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Polymer nanoparticles for drug and small silencing RNA delivery to treat cancers of different phenotypes

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Abstract

Advances in nanotechnology have provided powerful and efficient tools in development of cancer diagnosis and therapy. There are numerous nanocarriers that are currently approved for clinical use in cancer therapy. In recent years, biodegradable polymer nanoparticles (NPs) have attracted a considerable attention for their ability to function as a possible carrier for target-specific delivery of various drugs, genes, proteins, peptides, vaccines, and other biomolecules in humans without much toxicity. This review will specifically focus on the recent advances in polymer-based nanocarriers for various drugs and small silencing RNA's loading and delivery to treat different types of cancer.

Keywords

Polymer nanoparticles; Nanocarriers; Nanobiotechnology; PLGA; Small Silencing RNA; Cancer therapy; Drug delivery

INTRODUCTION

Cancer is one of the major worldwide diseases, with 12.4 million new cancer cases and 7.6 million cancer deaths reported in 2008^{1,2}. More than 10 million people are diagnosed with various cancers each year worldwide. Nearly 1.6 million new cancer cases are expected to be diagnosed and about 580,000 cancer deaths projected in the United States in 2013³. Lung, liver, stomach, colon, and breast cancers are the leading causes of cancer-related deaths each year^{1,2}. Cancer arises from single cell that is unable to repair mutations produced in DNA while avoiding normal elimination through apoptosis, and experiences abnormal cell growth and division, which later leads to invasion and metastasis. Normal cells can transform into tumor cells due to various reasons, such as genetic factors, external influences from agents like physical carcinogens (radiation), chemical carcinogens (asbestos, tobacco smoke, BPA, etc.) and biological carcinogens (infections from viruses, bacteria, and parasites)³. Cancer treatment requires careful screening and selection of therapies such as radiotherapy, chemotherapy, and surgery. Although radio- and chemotherapeutic drugs are capable to shrink the tumor growth for a short period of time, they require multiple schedules of treatments. In addition, chemotherapeutic drugs not only kill the cancerous cells but also the healthy normal cells, presenting a significant toxic side-effect to the patients. Hence, it would be a great benefit to develop drug delivery systems

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that actively or passively target only cancerous cells⁴. Nanobiotechnology could offer an ideal solution for many difficulties associated with current chemotherapy, and could provide as a new platform that may contain controlled, sustained, and targeted release of drugs and genes for cancer therapy.

Nanoparticles research has become a key area in drug delivery systems for the treatment of cancer and several other metabolic disorders⁵. Nanoparticles have the capability to deliver numerous drugs and genes into the various specific parts of the body for a sustained period of time. Over the past few decades, large numbers of nanoparticle-based drug delivery systems have been developed^{6,7}. Polymer-based nanoparticles are preferred for drug delivery because of their low toxicity, sustained release, biodegradable, and available sites for various modifications. Most of the polymer-based nanoparticles developed for drug delivery systems were around 100–200 nm mean size, and were prepared from either natural or synthetic polymers. The biodegradable polymer nanoparticles are considered one of the best platforms for targeted and controlled drug delivery systems to treat cancer of different phenotypes. These polymer nanoparticles displayed excellent biocompatibility, prolonged circulation time, and enhanced accumulation in tumors, with fewer side effects⁸. In most of the current studies, various polymer-based nanoparticles have been used mainly as controlled release agents in specific target sites for drug delivery processes.

PREPARATION AND CHARACTERIZATION OF POLYMER NANOPARTICLES

Several methods for the preparation of polymer nanoparticles were developed based on the drugs to be encapsulated and requirement of its application^{7,9,10}. Most commonly used methods are emulsion-solvent evaporation or diffusion, double emulsion, nanoprecipitation, salting out, dialysis and supercritical fluid techniques¹⁰. Usually water-in-oil-in water (W/O/W) double emulsion solvent evaporation method is widely used for encapsulation of hydrophilic drugs, and nanoprecipitation technique is used for encapsulation of hydrophobic drugs in most of polymeric nanoparticles preparations.

