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Folic Acid Conjugated Nano Systems: A Systematic Review

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Abstract

Cancer is the leading cause of death worldwide though it can be treated by the common ways of chemotherapy, radiotherapy, and surgery. The major limitation of conventional chemotherapy is the non-selective action of chemotherapeutic agents which leads to serious side effects such as damage to normal cells that hampers the immunity of the patient to fight against the disease. Active targeting mechanism is one of the approaches through which a chemotherapeutic agent can be delivered to malignant cells more selectively to the tumor-specific tissue with the help of ligands including proteins, peptides, hyaluronic acid, folic acid, antibodies, antibody fragments, aptamer, carbohydrates, and polysaccharides, etc. Folic acid conjugated nano systems have proved their efficiency in site-specific targeting of chemotherapeutic agents with reduced side effects as folic acid has an affinity for folate receptors which are overexpressed on several cancer cell surfaces. Various polymers have been utilized to prepare such nanomicelles in an active targeting approach including chitosan, Poly lactic-co-glycolic acid, alginates, human serum albumin, etc. In this review, active targeted nano systems of vincristine, methotrexate, mitoxantrone, doxorubicin, genistein, 5-aminovalaevulinic acid (5-ALA), carboplatin, 6-mercaptopurine, and gemcitabine, kaempferitrin, curcumin, paclitaxel, saquinavir, 5-Flurouracil, tamoxifen, resveratrol, isoalantolactone, and cabazitaxel were discussed which are successfully prepared using various polymers.

Keywords: Folic acid Conjugates; Folate receptor targeted systems; Polymeric nano systems; Active targeted nano systems; Chitosan; PLGA; Alginates; Human Serum Albumin

Introduction

Cancer is one of the leading causes of mortality and morbidity all around the globe regardless of developments in the tools of disease diagnosis, treatment, and prevention measures however the number of cases is constantly increasing and is estimated to be 21 million by 2030 [1]. Most patients treated with conventional chemotherapy suffer from serious side effects due to the non-selective action of chemotherapeutic drugs on normal cells. The fast-growing cells are killed using a single or a combination of drugs. Also, chemotherapeutic agents are cytotoxic by the way of interfering with the mitosis or cell division of cancer cells and inducing stress, and initiating apoptosis which results in the damaging of cells. Normal cells are also found to be susceptible to these effects, in particular cells of bone marrow, digestive tract, and hair follicles [2, 3]. An ideal delivery carrier for anticancer drugs must be capable of transporting the drug specifically to the cancerous tissues and releasing the drug molecules inside the tumor cells [4].

New technologies include nanoparticles for nano-medicines which aim to enhance anticancer activities of plant-derived drugs by controlling the release of the compound and investigating new methods for site-specific administration [5]. Nanoparticle-based targeted drug delivery systems have been extensively investigated for biomedical applications [6]. The unique features of nanoscale carriers are their huge surface area versus volume, ease of surface modification, prolonged circulation in the blood through avoidance of the reticuloendothelial system, and small size that facilitates specific interaction with cell surface receptors, enabling the targeting of specific cells or tissues having the disease in the specific organs. Furthermore, tumor-specific targeting of nanoparticles allows the killing of tumor cells selectively without harm to healthy tissues or cells [7].

Nanoparticles play an important role in increasing drug concentration in cancer cells by enhancing drug accumulation. Since nanoparticles themselves do not possess tumor specificity, various kinds of passive and active targeting mechanisms have been used to bestow tumor-specific drug targeting of nanoparticles [7]. The passive targeting nanoparticle is the mechanism by which the drugs leak from blood vessels supplying cancer cells and accumulate in the cells by enhanced permeability and retention (EPR) effect. The active targeting nanoparticles, on the other hand, various target ligands conjugated on the surface of nanoparticles, resulting in increased cellular uptake by receptor-mediated endocytosis (RME) and therefore increased drug accumulation in cancer cells. These tumor-specific ligands included proteins, peptides, hyaluronic acid, folic acid, antibodies, antibody fragments, aptamer, carbohydrates, and polysaccharides. Nanoparticles acting via both mechanisms have been shown to increase drug concentration in cancer cells resulting in the more efficient delivery of drugs to cancerous cells.

