU) is a medication that has been employed in innumerable tumour treatments for more than 20 years, and is still deliberated as an active antineoplastic drug in advanced colorectal cancer and malignancies of the head and neck. The specific cell targeting of PLGA-1,3-diaminopropanefolic acid and its nanoparticles loaded with 5-FU for HT-29 cells was boosted by increasing the conjugating ratio of folic acid. The conjugation ratio of 46.7% was attained by means of 1,3-diaminopropane as a cross-linker. The non-motility of folic acid into PLGA-based drug carriers due to 1,3-diaminopropane, revealed that the formulation can be very well employed as a specific drug delivery system for cancer cells[48]. Manganese-doped zinc sulphide quantum dots were engaged to track the path of 5-fluorouracil encapsulated in folate carboxymethylchitosan (CMC) nanoparticles. The breast cancer cell line MCF-7 was employed to study the specific targeting and cytotoxicity of the drug loaded nanoparticles, while another cell line, L929 was used to prove non-toxicity to non-cancerous tissue. Nanoparticles established cytotoxicity to MCF-7 cells and were non-toxic to mouse fibroblast L929 cells. Additionally, nanoparticles exhibited a specific attachment to the MCF-7 cells expressing folate receptors; at the same time, non-folate conjugated nanoparticles exhibited no specific cell attachment[49]. The drug encapsulation efficiency (EE) of 5-FU-NPs was explored by LC–MSMS (liquid chromatography–mass spectroscopy mass spectroscopy) and it was found to be 29.3%. These experiments revealed that 5-FU loaded chitosan nanoparticles can be propelled as smart drug delivery mediators for cancer treatments[50]. The inorganic multifunctional nano vehicle α-zirconium phosphate@folate acid–chitosan–rhodamine6G (5-FU/Fe_3O_4/α-ZrP@CHI-FA-R6G) for cancer treatment was successfully fabricated and directed towards the cancer cell. The cancer cells were recognized by folic acid through its biological affinity. Cell culture studies revealed the ability of drug-release system as an operational twin nano-carrier for the delivery of anticancer drug into cancer cells. Drug release *in vitro* on the attained nanocomposites displayed a sustained drug release profile[51]. Folic acid conjugated to carboxymethyl chitosan (CMCS) using PEG was employed in the targeted delivery of 5-FU to the cancer cells. The potential ability and cytotoxicity of (CMCS-5-FU)-PEG-FU were investigated in HeLa and A549 cells. The MTT assay results suggest that the cytotoxicity of the proposed drug carrier system was higher than the non-folate CMCS-5-FU which in turn revealed that the cellular uptake efficiency of 5-FU loaded CMCS-PEG-FA was affected by folate–receptor-mediated endocytosis. The solubility of CMCS-PEG-FA at physiological pH, targeting efficiency and its potential in carrying anticancer drugs makes it a promising candidate for the targeted delivery of chemotherapeutic agents[52]. Targeted folate receptor modified with chitosan as the carrier material for 5-fluorouracil was fabricated. *In vitro* drug release investigation indicated chitosan drug-loaded microspheres showed good slow release effect with 70% and 40% release rate after 24 h in simulation of gastric and intestinal juice respectively, which revealed that the drug release rate was relevant to medium pH value[53]. The folated poly[(*p*-nitrophenyl acrylate)-co-(N-isopropylacrylamide)] submicrogels (F-SubMGs) was prepared and used as a delivery system of anticancer drug 5-FU. The cytotoxicity of unloaded and 5-FU loaded F-SubMGs was studied in MCF7 and HeLA cells. The results showed that the cytotoxicity of unloaded F-SubMGs was low, compared with FU loaded F-SubMGs; however toxicity increased with increase in F-SubMGs concentration. As the HeLa cells are folate receptor-positive, the cellular uptake efficiency of F-SubMGs was higher in HeLa cells than MCF7 cells. These results demonstrate that the mean residence time of the drug was increased to 60 days and, therefore, the folate-conjugate submicrogels have great efficiency in controlling the release of 5-FU, which may lead to an innovative choice for treating numerous malignancies[54].