Polymer nanoparticles physiochemical characteristics such as size, shape, and zeta potential were characterized by using imaging methods such as dynamic light scattering (DLS) technique, scanning electron microscopes (SEM), transmission electron microscope (TEM), and atomic force microscopy (AFM)¹¹. DLS measures NPs hydrodynamic size, size distribution, and polydispersity. Though NPs characterizations using DLS is fast, reliable, repeatable, and precise for monomodal suspensions, but small percentage of larger NPs scatter much more even and obscure the contribution from small NPs. TEM is an excellent technique for visualizing the NPs and provides information about its size, shape, and size distribution, but sample preparation is quite challenging and time consuming and provide poor statistical representation of NPs sample. Zeta potential is a physical property demonstrated by any particle in suspension. It shows the overall charge of a particle acquired in a particular medium¹².

POLYMER NANOPARTICLES FOR DRUG DELIVERY

Biodegradable polymer nanoparticles for controlled drug delivery have already shown important improvements in cancer therapy¹³. These polymer nanoparticle-based delivery systems have been shown capable of delivering wide range of drugs for a sustained period of time in the target sites in a slow release manner to provide enhanced antitumor efficacy with reduced systemic side effects (see figure 1). Moreover, these nanocarriers can protect drugs from their rapid metabolism while in the systemic circulation, and clearance by liver, kidney, and reticuloendothelial system, which further enhances drugs' stability and target

specificity^{14, 15}. Several polymer-based nanoparticles such as poly(lactic-co-glycolic acid) or poly(lactide-co-glycolide) (PLGA), polylactic acid or polylactide (PLA), poly glycolic acid or polyglycolide (PGA), Polycaprolactone (PCL), poly (D,L-lactide) (PDLLA), chitosan, and PLGA-PEG have been developed for the drug delivery and are in various stages of clinical trials, with a few of them having been approved by FDA for clinical use (Table 1).

POLYMER NANOPARTICLES FOR DRUG LOADING AND DELIVERY

PACLITAXEL AND DOCETAXEL LOADED POLYMER NANOPARTICLES

Paclitaxel (PTX) loaded pegylated PLGA nanoparticles was developed by Danhier et al.¹⁶ They prepared the paclitaxel-loaded pegylated PLGA-based nanoparticles using simple emulsion and nano-precipitation techniques, with subsequent *in vitro* and *in vivo* testing against human cervical carcinoma cells (HeLa). The drug release behavior of Paclitaxel-loaded nanoparticles showed a biphasic pattern with an initial burst release followed by a slower and continuous release. Exposure of HeLa cells to paclitaxel-loaded nanoparticles induced the same percentage of apoptotic cells *in vitro*, and showed a greater tumor growth inhibition effect *in vivo* compared with free Paclitaxel¹⁶. Cheng et al. reported the synthesis, preparation, and size optimization of ¹⁴C-Paclitaxel and Docetaxel (DTXL) loaded carboxy-terminated PLGA-*b*-PEG-COOH block co-polymer nanoparticles using nanoprecipitation method. They also conjugated these NPs to A10 RNA-aptamer for *in vivo* drug delivery for prostate cancer (see figure 2). A 10-aptamer binds to prostate-specific membrane antigen (PSMA) protein on the surface of the prostate cancer (PCa) cells. NP and NP-aptamer bio-distribution was estimated in a LNCaP (PSMA+) xenograft mouse model for prostate cancer. A 10 RNA-aptamer conjugated NPs showed enhanced NP-delivery to the prostate tumors compared to that of corresponding non-targeted NPs¹⁷. In a similar study, the same group reported synthesis of the Docetaxel-loaded PLGA-*b*-PEG-COOH NPs conjugated with A 10 2'-fluoropyridine RNA-aptamer. These NPs showed remarkable efficiency, reduced toxicity, and more tumor reduction in LNCaP xenograft nude mice¹⁸. The same group reported a preparation of combinatorial library of DTXL-loaded PSMA ligand-targeted NPs of varying polymer compositions, concentrations, sizes, targeting ligand density, surface hydrophilicity, and other processing parameters using the single emulsion method¹⁹. These targeted NPs contain the poly(D,L-lactide) (PLA) or PLGA and PEG in which DTXL was physically entrapped, and a targeted ligand that mediated the molecular interactions between NPs and PSMA. They found that optimized DTXL PSMA ligand loaded NPs could release chemotherapeutic DTXL in a controlled manner *in vitro* as well as in live mice, rats, and non-human primates against the prostate cancer cells without any cytotoxicity to the animals. These DTXL NPs also slowed the inhibition of prostate tumor growth of up to 26%, compared with the free DTXL delivered animals in which tumor grew to 100% in a 7-week period in mouse. These NPs were used in treatment of 12 cancer patients (phase 1 clinical trials), and demonstrated clearance results similar to those in animals¹⁹.