Folic Acid

Water-soluble vitamin B9 can occur as 'folate' when enriched in dark leafy vegetables whereas 'folic acid' is when a synthetic folate compound is used as a vitamin supplement. [8]. Folate is a basic component of cell metabolism and DNA synthesis as well as repair and rapidly dividing cancer cells have an increased requirement for folate to maintain DNA synthesis [9]. Folate is transported across the cellular membrane in three ways. The main route of uptake is through the reduced folate carrier (RFC), which is ubiquitously distributed and aids the uptake of dietary folate. The second route is through the proton-coupled folate transporter (PCFT), which utilizes the Trans

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membrane proton gradient to mediate folate transport into the cells. Finally, Folates are also transported by high-affinity FRs in humans namely FRα, FRβ, FRγ, and FRδ, with molecular weights ranging from 38 to 45 kDa [8]. Out of these four isoforms FRα, FRβ, and FRδ are attached to the cell surface by a glycosylphosphatidylinositol anchor, while FRγ is a secreted protein. The folate receptor 4 (FOLR4) genes encoded a protein unable to bind folic acid or reduced folate, demonstrated in recent studies [10]. The alpha isoform, Folate Receptor α (FRα), also known as FOLR1 or folate binding protein (FBP), is a glycosylphosphatidylinositol (GPI)-anchored membrane protein with a high affinity for binding and coordinating the transport of the active form of folate, 5-methyltetrahydrofolate (5-MTF) [8].

In the non-malignant context, FRα tissue distribution is restricted to a limited number of polarized epithelia with expression localized at the apical/luminal surface of polarized cells and is accessible to circulation while in contrast the context of malignancy, FRα is overexpressed in the majority of tumors of the ovary, uterus, or ependymal brain and malignant pleural mesotheliomas, lung, kidney, breast, and colon carcinomas FRα loses its polarized cellular location and instead, the entire cell surface is covered with FRα proteins and are accessible to circulation. [10].

The folic acid receptor is a high-affinity receptor that mediates the cellular uptake of folic acid and its derivative in the eukaryotic cells [11,12] as it is overexpressed on several cancer cell surfaces including ovarian, cervical, breast, lung, kidney, colorectal, pleura, endometrium, kidney, bladder and brain cancers whereas barely detects on healthy tissues and cells [7,8,11]. To enhance the delivery of the drug to the therapeutic location by decreasing the delivery of the drug to the other site, polymeric micelles are conjugated with ligands for the active targeting of tumor cells [2]. Examples of such polymeric micelles are chitosan, MPEG, PLGA, PEG, carboxymethyl chitosan, alginates, bovine serum albumin, etc. These are used alone with a ligand or in combination with each other the percent expression of folate receptors in various types of cancer is depicted in [8,13,14] (Table 1).

As FRα is expressed on the cell surface in a tumor-specific manner and its ability to bind with folic acid or reduced folate, a relatively innocuous, small molecule that is amenable to chemical conjugation with other molecules, can rapidly penetrate solid tumors, the cell-surface receptor-ligand complex is transported into the cell via receptor-mediated endocytosis, Folic acid or its conjugates combine with folate receptor situated at the surface of cancer cells and are internalized to intracellular compartments to form endosomes. As the conjugation between folate receptors and folate conjugates separates in an acid environment (pH=5.0~5.5) folate receptors return to the cell surface after dissociation, and folate conjugates are degraded by lysosome or released into the cytosol [15] which provides the potential to allow not only tumor localization, but also selected delivery of therapeutic agents to the malignant tissue, minimizing collateral toxic side-effects [10].

One specialized route, pinocytosis, was proposed from the observation that FRa recycled between an acid-resistant (intracellular) and acid-sensitive (extracellular) pool where it has been proposed that FRa was concentrated in clusters in invaginations of the cell membrane surface called caveolae, whereby the membrane would transiently close and internalize the folate-bound receptor complex. Increased acidification of the internal compartment would dissociate folate from the receptor and move it across the membrane into the cytoplasm of the cell using the energy generated by the acidic gradient. The cell surface membrane would then unseal and expose the receptors for the next cycle [9].

Table 1: Percent expression of folate receptors in various types of cancer.

Type of cancer	% Expression of Folate receptor
Ovarian cancer	80%
Lung cancer	72%
Breast cancer	74%
Bladder Cancer	58.8%
Cervical Cancer	92%
Pancreatic	45%

Polymeric nanomicelles have been widely used in medicine as drug delivery carriers by an active targeting approach. They can improve hydrophobic drug delivery, reduce metabolic drug degradation, specific targeting of cancer cells by surface modification using targeted ligand, and display sustained and triggered drug release. In addition to the ability to enhance the aqueous solubility of hydrophobic drugs, nanomicelles also can target cancer cells by two approaches: passive and active. In the active targeting approach, nanomicelles are typically conjugated/ decorated with a targeting moiety, thereby facilitating the preferential accumulation of the drug in selected tissues, individual cancer cells, or intracellular organelles that are associated with specific recognition molecules in cancer cells [4].