Paclitaxel

Paclitaxel (PTX) is a hydrophobic antitumour drug used to treat different kinds of tumour with notable anticancer activity. Nano carriers which can enrich PTX solubility, advance PTX pharmacokinetic profiles *in vivo*, reduce its side-effects, passively or actively target tumour sites because of EPR effect[55]. The active targeting system to tumour cells, folate linked stearic acid grafted chitosan oligosaccharide (Fa-CSOSA) was fabricated by You *et al.*[56] A549 and HeLa cells were used to study the targeting efficiency of Fa-CSOSA micelles. The results showed that Fa-CSOSA micelles exhibited higher expression of folate receptors in less than 6 h. Further, for Taxol (a clinical formulation containing PTX) the obtained values of IC_{50} on A549 and HeLa cells were found to be 7.0 and 11.0 μg ml^{-1} respectively. Moreover, the cytotoxicity of PTX-loaded micelles was enhanced abruptly, indicating improved intracellular delivery of the drug[56]. PTX-loaded folate-conjugated chitosan (FA-CTS/PTX) was fabricated and its *in vitro* cytotoxicity against HeLa cells was examined. The PTX-loaded FA-CTS nanoparticles exhibited potent cytotoxicity against HeLa cells, an effect 2- to 3-fold stronger than that of PTX-loaded CTS nanoparticles[57]. The grafting of folic acid to N-octyl-N-phthalyl-3,6-O-(2-hydroxypropyl) chitosan (OPHPC) resulted in an average molecular weight of 70 kDa. The PTX micelle formulation amplified its apparent solubility by 4000-fold than the free PTX. The cellular uptake studies emphasized that a considerably greater quantity of PTX accumulated in a human breast adenocarcinoma cell line (MCF-7) compared with free Taxol®[58]. Deoxycholic acid-O-carboxy methylated chitosan conjugated with folic acid and formulated PTX loaded micelles was synthesized. The micelles were of spherical shape, sized <200 nm, and had a negative zeta potential (–21 mV) that increased its uptake by the liver, spleen and lung. Particles were synthesized to prolong blood circulation and consequently change the bio-distribution of PTX than Taxol® injection. The drug loading efficiency and encapsulation efficiency were found to be 26.5% and 90.3%, respectively. An increased localization of PTX in the spleen, lung and liver, and diminished accumulation in the heart and kidney were observed. The micelles were more effective in delivering PTX to the spleen, lung, and liver in comparison with Taxol® injection. Additionally, they decreased the distribution of drug in heart and kidney, enabling a reduction of PTX adverse side-effects in clinical practice, particularly regarding cardiotoxicity and renal toxicity[59]. PTX-loaded FA–CH–PLA nanoparticles possess an encapsulation efficiency of 90% and release PTX in a controlled manner. The uptake of PTX loaded FA–CH–PLA nanoparticles was found to be 11 g/ml which was greater than 6-fold improving capabilities compared to the free PTX. PTX-loaded FA–CH–PLA nanoparticles had more than three-fold enhancing capacities to prompt MCF-7 cell apoptosis than free PTX[60]. The stearic acid grafted carboxymethyl chitosan (molecular weight of 230 ± 40 kDa and degree of deacetylation (DD) 85%) was modified with folic acid to form amphiphilic nanoparticles possessing pH-sensitive dissolution (pH 5.6), low cytotoxicity and high amount of drug encapsulation. PTX-loaded nanoparticles (5–25 μg/ml) exhibited a noteworthy viability inhibiting effect on tumour cells (HeLa) over-expressing FR in comparison to non-over-expressing NIH/3T3 cells, with IC_{50} values of PTX loaded nanoparticles of 10.5 and 25 μg/ml respectively[61].

Mitomycin C

Mitomycin C (MMC) is a chemotherapeutic mediator and utilized as a therapy for esophageal carcinoma, breast, anal and bladder cancers. But it causes numerous side-effects such as nausea, vomiting, hair loss, skin rash, lung damage and shortness of breath when administered as a free drug. Hence it is conjugated with chitosan to treat cancer with reduced side-effects. The chitosan nanoparticles were modified with mPEG and folate to selectively deliver the anticancer drug MMC to cancer cells. The use of both PEG and folic acid led to enhanced nanoparticle uptake by tumour cells and the blood circulation time was increased. The *in vitro* study demonstrated a biphasic behaviour with a slower release after an initial burst release. *In vivo* studies of chitosan nanoparticles modified with both mPEG and folate, coated with MMC, were carried out on 4-week-old mice. The nanoparticles were implanted subcutaneously with hepatoma-22 cells of a mouse. Bio-distribution was studied using rhodamine B-labeled particles when tumours reached 0.2–0.5 cm in diameter. Both folated nano-carriers (FA-NPs and mPEG-FA-NPs) displayed higher level of tumour tissue accumulation than the non-folated (mPEG-NPs) carrier system[62]. PEG-modified chitosan nanoparticles (CS-NPs) were loaded with both mitomycin C and methotrexate (MTX) as a multi-drug delivery system in which folic acid acts as a targeting ligand. *In vitro* cell viability analysis specified that the (MTX + MMC)–PEG–CS–NPs presented a concentration- and time-dependent cytotoxicity. Furthermore, *in vitro* cellular uptake recommended that (MTX + MMC)–PEG–CS–NPs could be proficiently taken up by cancer cells via FA receptor-mediated endocytosis. In contrast, (MTX + MMC)–PEG–CS–NPs can co-deliver MTX and MMC to not only attain the highest accumulation at tumour site but also powerfully reduce the growth of tumour cells compared with the delivery of a single drug[63]. The derivatives of *N*-succinyl-chitosan were loaded with MMC and the conjugate is soluble in water when the MMC content is less than 12%, due to the hydrophilic property of *N*-succinyl-chitosan. These conjugates possessed excellent anti-cancer activity against a number of cancers such as murine leukemias (L1210 and

P388), and a murine hepatic cell carcinoma (MH134)[64]. The folate functionalized MMC-SPC (soybean phosphatidyhlcholine) phospholipid complexes when loaded with 10-hydroxycamptothecin (HCPT), possessed nano size, well-regulated drug loading efficiency, and pH-dependent drug release. The *in vitro* study revealed that MMC/HCPT loaded FA micelles presented an increased uptake by tumour cells through receptor-mediated endocytosis. The result of *in vitro* cell viability study presented that the MMC/HCPT loaded FA-micelles demonstrated time- and concentration-dependent cytotoxicity. The cytotoxicity of combined drugs is significantly enhanced compared to both the free drugs and necessarily deters tumour growth than free drugs[65] as represented in Figure 5. MMC–SPC complex loaded phytosomes (MMC-loaded phytosomes) as drug carriers were surface-modified with folate-PEG (FAPEG) to attain reduced toxicity and a superior MMC mediated therapeutic effect. The outstanding properties of FA-PEG-MMC-loaded phytosomes comprise better cellular uptake in HeLa cells and developed accumulation in H22 tumour-bearing mice over that of the PEG-MMC-loaded phytosomes. Moreover, FA-PEG-MMC-loaded phytosomes were accompanied with superior cytotoxic activity *in vitro* and an enhanced antitumour effect *in vivo* compared to that resulting from free MMC injection[66].