CISPLATIN LOADED POLYMER NANOPARTICLES

Cisplatin is one of the most widely used anticancer agents, particularly for the ovarian, breast, prostate, and testicular cancers. But their therapeutic effects are dose limiting because of many serious side-effects, notably liver and kidney toxicity, when administered directly as a free drug. To overcome these side-effects, a more selective administration to cancer cell is required. For this purpose, several Cisplatin-entrapped NPs were developed for slow delivery of drug, without loss of activity^{20–24, 54}. Gryparis reported a preparation of PLGA-*m*-PEG NPs of Cisplatin using the double emulsion method⁵⁴. These Cisplatin-loaded NPs showed *in vitro* anticancer activity against human prostate cancer LNCaP cells similar to

that of a free drug ²⁰. Cheng et al. reported that Cisplatin-loaded carboxymethyl cellulose (CMC) core PLGA-*m*-PEG NPs synthesized using the double emulsion method showed activity against the IGROV1-CP ovarian cancer cells *in vitro* and *in vivo* in nude mice (see figure 3) ²¹.

Dhar et al. reported the aptamer functionalized Pt(IV) (*c,t,c*-[Pt(NH₃)₂(O₂CCH₂CH₂CH₂CH₂CH₃)₂ Cl₂]) prodrug loaded PLGA-*b*-PEG NPs against human prostate PSMA-over-expressing LNCaP cancer cells. The inactive pro-drug Pt(IV) releases the active cisplatin after reduction inside the cell (see figure 4). The aptamer functionalized Pt(IV) entrapped NPs showed considerably superior activity compared to that of free cisplatin or non-targeted NPs against LNCaP cancer cells ²². The *in vivo* pharmacokinetics (PK), tolerability, bio-distribution and efficacy of Pt(IV) pro-drug entrapped PLGA-*b*-PEG-aptamer-NPs against xenograft of prostate cancer in rats and mice showed significant improvement in the drug therapeutic index ²³. $\alpha_v\beta_3$ integrin targeted Pt(IV) PLGA-PEG NPs conjugated with cyclic RGD peptide inhibited the growth of breast and prostate cancer cells *in vitro* at micromolar concentrations and improved anticancer activity *in vivo*, compared to free drugs in nude mice ²⁴.

Moreno et al. reported the cisplatin-entrapped PLGA NPs the using double emulsion method. These NPs showed a controlled release of cisplatin that induced more apoptosis than free cisplatin against colon adenocarcinoma cells *in vitro*, and also demonstrated good anticancer activity *in vivo* ^{25, 26}.

5-FLUOROURACIL (5-FU) LOADED POLYMER NANOPARTICLES

5-fluoro uracil (5-FU) is a pyrimidine analog that is one of the widely used cytotoxic drugs. It is a thymidylate synthase inhibitor that ultimately stops tumor cell growth. 5-FU is used in the treatment of malignant tumors such as glioblastoma, liver, and breast cancers. Like other chemotherapeutic drugs, 5-FU also has several side-effects when administered as a free drug.

5-fluorouracil loaded PLGA nanoparticles—Lekha et al. reported a preparation of 5-FU encapsulated PLGA NPs using the double emulsion method with 66% encapsulation efficiency. The 5-FU loaded PLGA NPs showed better cytotoxicity against glioma (U87MG) and breast adenocarcinoma (MCF7) cell lines than free drug ²⁷. Yadav et al. reported a preparation of 5-FU loaded hyaluronic acid-poly(ethylene glycol)-poly(lactide-co-glycolide) (HA-PEG-PLGA) NPs and tested them against Ehrlich ascites tumor (EAT) cell lines *in vitro* and *in vivo* in mice. These NPs were able to deliver a higher concentration of 5-FU in the tumor mass and found to be less hemolytic but more cytotoxic, and reduced the tumor volume significantly compared to free 5-FU ³⁰.

5-fluorouracil loaded chitosan nanoparticles—Cheng et al. reported a preparation of the 5-FU encapsulated galactosylated chitosan (GC) nanoparticles and tested their effects on hepatocellular carcinoma (HCC) *in vitro* and *in vivo*. These 5-FU loaded GC NPs extensively inhibited tumor growth and were more effective at targeting HCC than 5-FU free drug by activating the p53 pathway in orthotropic liver cancer mouse model ^{28, 29}.