Chitosan

Chitosan is a linear polysaccharide composed of β-(1-4)-linked 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose units. Chitosan is obtained from chitin by acetylation of the D-glucose, 2-amino-2-deoxy- unit of chitin in alkali media. It is the second most common natural polysaccharide after cellulose [16]. The chemical structure of chitin and chitosan is very similar to that of cellulose which consists of several hundred to more than a thousand b-(1-4) linked D-glucose units [17]. The amine and -OH groups of chitosan represent a great advantage because it enables distinctive biological functions as well as the application of modification reactions [18]. Furthermore, the excellent properties of these polysaccharides such as biocompatibility, biodegradability, bioactivity, bio resorptivity, non-toxicity and good adsorption properties make these materials very suitable and essential biomaterials with a great deal of industrial attention as probable alternatives to synthetic polymers [19]. Chitosan is stable in neutral environments, solubilizes in acidic environments, transports a drug to an acidic environment and helps in discharging the drug to the preferred site. It alone exhibits comparatively elongated blood circulation time and low uptake by the reticuloendothelial system (RES) [2]. To increase the target specificity, folic acid can be conjugated on the surface of chitosan nanoparticles [20].

Vincristine is an anticancer drug used to treat different types of cancer was reported to become resistant against small cell lung cancer cell lines because of decreased uptake and increased drug efflux. To increase its uptake, vincristine-loaded folic acid-chitosan conjugated nanoparticles were prepared using sodium tripolyphosphate and a polyanion as a cross-linking agent by ionic gelation method with varying ratios. The formulation with a ratio of 4:25 was found with Maximum encapsulation efficiency of 81.25% and loading capacity of 10.31% which is confirmed by Fourier Transform Infrared Spectroscopy (FTIR) and Transmission Electron Microscopy (TEM). Scanning Electron Microscopy (SEM) analysis resulted in spherical structure and rough surface of nanoparticles where High-temperature stability analysis confirmed the stability of vincristine loaded nanoparticles at pH 7.2 and 7.6. Positive zeta potential favored the efficient delivery of loaded vincristine through folic acid–chitosan conjugated nanoparticles to cancer cells with high encapsulation efficiency and loading capacity of vincristine [21].

Methotrexate is one of the classic anticancer agents and is used as monotherapy as well as in combination with other anticancer drugs in multidrug chemotherapy regimens but it is associated with several undesirable side effects like normal cell toxicity, drug resistance, renal toxicity, bone marrow inhibition, liver toxicity, and acute and chronic obstructive pulmonary diseases because of nonselective action of the drug. To overcome such side effects and impart site-specific targeting to tumor cells folic acid-chitosan-methotrexate nanoparticles (FA-Chi-MTX NPs) were prepared by coaxial electrospray atomization method. The chemical conjugation between folic acid and chitosan backbone was confirmed by FTIR analysis whereas the feasibility of prepared (FA-Chi-MTX NPs) to target tumors at extracellular pH was proved by particle size and particle size distribution analysis as drug release was greater in the pH of endosomes (acidic medium). The field emission scanning electron microscope (FE-SEM) analysis results showed the mean diameter of the core-shell NPs was around 304 nm while about 30% of the produced NPs were found in the desirable range. Drug loading and Encapsulation efficiency were found as 4.56 ± 0.15% and 89.6 ± 3.8%, respectively. An increased intracellular drug delivery among the endosome's sites increased the MTX release at pH 5.0 revealed by in vitro drug release studies. The Cell cytotoxicities of Methotrexate (MTX) and FA-Chi-MTX NPs were evaluated by using an MTT assay which revealed the less drug requirement of FA-Chi-MTX NPs in comparison with free MTX for the same therapeutic effect and better treatment of tumor cells in human epithelial cervical cancer [22].

Mitoxantrone is a synthetic antineoplastic drug related to the anthracycline antibiotic group widely used in the treatment of various types of cancer, specifically for metastatic breast cancer, acute myeloid leukemia, and non-small cell lung cancer, non-Hodgkin's lymphoma, and prostate cancer but it is associated with cardiotoxicity in higher doses [23]. The said limitation of the drug was overcome by preparing Folic acid-chitosan conjugated mitoxantrone nanoparticles (FA-CSNP/MTX) using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) as a cross-linking agent by CCK-8 method where the controlled-release capability of formulation in different situations and the specific uptake by HONE1 cells was confirmed by confocal microscopy. The particle size and particle size distribution analysis revealed uniform size distribution of prepared nanoparticles in the range of 48-58 nm. The highest encapsulation efficacy, as well as drug loading efficiency of the particles, was found as 77.5±1.9% and 18.4±0.4% respectively. The potential of targeted delivery of anticancer drug with by Folic acid -chitosan conjugated, mitoxantrone nanoparticles (FA-CSNP/MTX) were proved with sustained release and cell growth inhibition [24].