Folic acid conjugated chitosan for the delivery of genes

The usage of chitosan and its derivatives as gene transporters displays good biocompatibility and biodegradability, but the low transfection efficiency of DNA and low cell specificity, should be upgraded before testing in clinical trials. Folic acid modified chitosan is one of the most universally engaged carriers for specific cell targeting not only for drug delivery but also for gene delivery. These systems possibly encourage internalization of nucleic acids into the cell through receptor-mediated endocytosis with the overall result of improving the transfection efficiency. The synergism of formulation of folic acid-g-chitosan/DNA complexes as nanoparticulates and targeting ability can lead to significant advancements in gene delivery[67]. In a study, the FA and PEG were linked to chitosan-graft-polyethylenimine (CHI-g-PEI) to improve solubility and transfection efficiency. The FA-PEG-grafted CHI-g-PEI (FA-PEG-CHI-g-PEI) successfully decomposed the plasmid DNA (pDNA) into nanoparticles with a positive surface charge under an appropriate nitrogen/phosphorus (N/P) ratio. The transfection efficiency of FA-PEG-CHI-g-PEI/pDNA complex in 293T cells and LoVo cells, which are the folate receptors-over expressing cell lines, increased with increasing N/P proportion, but there was no noteworthy change in human lung carcinoma cells (A549), which are the folate receptors-deficient cell lines. The copolymer presented less cytotoxicity and showed high FA-media receptor specificity *in vitro* than the 25 kDa PEI and CHI-g-PEI. FA–PEG–CHI-g-PEI displayed enhanced transfection efficiency *in vivo* that represents the ability of FA–PEG–CHI-g-PEI to be a harmless as well as an effective gene carrier[68]. Folated poly(ethylene glycol)-chitosan-graft-polyethylenimine (FPCP) was fabricated and characterized. The results proposed that FPCP has low cytotoxicity in different cells, and FPCP–DNA complexes exhibited better specificity and increased transfection efficiency. Additionally, FPCP complexes reduced tumour growth more efficiently than PEI in H-ras12V liver cancer mice. From the observed results, it is evident that enhanced transfection efficiency and specific cell targeting is beneficial in gene therapy for liver cancer[69]. The physicochemical characteristics of folated-PEG and dual amino acid-modified chitosan (CM–PFA) complexed with DNA were evaluated by FTIR. The CM–PFA nanocarriers presented better biocompatibility and were internalized by target cells, attaining a 3.7 times rise in gene expression. *In vivo*-mimicking 2D co-cultures established a genuine attraction to tumour cells and an insignificant uptake in non-cancerous cells[70]. Nanoparticles containing an Au–Ag bimetallic core and a folated-chitosan shell (Au–Ag@CS-FA) NPs were consequently premeditated by numerous techniques such as scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM) and UV-visible spectra. DNA binding capability was also assessed. The results indicate that Au-Ag@CS-FA NPs are stable with better biocompatibility and strong DNA binding capacity that make them outstanding transportater of genes[71]. A chitosan-poly(ethylenimine) linked with folic acid (CP1.3K-FA) possesses reduced cytotoxicity with enhanced transfection efficiency. CP1.3K-FA demonstrated higher transfection ability than the unmodified chitosan-poly(ethylenimine) copolymer and it was comparable with the transfection efficiency of Fugene HD in B16 and U87 cells, which suggests that CP1.3K-FA can be used as a gene carrier for cancer treatment[72]. Yan *et al.*[73] synthesized

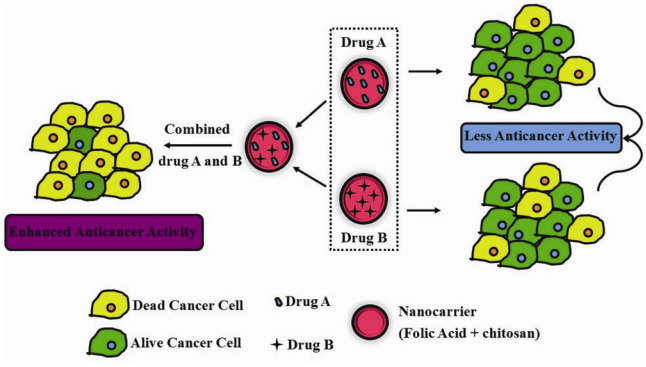


Figure 5. Multi-drug delivery utilizing folic acid-chitosan drug delivery system.

Table 2. Overview of cancer drug delivery using folic acid–chitosan conjugation

Drug	Type of cancer	Key findings	Reference
Doxorubicin (DOX)	Retinoblastoma	Better cytotoxic effect Improved intracellular uptake	34
	Liver cancer	Enhanced the cell uptake	35
	Liver cancer	High drug loading capacity Folate receptor mediated endocytosis	36
	Lung cancer	Higher cell uptake	37
	Cervical cancer	Excellent loading capacity pH dependent drug release	38
	Liver cancer	Increased cytotoxicity	25
	Lung cancer	pH dependent and closely related with the grafting proportions of two hydrophobic ingredients	39
	Breast cancer	Controlled drug release More effective folate-mediated endocytosis	40
	Oral cancer	Effective suppression of tumour growth	43
	Liver cancer	Efficient cell uptake More stable	44
5-Fluorouracil (5-FU)	Colon cancer	Improved specific cell targeting	45
	Breast cancer	Non-toxicity to non-cancerous tissue	46
	Cervical cancer	Sustained drug release profile	47
	Lung cancer	Increased solubility of drug Enhanced targeting efficiency	49
	Breast cancer	Increased cytotoxicity Increased mean residence time of drug	51
Paclitaxel (PTX)	Lung cancer	Higher expression of folate receptor Enhanced cytotoxicity Improved intracellular delivery	53
	Cervical cancer	Improved cytotoxicity	54
	Breast cancer	Enhanced solubility and cellular uptake	55
	Breast cancer	Increased apoptosis induction Controlled drug release	57
	Cervical cancer	pH dependent Improved drug encapsulation efficiency	58
Mitomycin C (MMC)	Liver cancer	Enhanced cellular uptake Increased blood circulation time Higher level of tumour tissue accumulation	59
	Cervical cancer	Concentration and time dependent cytotoxicity Powerfully suppress the growth of tumour cells	60
	Cervical cancer	Well-controllable drug loading efficiency Sustained and pH-dependent drug release	62
	Liver cancer	Better cellular uptake Superior cytotoxicity Enhanced antitumour effect	63