5-fluorouracil loaded PBLG-PEG-nanoparticles—5-FU entrapped poly(γ -benzyl-L-glutamate-poly(ethylene glycol)) (PBLG-PEG) NPs displayed promising pharmacokinetic characteristics, including sustained drug release, prolonged drug half-life, and increased tissue appetency. These NPs showed superior inhibition of tumor growth on human colon cancer (LoVo) cell xenografts and human oral squamous carcinoma (Tca 8113) cell xenografts ³¹.

DOXORUBICIN-LOADED POLYMER NANOPARTICLES

Doxorubicin (DOX) is an anthracycline antibiotic that inhibits the DNA synthesis, and is a widely used drug for cancer chemotherapy. Doxorubicin has been used in the treatment of acute leukemia, lymphoma, breast, bladder, ovarian, lung, gastric, and testicular cancers ^{7, 55}. DOX has many adverse effects when administered as a free drug. Efficient drug delivery systems for the doxorubicin are under development ^{7, 55}.

Doxorubicin-loaded PLGA nanoparticles

Betancourt et al. reported a preparation of doxorubicin-encapsulated PLGA NPs using an oil-water nanoprecipitation technique with the resulting average size of 174 nanometers (nm). These NPs showed therapeutic efficiency against the MDA-MB-231 breast cancer cells comparable with free drug *in vitro* ³². Poloxamer 188 coated Lecithin-PLGA-Human serum albumin (HSA) DOX-loaded (DOX-L-PLGA-HSA) NPs enabled delivery of DOX across the blood-brain barrier in the therapeutically effective concentrations in rat glioblastoma ³⁴.

DOX-loaded Cyclo-(1,12)-PenITDGEATDSGC (cLABL) peptide conjugated PLGA nanoparticles (DOX-cLABL-PLGA-NPs) showed more rapid cellular uptake by A549 lung epithelial cancer cells compared to non-peptide conjugated nanoparticles by targeting encapsulated doxorubicin to ICAM-1 expressing cells ³⁵. Doxorubicin-entrapped poly(lactic-co-glycolide)-gold half-shell nanoparticles (DOX-PLGA-Au-H-S NPs) can deliver both doxorubicin and heat simultaneously to a selected tumorigenic region when irradiated with lights in the near infrared (NIR) region with higher cytotoxicity and more controlled delivery in HeLa cells ³⁶.

DOX-loaded PLGA nanoparticles showed sustained drug release rate in fibroblast cells *in vitro* without initial burst ³⁷. DOX-loaded peptide-conjugated PLGA-PEG-NH₂ nanoparticles containing an amino end group displayed three-fold higher uptake than corresponding peptide-free PLGA-PEG-NH₂ nanoparticles in a human ovarian cancer SKOV3 cell line with high expression of epidermal growth factor receptor (EGFR) ³⁸.

Doxorubicin-loaded polycaprolactone (PCL) polymer nanoparticles

DOX- entrapped biodegradable amphiphilic diblock copolymer methoxy poly(ethylene glycol)-b-poly(ε-caprolactone-co-γ-hydroxyl-ε-caprolactone) (mPEG-*b*-P(CL-co-HCL)) bearing pendant hydroxyl groups NPs showed good cytotoxicity against HepG2 cells *in vitro* ³³.

TAMOXIFEN (TMX) AND 4-HYDROXYTAMOXIFEN (4-OHT) LOADED POLYMER NANOPARTICLES

Tamoxifen is an estrogen receptor antagonist that acts via its metabolite hydroxytamoxifen; its more active form is 4-hydroxytamoxifen (4-OHT), a triphenylethylene derivative. Tamoxifen binds to the estrogen receptor in breast tumors, releasing a nuclear complex that reduces the DNA synthesis and inhibits estrogen's effects. Tamoxifen is a widely used drug in the anti-estrogen treatment for advanced or metastatic breast cancers that are positive for estrogen receptor expression ⁵⁶. Due to tamoxifen's relatively poor oral bioavailability and several side- effects, polymer nanoparticles have gained much interest for its drug delivery.