Folic acid-chitosan conjugated nanoparticles (FA-CS NPs) of doxorubicin hydrochloride (Dox) were prepared to evaluate their targeting specificity on tumor cells by the ionic cross-linking method. Monodisperse Dox loaded FA-CS NPs were found to have an average size of 38 ± 2 nm and surface potential of 13.6 ± 4.8 mV as well as higher cell up taking ability due to the known folate-receptor mediated endocytosis compared to the lower uptake of CS NPs in tumor cells while Dox loaded to FA-CS NPs was found with the encapsulation efficiency and Loading efficiency of 45.4 ± 3.2wt% and 30.5 ± 1.2wt% respectively. The drug release data revealed Dox-loaded FA-CS NPs had a long and biphasic release behavior with the potential as a long-lasting and effective drug delivery system with a promising tumor-targeting [15].

Genistein (4′, 5, 7-trihydroxyisoflavone) (GEN) is a potentially effective bioflavonoid with potent anticancer properties with major drawbacks like limited solubility (~1.45 µg/ml) and limited bioavailability. To improve the physicochemical as well as anti-cancer properties a novel folic acid-conjugated chitosan nanoparticle was formulated for specific delivery of Genistein (GEN) to the cervical cancer cells. Dynamic light scattering (DLS) analysis confirmed the cellular internalization and systemic performance of GEN-loaded nanoparticles with an average particle size of ~140 nm, excellent polydispersity index of ~165 nm, and an average hydrodynamic diameter of less than 200 nm which proved sufficient penetration of drug the tumor tissues. Furthermore, it showed a high entrapment efficiency of more than >95% making it suitable for systemic applications. Spherical shaped morphology and uniform distribution of prepared nanoparticles was confirmed by Transmission Electron Microscopy (TEM). The IC50 value of GEN decreased from 33.8 -14.6 µg/ml when treated with FGCN after 24 h incubation which indicated that Folic acid-targeted nano formulations exhibited superior cytotoxic effect compared to that of non-targeted formulations and proved beneficial for overall cancer treatment [20].

5-aminolaevulinic acid (5-ALA) loaded folic acid-chitosan conjugates were successfully prepared for the treatment of colorectal cancer which is one of the leading causes of malignant death. The loading efficiency of 5-ALA in fCNA particles was found to be in the range of 35-40% with a z-average diameter of 100 nm and the zeta-potential of 20 mV indicating stable nanoparticles without aggregation. The short-term uptake studies revealed that fCNA can be taken up more easily by HT29 and Caco-2 cell lines via receptor-mediated endocytosis and the PpIX accumulates in cancer cells as a function of the folate receptor expression and the folic acid modification appears an ideal vector for colorectal-specific delivery of 5-ALA [25].

5-fluorouracil (5-FU) is a thymidylate synthase inhibitor that is extensively used in the treatment of solid tumors associated with several limitations like poor bioavailability, short plasma half-life, and coupled off-target cytotoxicity. 5-fluorouracil (5-FU) gold nanoparticles with Folic acid (FA) conjugated Chitosan (CS) (FA-CS-GNP-5-FU) were prepared by ionic complexation method for improved drug efficacy with minimal side effects. The results revealed that the obtained nanoparticles were mono dispersed with suitable average sizes and positive surface charges which contributed to excellent stability and pH-dependent and sustained drug release of 5-FU while the Cell toxicity assay confirmed the potential for better efficacy of FA-CS functionalized GNPs with reduced dosage regimen and subsequent side effects [26].

Carboplatin (CRB) possesses superior anticancer activity in cervical cancer cells however suffers from severe side effects like low therapeutic efficacy due to undesirable tissue distributions. To impart the selective delivery of carboplatin to the cervical cancer cells, a unique folic acid-conjugated chitosan-coated poly (DL-lactide co-glycolide) (PLGA) nanoparticles (FPCC) were prepared. The nano sized spherical shaped particles with a size less than <200nm with a narrow size distribution were obtained by particle size and zeta potential analysis as well as dynamic light scattering (DLS) technique which was further confirmed by transmission electron microscopy (TEM) analysis indicating intracellular uptake of nanoparticles in tumor tissue. FPCC showed high entrapment efficiency (EE) of more than 90% with an effective drug loading of 11.85 % while whereas the IC50 value of 0.65 µg/ml. The cellular uptake efficiency analysis indicated that FPCC had a specific affinity for the cancerous, Hela cells owing to ligand-receptor (FA-FR-α) recognition while the Apoptosis assay confirmed the superior cytotoxic effect due to the higher internalization of FPCC inside the cells due to the surface modification of folic acid and continuous exposure as well

as the sustained release of the drug for a prolonged period at the site of action proving the great impact of folate conjugation the cervical cancer cells [27].