a new kind of Tat tagged and folate-modified N-succinyl-chitosan (Tat–Suc–FA) nanoparticles, for delivery of genes to cancer cells. The Tat–Suc–FA NPs exhibited less toxicity than chitosan displayed by cytotoxicity assay. The particle size of Tat–Suc–FA/DNA complexes exhibited a spherical and compact morphology and was found to be in the range of 54 and 106 nm. Zeta potentials of these complexes varied from 3 to 44 mV as the weight ratio changed. Overall, the results propose that low toxic Tat–Suc–FA cationic polymers could be considered for gene delivery vectors[73]. The designed folic acid modified with Schiff-base conjugated imidazolechitosan (FA–SLICS) having pH sensitive Schiff-base moieties along chitosan backbones resulted in controlled and targeted release of loaded pDNA in the endosomal microenvironment. FA–SLICS has insignificant cytotoxicity to non-cancerous cells, but presents slight toxicity to cancer cells (HeLa and HepG2)[74]. Further, for effective folate receptor-expressing ovarian cancer cells transfection, the siRNA/folic acid–poly(ethylene glycol)–chitosan oligosaccharide lactate (FA–PEG–COL) nanoparticles were fabricated. FA–PEG–COL nanoparticles exhibited greater blood compatibility and enhanced cell viability than COL nanoparticles. *In vitro* transfection and gene knockdown efficiency of HIF-1α were found to be superior than COL nanoparticles in the range of 76%–62%. Further, active

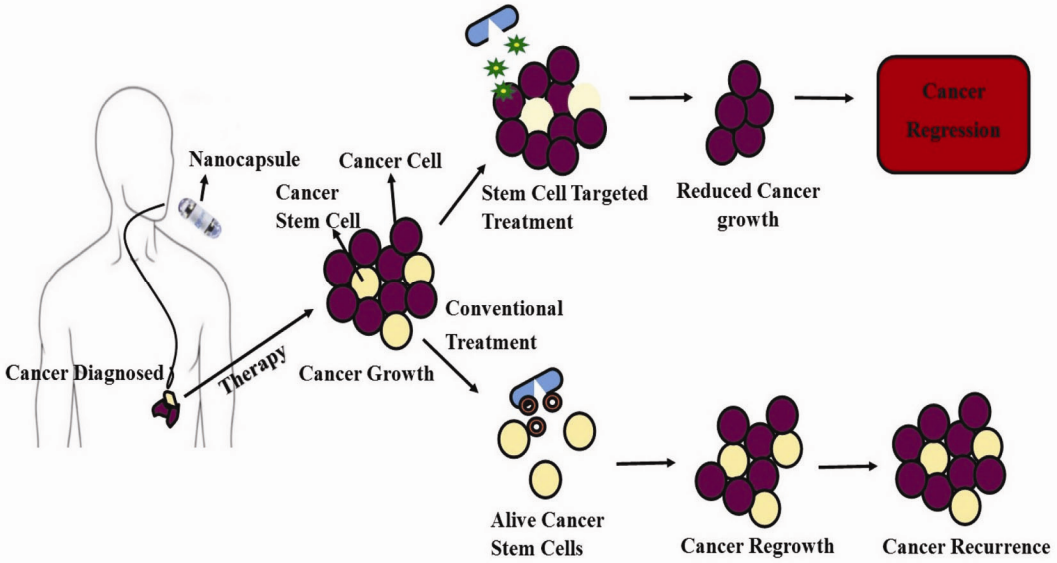


Figure 6. Application of nanotheranostics for active targeting of cancer stem cells.

Table 3. Outline of gene delivery using folic acid–chitosan conjugation

Nanocarrier	Findings	Reference
FA–PEG–CHI–g-PEI	Increased transfection efficiency in folate receptor positive cells (293T cells and LoVo cells) No noteworthy changes in folate receptors deficient cell lines (A549)	65
FPCP	Low cytotoxicity Good cancer cell specificity Increased transfection efficiency	66
CM–PFA	Better biocompatibility Enhanced gene expression Insignificant uptake in normal cells	67
Au–Ag@CS–FA	Strong DNA binding ability Stable with good biocompatibility	68
CP1.3K–FA	Reduced cytotoxicity Enhanced transfection efficiency	69
Tat–Suc–FA	Less toxicity Efficient condensation of DNA	70
FA–SLICS	Controlled and targeted delivery Insignificant cytotoxicity to normal cells Efficiently transfect folate receptor positive cells	71
FA–PEG–COL	Greater compatibility with erythrocytes Superior gene knockdown efficiency	72
FA–LA–PMLA–LMC	Highlighted the importance of hydrophobic and hydrophilic grafting degrees	73
FPCPHDs	Enhanced gene transfection and expression in KB cells	74
F–PEG–g-TMC and mPEG–g-TMC	Improved cellular uptake Increased transfection efficiency	75

targeting FA–PEG–COL nanoparticles indicated higher accumulation than passive targeting COL nanoparticles[75]. FA modified amphiphilic linoleic acid (LA) and poly (β-malic acid) (PMLA) double-grafted chitosan (LMC) nanoparticles (NPs) were prepared for the co-delivery of PTX and surviving shRNA-expressing plasmid (iSur-pDNA). PTX loading, cellular uptake, nuclear accumulation of pDNA, *in vitro* gene silencing efficiency, and cell growth inhibition were stimulated through FA functionalization and greater grafting degree of LA, but inhibited