Tamoxifen loaded poly(D,L-lactide-co-caprolactone) (PLC) co-polymer and poly(D,L-lactide) (PDLLA) nanoparticles

Tamoxifen-loaded mixture of poly(D,L-lactide-co-caprolactone) (PLC) co-polymer and poly(D,L-lactide) (PDLLA) nanoparticles showed good cytotoxicity against cervical cancer (HeLa) cells and effectiveness against breast cancer (MCF-7) cells ³⁹.

Tamoxifen loaded poly(amidoamine)-cholesterol nanoparticles

Tamoxifen-entrapped poly(amidoamine)-cholesterol (PAA-Cholesterol) conjugated nanoparticles showed slow release and enhanced cytotoxicity against MCF-7 cells in comparison with the free drug ⁴⁰.

Tamoxifen loaded PLGA nanoparticles

Tamoxifen citrate- encapsulated PLGA nanoparticles with mean size of 165 nm showed enhanced activity against breast cancer (C1271) tumor when administered orally in a murine model. Oral bioavailability of tamoxifen from PLGA NPs was increased by 11.19 times as compared to the free drug *in vivo*. The tumor size was reduced up to 41.56% as compared to untreated animal, which showed an increased tumor size of up to 158.66% in a DMBA-induced breast tumor model. These tamoxifen-loaded NPs exhibited important value for oral chronic breast cancer therapy ⁴¹. 4-OHT-loaded PLA-PEG nanoparticles arrested estradiol-induced tumor growth, inducing apoptosis and inhibiting angiogenesis in breast cancer (MCF-7) cells *in vivo* in mice ⁴².

Tamoxifen and paclitaxel co-loaded polyethyleneoxide-polycaprolactone (PEO-PCL) nanoparticles

As tamoxifen is more cytostatic than cytotoxic, paclitaxel and tamoxifen combination drugs loaded in polyethyleneoxide-polycaprolactone (PEO-PCL) nanoparticles has been used and found to have enhanced antitumor efficacy in ovarian adenocarcinoma (SKOV3 and MDR-1-positive SKOV3TR cell lines) *in vivo* in nude mice. The results demonstrate that biodegradable PEO-PCL nanoparticles can serve as an effective approach to overcome multidrug resistance in ovarian adenocarcinoma ⁴³.

GEMCITABINE LOADED POLYMER NANOPARTICLES

Gemcitabine is a nucleoside analog of deoxycytidine used as a chemotherapeutic agent to treat various types of cancers, including non-small cell lung carcinoma, pancreatic cancer, metastatic breast cancer, and ovarian cancer. Gemcitabine undergoes rapid enzymatic degradation following intravenous injection and exhibits various side effect, including fever, nausea, vomiting, fatigue, hair loss, and shortness of breath when administered as a free drug. To improve the delivery of gemcitabine and increase the antitumorogenic effect, various drug delivery systems were developed using polymer nanoparticles ⁴⁴⁻⁴⁸.

Gemcitabine loaded chitosan nanoparticles

Herceptin (HER2)-conjugated gemcitabine-loaded chitosan nanoparticles (HER2-Gem-CS-NPs) showed efficient targeted delivery of gemcitabine in pancreatic cancer, and displayed enhanced anti-proliferative activity. These nanoparticles also induced an improved S-phase arrest leading to apoptosis, in comparison with non-targeted gemcitabine-loaded nanoparticles and the free gemcitabine ⁴⁴. Intravenous injection of gemcitabine-entrapped chitosan nanoparticles demonstrated significantly enhanced antitumor activity compared to free gemcitabine against murine lymphocytic tumor (L1210wt cells) in mice (see figure 5) ⁴⁵.

Gemcitabine loaded PLGA and PDLLA nanoparticles

Anti-EGFR monoclonal antibody-conjugated gemcitabine-loaded PLGA nanoparticles were used for the selective delivery in pancreatic cancer cells *in vitro*⁴⁶. Gemcitabine-loaded PLGA nanoparticles showed significantly increased cytotoxicity, apoptosis, and distinct intracellular trafficking mechanisms in drug-resistant pancreatic cancer cells (PANC1) in comparison to free gemcitabine *in vitro*⁴⁷. Gemcitabine-loaded poly(ethylene glycol)-block-poly(D,L-lactide (PEG-PDLLA) nanoparticles displayed good performance for controlled drug release, and showed similar cytotoxic activity to free gemcitabine in human pancreatic cancer (SW1990) cell line *in vitro*⁴⁸.