6-mercaptopurine is a cytotoxic and immunosuppressant anticancer drug with major limitations of poor bioavailability and short plasma half-life [28]. Folate receptor-targeting and glutathione (GSH)-responsive polymeric pro drug was prepared for enhanced intracellular accumulation, improved the stability and solubility of drugs, to avoid premature leaking in the bloodstream and selective drug release enhancement in target tissues or cells with increased circulation time in the physiological environment of 6-mercaptopurine (6-MP) in leukemia. CMCS-g-PTA pro drugs were synthesized by attaching 6-MP to the carboxyl group of carboxymethyl chitosan via a GSH-sensitive carbonyl vinyl sulfide linkage owing to their amphiphilic structure in an aqueous solution with the advantage of a small amount of drug leakage in the simulated circulatory system and the large quantity of release in the tumor cell environment which resulted in spherical particles with relatively uniform size of about 186 nm confirmed by TEM. In-vitro drug release study revealed the potential of GSH stimulation to enhance the release of drug in a tumor-selective manner whereas the overall results revealed highly inhibited HL-60 cell proliferation with considerably lower cytotoxicity for normal L929 cells thus proving the potential of folate receptor targeting and GSH-responsive carboxymethyl chitosan nanoparticles containing 6-MP for the treatment of leukemia. [12].

Gemcitabine (Gem) is a standard chemotherapeutic drug used in the treatment of cancer with the major limitations of short plasma half-life and lack of targeting in pancreatic cancer therapy. To improve the half-life and targeting of the drug in pancreatic cancer cells, FA-Chi-Gem core-shell nanoparticles were synthesized by coaxial electrospray technology using polyethylene glycol (PEG) to reduce the contact chance of Gem with deoxycytosine deaminase shielding effect. The value of IC50 as 13.51 μg/mL in cytotoxicity analysis indicated no significant Cytotoxicity whereas the proliferation of COLO357 significantly inhibited the growth of human pancreatic cancer xenografts by FA-Chi-Gem nanoparticles resulting in an enhanced therapeutic effect minimized side effects with reduced damages to normal tissues [29].

Polylactic-co-glycolic Acid (PLGA)

Polylactic-co-glycolic acid (PLGA) is one of the extensively researched synthetic biodegradable polymers due to its favourable properties as well as a copolymer of lactic acid (a-hydroxypropanoic acid) and glycolic acid (hydroxy acetic acid) [30]. It is also known as a 'Smart Polymer' due to its stimuli-sensitive behavior [31]. It is a crystalline hydrophilic polymer with low water solubility and fast degradation rate under physiological conditions with several advantages of controlled properties, complete biodegradability, biocompatibility, well-defined formulation techniques, and easy processing [32, 33]. It is non-toxic, non-irritating, and fully biodegradable with good biocompatibility and human adaptability. In vivo, the final degradation product of PLGA is lactate, which can be metabolized by intravital cells and eventually decomposes to non-toxic products (H_2O and CO_2) that are eliminated from the body [32, 34].

Kaempferitrin is an anticancer drug with wide pharmacological activity but is associated with poor solubility in an aqueous solution. To overcome this limitation kaempferitrin was encapsulated with PLGA and KM+FA+PLGA-Nps by using the combined emulsion solvent evaporation method. Particle size analysis resulted in the mean particle size of 151.21 ± 8.4 with a polydispersity index of 0.092 ± 0.021 which indicated drug accumulation in cancer cells. The spherical morphology with the sizes ranging between 87.36 and 144.9 nm with ~68.57% entrapment efficiency and ~0.256% drug loading was confirmed by Scanning electron microscopy (SEM) and Transmission Electron microscopy (TEM) whereas the cytotoxicity analysis showed improved cytotoxic activity mediated by high selectivity and considerably induced cell apoptosis in colon adenocarcinoma HT-29 cell lines and thus proved potential drug carrier to deliver drugs for cancer therapy by improving the anti-cancer efficacy [35].

Folic-acid-conjugated pullulan-g-poly (DLlactide-co-glycolide) copolymers were prepared by using pullulan as a drug-carrying material while its derivatives pullulan/PLGA graft copolymer were considered an ideal vehicle with amphiphilic properties for fabrication of drug targeting carrier for targeted delivery of the anticancer drug by using Doxorubicin (DOX) as a model drug. HNMR analysis confirmed the successful synthesis of folic-acid-conjugated pullulan-g-poly (DLlactide-co-glycolide) also termed as (FAPuLG) copolymers. Small particle sizes <200nm allowed drug targeting to specific organs and tissues, prolonged blood circulation as well as virus-like behavior. Cell viability of nanoparticles in the presence of folic acid was increased 20% higher than that in the absence of folic acid indicating that FAPuLG is a promising candidate for active targeting of the folate receptor of tumor cells [36].