by increasing grafting degree of PMLA. In tumour-bearing mice, co-delivery of PTX and iSur-pDNA presented superior antitumour activity and extended survival period[76]. A pH-dependent core-shell system FA-PEG-CCTS/PAMAM/HMGB1/pDNA nanocomplexes (FPCPHDs), was synthesized and examined. FPCPHDs exhibited negligible toxic effects on HepG2 and KB cells and improved gene transfection and expression in KB cells displayed by luciferase activity assay and RFP fluorescence intensity analysis. In addition, gene transfection and expression in KB cells were subdued by free folic acid[77]. Folate-poly(ethylene glycol)-grafted-trimethyl chitosan (F-PEG-g-TMC) and methoxypolyethylene glycol-grafted-trimethyl chitosan (mPEG-g-TMC)/pDNA complexes were fabricated and tested. The cellular uptake of F-PEG-g-TMC/pDNA with N/P ratio of 20 in KB cells was amplified by 1.68 times than that of mPEG-g-TMC/pDNA (N/P ratio 20) resulting in 1.5-fold and 1.4-fold improved transfection efficiency in KB cells and SKOV3 cells respectively. F-PEG-g-TMC/pDNA showed increased cellular uptake and transfection efficiency when compared with folate-TMC/pDNA because of the stabilizing effect of PEGylation[78].

Conclusion

In this review, the application of smart carriers based on folated chitosan was reported. Among the chitosan nanoparticle synthesis techniques discussed, the most widely developed are ionic gelation and polyelectrolyte complex methods as these possess an advantage of easy synthesis of chitosan nanoparticle without the use of organic solvents and harmful conditions. Several studies have pointed out the possible application of folated chitosan nanoparticles for tumour-specific delivery of antitumour agents and a number of illustrations presenting promising results are summarized in Tables 2 and 3. At present, the chitosan-based gene carrier is efficient in folate receptor positive cells like ovarian, breast, colon, renal, lung, etc. and increases transfection efficiency with low cytotoxicity[79], when compared with non-folate receptor cells. Hence, the codelivery of drug and gene to non-folate receptor cancer cells should be better exploited for superior anticancer activity.

An attention-grabbing aspect underlined by the described systems beyond targeted delivery can be related to the concept of nanotheranostics[80]. Nanotheranostics, a clever practice targets to observe the consequence of treatment given, enhances drug efficacy and safeguarding the patient. A comprehensive study on nanotheranostic techniques for cancer should be encountered to assist in personalizing chemotherapy. Cancer stem cells are not responsive to chemotherapy and subsequently incline to persist in the body even after a course of treatment was completed, and they can repeatedly prompt cancer recurrence or metastasis as displayed in Figure 6. The folated chitosan nanoparticles to deliver an anti-tumour drug, which kills both cancer cells and cancer stem cells considerably diminish cancer recurrence are to be fabricated and investigated in depth with more pre-clinical trials. Additional improvements are required to turn the concept of targeting both cancer cells and cancer stem cells based on folated chitosan nanoparticle technology into a realistic practical application as the next generation of drug delivery system.

1. https://www.cancer.org/content/dam/cancer-org/research/cancer-facts-and-statistics/annual-cancer-facts-and-figures/2017/cancer-facts-and-figures-2017.pdf (accessed on 31 May 2017).
2. https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/chemotherapy/how-chemotherapy-drugs-work.html (accessed on 31 May 2017).
3. http://www.understandingnano.com/cancer-treatment-nanotechnology.html (accessed on 31 May 2017).
4. Dand, N. M., Patel, P. B., Ayre, A. P. and Kadam, V. J., Polymeric micelles as a drug carrier for tumour targetting. *Chron. Young Sci.*, 2013, **4**, 94–101.
5. Saeed, S. E., Mahnaz, T., Mehdi, F., Javad, M. and Bahram, R., Effects of Levodopa loaded chitosan nanoparticles on cell viability and caspase-3 expression in PC12 neural like cells. *Neurosciences*, 2013, **18**(3), 281–283.
6. Torchilin, V., Tumour delivery of macromolecular drugs based on the EPR effect. *Adv. Drug Deliv. Rev.*, 2011, **63**, 131–135.
7. Maeda, H., Macromolecular therapeutics in cancer treatment: the EPR effect and beyond. *J. Control Release*, 2012, **164**, 138–144.
8. Tsume, Y., Hilfinger, J. M. and Amidon, G. L., Enhanced cancer cell growth inhibition by dipeptide prodrugs of floxuridine: increased transporter affinity andmetabolic stability. *Mol. Pharm.*, 2008, **5**(5), 717–727.
9. Song, H. *et al.*, Folic acid–chitosan conjugated nanoparticles for improving tumour-targetted drug delivery. *BioMed. Res. Int.*, 2013, 1–6.
10. Lu, Y. and Low, P. S., Folate-mediated delivery of macromolecular anticancer therapeutic agents. *Adv. Drug Deliv. Rev.*, 2002, **54**(5), 675–693.
11. Prabaharan, M., Chitosan-based nanoparticles for tumour-targetted drug delivery. *Int. J. Biol. Macromolec.*, 2015, **72**, 1313–1322.
12. Agarwal, M., Nagar, D. P., Srivastava, N. and Agarwal, M. K., Chitosan nanoparticles-based drug delivery: an update. *Int. J. Adv. Multidiscip. Res.*, 2015, **2**(4), 1–13.
13. Patel, M. P., Patel, R. R. and Patel, J. K., Chitosan mediated targetted drug delivery system: a review. *J. Pharm. Pharma. Sci.*, 2010, **13**, 536–557.
14. Jie, J., Wu, W. Z., Zhong, Z. R., Guang, X. T. X., Shu, L. Z. and Wang, L., Recent advances of chitosan nanoparticles. *Int. J. Nanomedicine*, 2011, **6**, 765–774.
15. Wu, J. *et al.*, Chitosan nano carriers loading anti-tumour drugs. *J. Nano. Res.*, 2015, **32**, 113–127.
16. Shia, J. *et al.*, Immunohistochemical expression of folate receptor-alpha in ovarian epithelial neoplasms bears clinical and pathological significance. *Mod. Pathol.*, 2009, **22**, 237a.
17. LeBlanc, J. G., de Giori, G. S., Smid, E. J., Hugenholtz, J. and Sesma, F., Folate production by lactic acid bacteria and other food-grade microorganisms. *Current Research and Educational Topics and Trends in Applied Microbiology* (ed. Méndez-Vilas, A.), 2007, pp. 329–339.
18. Amidi, M., Mastrobattista, E., Jiskoot, W. and Hennink, W. E., Chitosan-based delivery systems for protein therapeutics and antigens. *Adv. Drug Deliv. Rev.*, 2010, **62**(1), 59–82.