MITOMYCIN C (MMC) LOADED POLYMER NANOPARTICLES

Mitomycin C is an antineoplastic antibiotic. It is used as chemotherapeutic agent for the treatment of esophageal carcinoma, breast, anal, and bladder cancers. Mitomycin C has showed several side-effects, including nausea, vomiting, hair loss, skin rash, lung damage and shortness of breath when it is administered as a free drug.

Mitomycin C loaded chitosan nanoparticles

Mitomycin C entrapped cationic nanoparticles based on chitosan, poly-L-lysine or polycaprolactone showed effective intravesical delivery of mitomycin C in bladder tumors in a rat model^{57, 58}. MMC loaded with folic acid (FA) and methoxypoly(ethylene glycol) (mPEG) conjugated chitosan nanoparticles showed initial burst release and subsequent slower release in human adenocarcinoma HeLa cells. Laser confocal scanning imaging showed that these MMC-FA-m-PEG-chitosan nanoparticles could significantly increase drug uptake by HeLa cells. *In vivo* animal experiments demonstrated that an increased amount of MMC-FA-mPEG-NPs was accumulated in the tumor tissue relative to the mPEG-NPs in a nude mouse xenograft model⁴⁹.

EPIRUBICIN (EPI) LOADED POLYMER NANOPARTICLES

Epirubicin is an anthracycline chemotherapeutic drug, and is the 4'-epimer of Doxorubicin. Epirubicin intercalates within the DNA strands that leads to the complex which inhibits DNA replication and RNA transcription. Epirubicin is mainly used for the treatment of breast, lung, ovarian, and gastric cancers, and lymphomas.

Epirubicin loaded poly(butyl cyanoacrylate) nanoparticles

Epirubicin-loaded poly(butyl cyanoacrylate) nanospheres prepared by nanoprecipitation are found to be more cytotoxic than free drug to human carcinoma (HeLa and A549) cell lines *in vitro* due to enhanced cellular internalization of epirubicin⁵⁰.

Epirubicin loaded pullulan acetate nanoparticles

Epirubicin-loaded folic acid conjugated pullulan acetate (PA) (Epi-FA-PA NPs) nanoparticles showed higher cytotoxicity and greater extent of cellular uptake against human nasopharyngeal epidermal carcinoma (KB) cell lines over-expressing folate receptor. Epi-FA-PA NPs might be a possible targeted carrier for delivering drugs to folate receptor over-expressing cancer cells⁵¹.

CURCUMIN LOADED POLYMER NANOPARTICLES

Curcumin is a natural phenolic compound, a major component found in spice turmeric (*curcuma longa*). It is known to exhibit a variety of activities, including antioxidant, anti-inflammatory, anti-amyloid, ant-arthritic, and most importantly, antitumor activities.

Nevertheless, these benefits are limited by its poor oral bioavailability and aqueous solubility. To improve its bioavailability, several delivery approaches have been developed.

Curcumin loaded PLGA nanoparticles

Curcumin-loaded PLGA-NPs and PLGA-PEG-NPs showed 15.6- and 55.4-fold increases in curcumin oral bioavailability respectively, when compared to free curcumin in aqueous suspension in rats⁵⁹. Curcumin-loaded PLGA-NPs were shown to be more effective in arresting cell growth as compared to free curcumin in prostate cancer (LnCAP, PC3 and DU145) cell lines⁵³.

Curcumin loaded cellulose nanoparticles

Curcumin-encapsulated cellulose NPs displayed the highest cellular uptake and caused maximum ultra-structural changes related to apoptosis and enhanced anti-cancer efficacy compared to free curcumin against prostate cancer (C4-2, LnCAP, PC3 and DU145) cell lines *in vitro*⁵².