Curcumin is an anticancer agent having limitations to its potential because of its lack of solubility in aqueous solvents and poor oral bioavailability. To improve its bioavailability, the curcumin was encapsulated with a biodegradable nanoparticulate formulation based on poly (lactide-co-glycolide) (PLGA) and a stabilizer polyethylene glycol (PEG)-5000. The results indicated of Dynamic laser light scattering and transmission electron microscopy indicated a particle diameter of 80.9 nm. The *In-vitro* curcumin (NP) exhibited very rapidly (2 h vs. > 72 h) and more efficient cellular uptake as compared to curcumin. Esterase staining analysis revealed that curcumin (NP) was as potent as or more potent than curcumin in inducing apoptosis of leukemic cells and in suppressing proliferation of various tumor cell lines while electrophoretic gel shift mobility assay indicated that curcumin (NP) was more active than curcumin in inhibiting TNF-induced NF-κB activation and in suppression of NF-κB-regulated proteins involved in cell proliferation (cyclin D1), invasion (MMP-9), and angiogenesis (VEGF). Also when studied in vivo in mice, curcumin (NP) was more bioavailable and had a longer half-life than curcumin. The overall studies indicated that curcumin-loaded PLGA nanoparticles formulation has enhanced cellular uptake, increased bioactivity *In vitro* and superior bioavailability *In vivo* over curcumin [37].

Paclitaxel a potent naturally derived chemotherapeutic agent with a major limitation of its declining efficacy as an anticancer agent as cells become resistant to the normally administered dose and thus it needs exposure to higher doses of the drug leading to more deleterious side effects whereas curcumin, a powerful poly phenolic chemosensitizer with major limitation of poor aqueous solubility leading to fast clearance and poor bio availability at the target site. Thus, Co-administration of paclitaxel and curcumin in PLGA nanoparticles conjugated with folic acid was studied which resulted in the increased therapeutic potential of curcumin. The results revealed that encapsulation of curcumin in folic acid conjugated PLGA-PEG nanoparticles significantly improved the efficacy of curcumin in chemo sensitizing HeLa cells while PPF-curcumin was found more efficient in down regulating paclitaxel-induced activation of NF-κB, Akt and MAPK pathways as compared to free curcumin. Also, it has been concluded that Nano encapsulation and folic acid conjugation enhances the chemo sensitization potential

of curcumin towards paclitaxel causing a significant reduction of tumor growth in vivo with a reduction in NF-κB and AP-1 nuclear translocation and improving the cellular uptake [38].

Doxorubicin, a model anticancer drug was encapsulated into the rapidly biodegradable, biocompatible, thermo responsive, non-toxic, block copolymer (PEO–PLGA–PEO) to circulate nanoparticles in blood for a longer time without occluding arteries, veins, or capillaries by using poloxamer (pluronic F-127) for enhancing the uptake of particles by the reticuloendothelial system (RES). The structural, magnetic, and physical properties of the core were evaluated by X-ray diffraction, vibrating sample magnetometer, and transmission electron microscopy techniques, respectively while the hydrodynamic size of composite nanoparticles was determined by dynamic light scattering and was found to be 36.4 nm at 25℃. Fourier transform infrared spectroscopy was used to analyze the functionalization of a magnetic core with various polymeric chain molecules however the encapsulation efficiency and loading efficiency of doxorubicin into the polymeric magnetic nanoparticles were confirmed by UV–vis spectroscopy and the loading efficiency of the drug was found to be as 89%. Hence the results of the experiment concluded that drug release is mainly governed by the thermal response of polymeric magnetic particles while a static magnetic field could be useful for targeting drug-polymer composite to the sight of the action [39].

Saquinavir (SQV) is an effective antiviral drug with a significant antineoplastic effect but it induces cell death in prostate and breast cancer cells in a dose and time-dependent manner. SQV-loaded FA anchored PEGylated PLGA NPs were developed by using a single emulsion solvent evaporation method for tumor targeting employing PC-3 (human prostate) and MCF-7 (human breast) cancer cell lines. The results of the experiments showed that SQV-Fol-PEG-PLGA NPs showed a higher percentage of cell growth inhibition than non-targeted drug-loaded PLGA (SQV-PLGA) NPs due to folate receptor-mediated endocytosis (RME) on the tumor cell membrane in both cell lines. The MTT cytotoxicity study concluded that the developed targeted SQV-Fol-PEG-PLGA NPs were superior anticancer potential as compared to non-targeted SQV–PLGA NPs [40].

5-fluorouracil (5-FU) an anticancer drug is encapsulated in an FA-conjugated PLGA system using 1, 3-diaminopropane as a cross linker to achieve a high conjugation ratio. The 1, 3-diaminopropane facilitated the immobilization of FA into PLGA-based drug carriers, showing the formulation with great potential to be used as a cancer cell-specific delivery system for anticancer agents [41].