19. Prabaharan, M. and Mano, J. F., Chitosan-based particles as controlled drug delivery systems. *Drug Deliv.*, 2005, **12**, 41–57.
20. Tiyaboonchai, W. and Limpeanchob, N., Formulation and characterization of amphotericin B-chitosan-dextran sulfate nanoparticles. *Int. J. Pharm*., 2007, **329**, 142–149.
21. Lankalapalli, S. and Kolapalli, V. R. M., Polyelectrolyte complexes: a review of their applicability in drug delivery technology. *Indian J. Pharm. Sci*., 2009, **71**(5), 481–487.
22. Kumar, N., Patel, A. K., Kumari, N. and Kumar, A., A review on chitosan nanoparticles for cancer treatment. *Int. J. Nanomater. Bios*., 2014, **4**(4), 63–65.
23. Huang, H. Y., Shieh, Y. T., Shih, C. M. and Twu, Y. K., Magnetic chitosan/iron (II, III) oxide nanoparticles prepared by spray-drying. *Carbohydr Polym*., 2010, **81**(4), 906–910.
24. Goldberg, M., Langer, R. and Jia, X., Nanostructured materials for applications in drug delivery and tissue engineering. *J. Biomater. Sci. Polym. Ed*., 2007, **18**(3), 241–268.
25. Guaragna, A., Chiaviello, A., Paolella, C., D'Alonzo, D. and Palumbo, G., Synthesis and evaluation of folate-based chlorambucil delivery systems for tumour-targetted chemotherapy. *Bioconjug. Chem*., 2011, **23**(1), 84–96.
26. Vllasaliu, D., Casettari, L., Bonacucina, G., Cespi, M., Palmieri, G. P. and Illum, L., Folic acid conjugated chitosan nanoparticles for tumour targetting of therapeutic and imaging agents, *Pharm. Nanotechnol*., 2013, **1**, 184–203.
27. Chakraborty, S. P., Sahu, S. K., Pramanik, P. and Roy, S., Biocompatibility of folate–modified chitosan nanoparticles. *Asian Pac. J. Trop. Biomed*., 2012, **2**(3), 215–219.
28. Sahu, S. K., Maiti, S., Maiti, T. K., Ghosh, S. K. and Pramanik, P., Folate-decorated succinylchitosan nanoparticles conjugated with doxorubicin for targetted drug delivery. *Macromol. Biosci*., 2011, **11**(2), 285–295.
29. Jiang, H. L. *et al*., The suppression of lung tumourigenesis by aerosol-delivered folatechitosan-graft-polyethylenimine/Akt1 shRNA complexes through the Akt signalling pathway. *Biomater*., 2009, **30**(29), 5844–5852.
30. Bhattacharya, S., Li, X., Nyshadham, J. and Jasti, B., Folate receptor targetted delivery systems: a novel micellar drug delivery approach. *Curr. Trends Biotechnol. Pharm.*, 2010, **4**(1), 490–509.
31. Ke, J. H., Lin, J. J., Carey, J. R., Chen, J. S., Chen, C. Y. and Wang, L. F., A specific tumour-targetting magnetofluorescent nanoprobe for dual-modality molecular imaging. *Biomaterials*, 2010, **31**, 1707–1715.
32. Bahrami, B. *et al.*, Folate-conjugated nanoparticles as a potent therapeutic approach in targetted cancer therapy. *Tumour Biol*., 2015, **36**(8), 5727–5742.
33. Park, J. H., Lee, S., Park, K., Kim, K. and Kwan, I. C., Smart chitosan-based stimuli-responsive nanocarriers for the controlled delivery of hydrophobic pharmaceuticals. *Macromolecules*. 2011, **44**, 1298–1302.
34. Neha, M. D., Pranav, B. P., Anita, A. and Vilasrau, J. K., Polymeric micelles as a drug carrier for tumour targetting. *Chron. Young Sci.*, 2013, **4**(2), 94–101.
35. Goren, D., Horowitz, A. T., Tzemach, D., Tarshish, M., Zalipsky, S. and Gabizon, A., Nuclear delivery of doxorubicin via folate-targetted liposomes with bypass of multidrug-resistance efflux pump. *Clin. Cancer Res*., 2000, **6**(5), 1949–1957.
36. Yang, H. C. and Hon, M. H., The effect of the molecular weight of chitosan nanoparticles and its application on drug delivery. *Microchem. J*., 2009, **92**(1), 87–91.
37. Parveen, S. and Sahoo, S. K., Evaluation of cytotoxicity and mechanism of apoptosis of doxorubicin using folate-decorated chitosan nanoparticles for targetted delivery to retinoblastoma. *Cancer Nanotechnol*., 2010, **1**(1–6), 47–62.
38. Fan, L. *et al*., Co-delivery of PDTC and doxorubicin by multifunctional micellar nanoparticles to achieve active targetted drug delivery and overcome multidrug resistance. *Biomaterials*, 2010, **31**(21), 5634–5642.