POLYMER NANOPARTICLES FOR SMALL SILENCING RNA DELIVERY IN CANCER THERAPY

In 1993, the first small silencing RNA was discovered. Since then, numerous small RNAs, including small interfering RNA (siRNA), small hairpin-RNA (shRNA) and microRNA (miRNA) have been identified and used for various biological applications⁶⁰. siRNAs are 20–25 nucleotide double-stranded RNA (dsRNA) molecules that after binding to RNA-induced silencing complex enable the cleavage and degradation of recognized messenger RNA (mRNA). MicroRNAs are small single stranded RNAs of 18–23 nucleotides that are partially or fully complementary to the 3' non-coding region of mRNA sequence. MicroRNAs achieve gene silencing through blocking mRNAs translation or by degrading mRNAs. The ability to achieve sequence-specific gene silencing using RNA interference (RNAi) has been used in wide-range of applications, including the treatment of numerous diseases, particularly for the cancer therapy⁶¹. Nevertheless, *in vivo* systemic administration and delivery of these small silencing RNA-based therapeutics to cells and tumors remains a challenge owing to the limitations such as poor cellular uptake, degradation by serum nucleases, and rapid renal clearance after administration⁶². To overcome these limitations, the development of effective and safe nanocarriers for systemic delivery of small silencing RNAs is required for efficient cancer therapy. Biodegradable polymers nanoparticles have been found to be safe and effective nanocarriers for the delivery of small silencing RNAs (Table 2–4).

POLYMER NANOPARTICLES FOR MICRO-RNA (MIRNA) DELIVERY

MicroRNAs are endogenous small, highly conserved, non-coding RNAs of 18–24 nucleotides in length that are engaged in the post transcriptional regulation of gene expression through the RNA interference (RNAi) pathway in cells^{60–71}. Dysregulated expression of various miRNAs has been associated with various cellular diseases including cancer. MiRNAs are closely associated with cancer development due to their ability to promote growth of cancer cells^{60–71}. Based on their functions, they are categorized as oncogenic miRNAs, or oncomirs, and tumor suppressors miRNAs, or anti-oncomirs. Oncomirs promote tumor growth by inhibiting tumor suppressor genes or apoptotic genes, whereas anti-oncomirs are a group of microRNAs which block the function of cell cycle proteins and induce apoptosis by down-regulating the expression of the anti-apoptotic genes^{66, 67}. There are numerous miRNAs that are considered to be tumor suppressors, including miR-17-5p, miR-21, miR-29, miR-34, miR-127, miR-155, let-7, etc⁶⁵. Targeting

microRNAs is considered to be a new approach in development of a new class of molecularly targeted anticancer therapeutics (Table 2).

POLYMER NANOPARTICLES FOR ANTISENSE MICRO-RNA (ANTAGOMIR) DELIVERY

Effective overexpression strategies *in vivo* are needed to examine the effects of miRNA-directed regulation of gene expression. AntagomiRs or anti-miRs are small chemically modified antisense oligonucleotides that are used to block the function of endogenous miRNAs. They inhibit miRNA activity by irreversibly binding to the target miRNA⁷⁸. Specific miRNAs can be silenced by anti-miRNAs complementary to the mature miRNA sequences⁷⁹. Each miRNA controls the expression of numerous target genes by blocking translation of the target protein, and about 200 target genes may be regulated by a single miRNA⁸⁰. AntagomiRs are effective silencers of miRNA function expression in mice. AntagomiR-122 has been used to specifically silence the function of miR-122 in the lung, liver, skin, intestine, heart, and bone marrow of normal mice. The effect of targeted gene silencing was found to last for more than a week after a single intravenous injection^{78, 80}.

Anti-miR-155-loaded PLGA nanoparticles

MicroRNA-155 (miR-155) is an oncogenic microRNA. It is overexpressed in several cancers and regulates numerous pathways involved in cell division and immunoregulation. It is a key target for future therapies⁷². Babar et al. reported the synthesis of anti-miR-155 encapsulated PLGA nanoparticles by a double emulsion solvent evaporation method. NPs' surfaces were modified with cell penetrating peptides (CPP). Systematic delivery of these anti-miR-155-loaded PLGA polymer nanoparticles inhibited miR-155 and reduced the growth of pre-B-cell tumors *in vivo* in mice, indicating an encouraging therapeutic option for lymphoma and leukemia⁷².

Anti-miR-10b-loaded poly-L-lysine (PLL) nanoparticles

The wound-healing assay is generally used to present valuable information on cell migration, invasion, and proliferations. MicroRNA-10b positively controls breast cancer cell migration and invasion through inhibition of HOXD10 target synthesis, and directly inhibits breast cancer metastasis. Anti-miRNA-10b-loaded poly-L-lysine (PLL-anti-miR-10b) nanoparticles, when administered to MDA-MB-231 breast cancer cells, strongly inhibit the invasive property of cells as measured by the wound-healing assay for up to 4 weeks. Anti-miR-10b molecules delivered into the cytoplasm of the breast cancer cells in a concentration-dependent manner showed sustained efficiency. These PLL-anti-miR-10b nanoparticles may offer progressive applications for inhibiting breast cancer metastasis⁷³.