Alginates

Alginate is a naturally occurring anionic polymer obtained from brown seaweed investigated and used for many biomedical applications as it offers several advantages like biocompatibility, low toxicity, relatively low cost, easily controlled physical properties, and mild gelation by addition of divalent cations such as Ca2+ [42]. Furthermore it can be used in targeted drug delivery because of its high affinity for folic acid receptors, non-immunogenicity, and bio stability [43]. It consists of 1→4 linked α-L-guluronic acid (G) and β-D-mannuronic acid (M) pyranose residues in an un-branched chain [44]. The alginates can create complexes with other biomaterials by electrostatic interactions, chemical modification, or crosslinking because of which it can be exploited for building hybrid and more versatile DDSs. Alginate has carboxyl groups that are charged at pH values higher than 3-4, making alginate soluble in neutral and alkaline conditions to promote the widespread use of alginates [45]. However, Ca-alginate complexes suffer from poor stability in physiologically relevant media leading to extensive uncontrolled release of loaded drugs which limits the ability of Ca-alginate complexes as controlled release matrices, especially for oral administration. To overcome this limitation research is focused on preparing stable complexes either by switching to other metal cations such as zinc, aluminum, or iron or by synthetically modifying alginic acid to enhance the stability of its corresponding complexes [46].

5-aminolaevulinic acid (5-ALA) is an anticancer drug conjugated with folic acid and alginates nanoparticles to be incorporated into the cancer cells by receptor-mediated endocytosis. The prepared nanoparticles possessed good cytotoxicity, stability, and uniformity in shape and size. The results indicated that the release of 5ALA from the NPs was pH-sensitive and was caused by the hydrolysis of alginate in the acidic lysosomal environment of cancer cells. It was found that the system was not degraded by enzymes or other external factors before reaching the target site, the drug was stably delivered to the cancer cells preventing drug leakage during circulation which proved the active targeting efficiency of folate conjugated alginate nanoparticle-containing 5-aminolaevulinic acid (5-ALA) [43].

The alginate incorporated and folic acid-conjugated chitosan nanoparticles of 5-ALA were prepared for colorectal-specific delivery by fluorescent endoscopic detection method. The incorporated alginate molecules complexed stably with chitosan via electrostatic attraction. The results of average particle diameter and zeta potential were found to be 115 nm and 22 mV respectively which kept nanoparticles stable enough in aqueous suspension without aggregation. The loading efficiency of 5-ALA was found to be 27%. The prepared nanoparticles were readily taken up by colorectal cancer cells via folate receptor-mediated endocytosis and 5-ALA was released in the lysosome, this was promoted by the reduced attraction intensity between chitosan and 5-ALA via the deprotonated alginate, resulting in a higher intracellular PpIX accumulation for the photodynamic detection indicating excellent vectors for colorectal-specific delivery of 5-ALA [47].

Tamoxifen (TMX) loaded folate-targeted nanoparticles based on disulfide bond reduced bovine serum albumin (BSA-SH) and BSA-SH/alginate-cysteine (BSA-SH/ALG-CYS) mixtures were prepared with a mean size of 76-417 nm. The drug release studies in the presence of surfactant showed a gradual release of the drug between 4-7 h. Internalization of the systems was achieved and mediated by folate receptor which proved the efficacy as controlled TMX release of BSA-SH system compared to BSA-SH/ALG-CYS system [48].

Human Serum Albumin

Human Serum Albumin is a natural transport single-chain protein with multiple ligand binding sites, a protein with low molecular weight (66/5 kDa) containing 585 amino acids. It is a simple protein, non-glycosylated polypeptide, hydrophobic patches/cavities, and it lacks prosthetic groups with a long circulatory half-life of 19 days [49-51]. It represents an important carrier with great potential in controlled delivery applications which can lead to suitably targeted delivery of various drugs and endogenous molecules with several advantages like high drug loading and entrapment capacity, good biocompatibility and biodegradability, the high binding capacity of a wide variety of drugs, reliability and efficient carrier systems with features such as facile preparation, controllable, well-defined size, and desirable surface modification, half-life, stability, versatility, safety and ease of manufacture [52]. These inherent biochemical and biophysical properties make it an attractive drug delivery platform.

To maximize the proven antineoplastic activity of the paclitaxel against solid tumors folate-targeted cross-linked nanoparticles based on BSA and alginate mixtures were prepared to have nonmetric sizes ranging between 169 ± 28nm - 296 ± 57nm with negative Z-potential values between -0.12 ± 0.04 and -94.1± 0.4 released sustainably along 23 and 27h. The prepared folate-targeted PTX-loaded BSA/ALG Nanocarriers proved the effectiveness against cancer cells that overexpress folate receptors on their surface, reducing cell survival in a cervical carcinoma cell line, a breast adenocarcinoma ER+ cell line, and a TNBC cell line which are very aggressive cancer types with difficult treatment. The cell viability results confirmed a cyto compatibility of unloaded nanoparticle-Fol and a gradual decrease in cell viability after treatment with PTX-loaded nanoparticles-Fol due to the sustainable PTX release [53].