39. Shen, J. M., Tang, W. J., Zhang, X. L., Chen, T. and Zhang, H. X., A novel carboxymethyl chitosan-based folate/Fe_3O_4/CdTe nanoparticle for targetted drug delivery and cell imaging. *Carbohydr. Polym*., 2012, **88**(1), 239–249.
40. Hu, H., Tang, C. and Yin, C., Folate conjugated trimethylchitosan/graphene oxide nanocomplexes as potential carriers for drug and gene delivery. *Mater Lett.,* 2014, **125**, 82–85.
41. Yu, J. *et al*., Folic acid conjugated glycol chitosan micelles for targetted delivery of doxorubicin: preparation and preliminary evaluation *in vitro*. *J. Biomater. Sci. Polym. Ed*., 2013, **24**(5), 606–620.
42. Chen, D. *et al.*, pH responsive mechanism of a deoxycholic acid and folate comodified chitosan micelle under cancerous environment. *J. Phys. Chem. B*, 2013, **117**(5), 1261–1268.
43. Manaspon, C., Viravaidya-Pasuwat, K. and Pimpha, N., Preparation of folate-conjugated pluronic f127/chitosan core-shell nanoparticles encapsulating doxorubicin for breast cancer treatment. *J. Nanomater*., 2012, **2012**, 1–11.
44. Depan, D., Shah, J. and Misra, R. D. K., Controlled release of drug from folate-decorated and graphene mediated drug delivery system: Synthesis, loading efficiency, and drug release response. *Mater. Sci. Eng. C*, 2011, **31**(7), 1305–1312.
45. Huang, H., Yuan, Q., Shah, J. S. and Misra, R. D. K., A new family of folate decorated carbon nanotube-mediated drug delivery system: synthesis and drug delivery response. *Adv. Drug Deliv. Rev*., 2011, **63**(14–15), 1332–1339.
46. Lee, K. D., Choi, S. H., Kim, D. H., Lee, H. Y. and Choi, K. C., Self-organized nanoparticles based on chitosan-folic acid and dextran succinate-doxorubicin conjugates for drug targetting. *Arch. Pharm. Res*., 2014, **37**, 1546–1553.
47. Ji, Z. *et al.*, Targeted therapy of SMMC-7721 liver cancer *in vitro* and *in vivo* with carbon nanotubes based drug delivery system. *J. Colloid Interf. Sci*., 2012, **365**(1), 143–149.
48. Wang, Y., Li, P., Chen, L., Gao, W., Zeng, F. and Kong, L. X., Targeted delivery of 5-fluorouracil to HT-29 cells using high efficient folic acid-conjugated nanoparticles. *Drug Deliv*., 2015, **22**(2), 191–198.
49. Mathew, M. E., Mohan, J. C., Manzoor, K., Nair, S. V., Tamura, H. and Jayakumar, R., Folate conjugated carboxymethyl chitosan manganese doped zinc sulphide nanoparticles for targetted drug delivery and imaging of cancer cells. *Carbohydr. Polym*., 2010, **80**(2), 443–449.
50. Kadagi, M. *et al*., Synthesis, characterisation of 5-Fu loaded chitosan nanoparticles, *Glob. J. Res. Anal.*, 2014, **3**(9), 114–116.
51. Yu, S. *et al.*, Inorganic nanovehicle for potential targetted drug delivery to tumour cells, tumour optical imaging, *ACS Appl. Mater. Interf.,* 2015, **7**, 5089–5096.
52. Li, H. L., He, Y. X., Gao, Q. H. and Wu, G. H., Folate-polyethylene glycol conjugated carboxymethyl chitosan for tumour-targetted delivery of 5-fluorouracil. *Mol. Med. Rep*., 2014, **9**, 786–792.
53. Yang, Z. M., Peng, Z. and Zhou, M., Drug-loading chitosan polymer microsphere with targetted and slow-release function and its characteristics. *J. Funct. Mat*., 2013, **44**(12), 1703–1708.
54. Blanco, M. D., Guerrero, S. and Benito, M., *In vitro* and *in vivo* evaluation of a folate-targetted copolymeric submicrohydrogel based on *n*-isopropylacrylamide as 5-fluorouracil delivery system. *Polym*., 2011, **3**, 1107–1125.
55. Vasanti, S. and Preeti, S., Paclitaxel nanoparticles – an approach to improve the bioavailability. *Int. J. Pharm. Sci. Rev. Res*., 2014, **27**(1), 200–208.
56. You, J., Li, X., De Cui, F., Du, Y. Z., Yuan, H. and Hu, F. Q., Folate-conjugated polymer micelles for active targetting to cancer cells: preparation, *in vitro* evaluation of targetting ability and cytotoxicity. *Nanotechnol*., 2008, **19**(4), 1–9.

57. Lan, G. J., Sen-ming, W., Xi-gang, H., Man-ming, C. A. O. and Ji-ren, Z., Synthesis and characterization of folic acid-conjugated chitosan nanoparticles as a tumour-targetted drug carrier. *J. South Med. Univ*., 2008, **28**(12), 2183–2186.
58. Qu, D., Lin, H., Zhang, N., Xue, J. and Zhang, C., *In vitro* evaluation on novel modified chitosan for targetted antitumour drug delivery. *Carbohydr. Polym*., 2013, **92**(1), 545–554.
59. Wang, F. *et al.*, Tissue distribution and pharmacokinetics evaluation of DOMC-FA micelles for intravenous delivery of PTX. *J. Drug Deliv*., 2013, **21**(2), 137–145.
60. Huang, S., Wan, Y., Wang, Z. and Wu, J., Folate-conjugated chitosan–polylactide nanoparticles for enhanced intracellular uptake of anticancer drug. *J. Nanopart. Res*., 2013, **15**, 1–15.
61. Sahu, S. K., Maiti, S., Maiti, T. K., Ghosh, S. K. and Pramanik, P., Hydrophobically modified carboxymethyl chitosan nanoparticles targetted delivery of paclitaxel. *J. Drug Target*, 2011, **19**(2), 104–113.
62. Hou, Z. *et al.*, Both FA- and mPEG-conjugated chitosan nanoparticles for targetted cellular uptake and enhanced tumour tissue distribution. *Nanoscale Res. Lett*., 2011, **6**(1), 563–574.
63. Jia, M., Li, Y. and Yang, X., Development of both methotrexate and mitomycin c loaded pegylated chitosan nanoparticles for targetted drug codelivery and synergistic anticancer effect. *Appl. Mater. Interfaces*, 2014, **6**, 11413–11423.
64. Patel, M. P., Patel, R. R. and Patel, J. K., Chitosan mediated targetted drug delivery system: a review. *J. Pharm. Pharmaceut. Sci*., 2010, **13**(3), 536–557.
65. Lin, J., Li, Y. and Wu, H., Tumour-targetted co-delivery of mitomycin C and 10-hydroxycamptothecin via micellar nanocarriers for enhanced anticancer efficacy. *RSC Adv.*, 2015, **5**, 23022–23033.
66. Li, Y., Wu, H. and Jia, M., Therapeutic effect of folate-targetted and pegylated phytosomes loaded with a Mitomycin C-soybean phosphatidyhlcholine complex. *Mol. Pharmaceu*., 2014, **11**, 3017–3026.
67. Morris, V. B., Pillai, C. K. S. and Sharma, C. P., Folic acid-conjugated depolymerized quaternized chitosan as potential targetted gene delivery vector. *Polym. Int*., 2011, **60**(7), 1097–106.
68. Zhou, Y., Chen, J. and Wang, H., Synthesis and characterization of folate-poly(ethylene glycol) chitosan graft-polyethylenimine as a non-viral carrier for tumour-targetted gene delivery. *Afr. J. Biotechnol*., 2011, **10**(32), 6120–6129.
69. Kim, Y. K., Tehrani, A. M., Lee, J. H., Cho, C. S., Cho, M. H. and Jiang, H. L., Therapeutic efficiency of folated poly(ethylene glycol)-chitosan-graft-polyethylenimine-Pdcd4 complexes in H-ras12V mice with liver cancer. *Int. J. Nanomed*., 2013, **8**, 1489–1498.
70. Gaspar, V. M., Costa, E. C., Queiroz, J. A., Pichon, C., Sousa, F. and Correia, I. J., Folate-targetted multifunctional amino acid-chitosan nanoparticles for improved cancer therapy. *Pharm. Res*., 2015, **32**, 562–577.
71. Guana, Q. and Wang, M., Fabrication and characteristics of gene-delivering nanodevices based on Au-Ag@CS-FA hybrid particles. *Mater. Sci. Forum*, 2015, **815**, 401–406.
72. Lai, W. F. and Lin, M. C., Folate-conjugated chitosan-poly(ethylenimine) copolymer as an efficient and safe vector for gene delivery in cancer cells. *Curr. Gene Ther*., 2015, **15**(5), 472–480.
73. Yan, C. Y., Gu, J. W. and Hou, D. P., Synthesis of tat tagged and folate modified *N*-succinyl-chitosan self-assembly nanoparticles as a novel gene vector. *Int. J. Biol. Macromol.*, 2015, **72**, 751–756.
74. Shi, B., Zhang, H., Bi, J. and Dai, S., Endosomal pH responsive polymers for efficient cancer targetted gene therapy. *Colloids Surf. B*, 2014, **119**, 55–65.
75. Li, T. S., Yawata, T. and Honke, K., Efficient siRNA delivery and tumour accumulation mediated by ionically cross-linked folic acid-poly(ethylene glycol)-chitosan oligosaccharide lactate nanoparticles: for the potential targetted ovarian cancer gene therapy. *Eur. J. Pharm. Sci*., 2014, **52**, 48–61.
76. Yu, B., Tang, C. and Yin, C., Enhanced antitumour efficacy of folate modified amphiphilic nanoparticles through co-delivery of chemotherapeutic drugs and genes. *Biomaterials*, 2014, **35**(24), 6369–6378.
77. Wang, M., Hu, H. and Sun, Y., A pH-sensitive gene delivery system based on folic acid-PEG-chitosan-PAMAM-plasmid DNA complexes for cancer cell targetting. *Biomaterials*, 2013, **34**(38), 10120–10132.
78. Zheng, Y., Song, X. and He, G., Receptor-mediated gene delivery by folate-poly(ethylene glycol)-grafted-trimethyl chitosan *in vitro*. *J. Drug Target*, 2011, **19**(8), 647–656.
79. Parker, N., Turk, M. J., Westrick, E., Lewis, J. D., Low, P. S. and Leamon, C. P., Folate receptor expression in carcinomas and normal tissues determined by a quantitative radioligand binding assay. *Anal. Biochem*., 2005, **338**, 284–293.
80. Bwatanglang, I. B., Mohammad, F. and Yusof, N. A., Folic acid targetted Mn : ZnS quantum dots for theranostic applications of cancer cell imaging and therapy. *Int. J. Nanomed.*, 2016, **11**, 413–428.

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