The anticancer drug resveratrol is associated with low solubility and low bioavailability. To encounter this problem Folic Acid-Human Serum Albumin- resveratrol Nanoparticles (FA-HSA-RES-NPs) were successfully prepared by using a high-pressure fluid nano-homogeneous emulsification method. As a result of this, the average particle size of nanoparticles was obtained as 102.1 ± 4.9 nm with the drug capsulation efficiency and drug loading efficiency of 98.36 and 14.66%, respectively. The characteristics of the FA-HAS-RES-NPs were examined by SEM, TEM, FTIR spectroscopy, TGA, XRD, and DSC which indicated amorphously encapsulated resveratrol, approximately spherical with low crystallinity and hence improved in solubility with improved bioavailability of intravenous administration of FA-HSA-RESNPs by 5.95 times higher than that of the original RES compared to the original RES and also with slow and continuous release no toxic effects on rat organs. Moreover, this study proved the excellent targeting and sustained release characteristics that FAHSA-RESNPs have for liver tumor cells [54].

To reduce the side effects and improve the therapeutic effect of antitumor drugs like doxorubicin targeted folic acid-conjugated, doxorubicin-loaded, magnetic iron oxide bovine serum albumin nano spheres (FA-DOX-BSA MNPs) were prepared using a desolvation cross-linking method in which the activated folic acid i.e. N-hydroxy succinimide ester of folic acid was conjugated to the surface of albumin nano spheres via amino groups. It resulted in a uniform size average diameter of 180 nm with 80% entrapment efficiency. A satisfactory heat treatment temperature at a significantly lower dose, when placed in a high-frequency alternating magnetic field, imparted more efficient antitumor activity with controlled and targeted delivery of drugs [55].

Cabazitaxel is an anticancer agent with the major issue of vehicle-related toxicity due to it being based on Tween-80 (Cbz-Tween). To overcome this problem Cabazitaxel -loaded human serum albumin nanoparticles (Cbz-NPs) were synthesized using the salting-out method. The results of which revealed that Cbz-NPs have significant safety advantages compared to Cbz-Tween with superior blood biocompatibility, prolonged blood circulation, and enhanced accumulation of Cbz in tumors along with reduced toxicity in prostate cancer [56].

Isoalantolactone (IAL) is a drug with a variety of pharmacological activities in vivo and in vitro, including cytotoxic, diuretic, and immunosuppressive activity. The folic acid-conjugated human serum albumin nanoparticles for IAL (FHNs-IAL) were developed by desolvation and stabilized by chemical cross-linking with glutaraldehyde in which Folic acid was covalently coupled to amino groups on the surface of HNs-IAL by carbodiimide reaction to improve the targeted activity, water solubility and to reduce untoward effects. The results of the study revealed the average diameter of spherical FHNs-IAL as 118.7 ± 11.6 nm with 36.1 ± 3.3% of encapsulation efficiency. The cytotoxic activity in vitro and the cellular uptake of FHNs-IAL examined by HeLa cells indicated increased IAL uptake into cancer cells with the superior anti-tumor effect of FHNs-IAL by human tumor xenograft animals [57].

Discussion

In this review, active targeted nano systems of vincristine, methotrexate, mitoxantrone, doxorubicin, genistein, 5-aminovalaevulinic acid (5-ALA), carboplatin, 6-mercaptopurine, and gemcitabine, kaempferitrin, curcumin, paclitaxel, saquinavir, 5-Flurouracil, tamoxifen, resveratrol, isoalantolactone and cabazitaxel were discussed which are successfully prepared using various polymers.

Conclusion

Overall, the folate receptors are highly overexpressed on surfaces of malignant cells in most malignancies. This advantage has been utilized for drug targeting tumor-specific cells. Various polymers were effectively utilized to prepare such folate conjugates. Chitosan was successfully utilized to prepare folic acid conjugated nano systems of vincristine, methotrexate, mitoxantrone, doxorubicin, genistein, 5-aminovalaevulinic acid (5-ALA), 5-flurouracil, carboplatin, 6-mercaptopurine, and gemcitabine, etc., Polylactic-co-glycolic acid (PLGA) for kaempferitrin, doxorubicin, curcumin, paclitaxel, saquinavir, 5-Flurouracil, etc., alginates for 5-Aminovalaevulinic acid (5-ALA) and tamoxifen whereas as Human serum albumin for paclitaxel, resveratrol, Isoalantolactone, doxorubicin, and Cabazitaxel. All above systems proved the efficacy of Folic acid conjugated systems as promising targeted drug delivery for cancer imaging and treatment with reduced side effects.

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Conflicts of interest

The authors have no financial or non-financial conflicts of interest for this work.

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