Co-delivery of anti-miR-21 and 5-FU by poly(amidoamine) dendrimer nanoparticles

MiR-21 is an important player in a majority of cancers. Anti-miR-21- and 5-FU-loaded poly(amidoamine) dendrimer nanoparticles significantly enhanced the cytotoxicity of 5-FU and strongly increased the apoptosis of U251 glioma brain tumor cell line, and the migration ability of tumor cell was significantly decreased. The co-delivery of anti-miR-21 might have significant applications in the treatment of miRNA-21-overexpressing glioblastoma tumors⁷⁴.

MiRNA-26a expression vector (pDNA) loaded PLGA-PEI nanoparticles

Liang et al. reported a preparation of cationic PLGA-based nanoparticles of about 60 nm sizes by coating PLGA core with a cationic polyethylenimine (PEI) shell for gene delivery by the emulsion evaporation method. These nanoparticles have overcome the limitations of

cationic liposome and PEI based nanoparticles. These cationic PLGA-PEI nanoparticles were loaded with miRNA-26a expression vector (pDNA) and studied in HepG2 hepatocellular carcinoma cells. The results showed efficient delivery and expression of miRNA in hepatocellular carcinoma (HepG2) cells, and improved the effect of functional miRNA-26a delivery. The results showed that these PLGA-PEI nanoparticles are promising non-viral vectors for gene delivery ⁷⁵.

Cell-penetrating peptide-coated PLGA nanoparticles have been used to efficiently deliver chemically modified oligonucleotide analogues to compete gene regulation in blocking the activity of the oncogenic miR-155, and to modulate splicing to decrease the expression of the proto-oncogene, Mcl-1. Regulation of these genes in cancer cells decreased cell viability and produced pro-apoptotic effects ⁷⁶.

Anti-miR-296 loaded PEGylated liposome-polycation-hyaluronic acid nanoparticles

Cyclic RGD peptide (cRGD) conjugated PEGylated liposome-polycation-hyaluronic acid (PEG-LPH) targeted nanoparticles efficiently delivered the anti-miR-296 oligonucleotides (AMOs) to the cytoplasm and down-regulated the target mRNA in human umbilical vein endothelial cells (HUVECs) by blocking the function of endogenous miT-296. However, without cRGD peptide conjugation, these nanoparticles showed only little AMO uptake and miRNA silencing activity, whereas cRGD peptide-conjugated nanoparticles efficiently inhibited endothelial cell migration and blood tube formation, due to substantial upregulation of hepatocyte growth factor-regulated tyrosine kinase substrate (HGS). These targeted cRGD peptide-conjugated PEG-LPH nanoparticles also showed potential anti-angiogenesis activity with the delivery of anti-miR-296 oligonucleotides to the targeted site *in vivo*, as assessed by the Matrigel plug assay ⁷⁷.

POLYMER NANOPARTICLES FOR SMALL INTERFERING RNA (SiRNA) DELIVERY

Small interfering RNA is double-stranded RNA (dsRNA) of 20–25 base pairs long. Sequence-specific post transcriptional gene silencing was found to be induced by synthetic small silencing RNA via the RNA interference (RNAi) pathway in mammalian cells ⁶⁷. After finding the way to regulate the gene expression, siRNA has quickly been developed as a powerful tool in selectively silencing gene expression ^{67, 68}.

SiRNA loaded PLGA nanoparticles

Woodrow et al. reported the development of PLGA nanoparticle formulations with a higher siRNA loading capacity using the double-emulsion solvent evaporation technique. Topical administration of a single dose of siRNA-loaded PLGA nanoparticles causes efficient and sustained gene silencing throughout the female mouse reproductive tract when applied externally. The knockdown of gene expression was also observed to be proximal and distal to the site of topical delivery. These siRNA-loaded PLGA nanoparticles penetrated deep into the vaginal tissue after topical administration and demonstrated effective delivery capability for siRNA to the vaginal mucosa (Table 3) ⁸¹.

A codon-optimized plasmid-DNA (p-DNA) containing the eGFP and SiRNA targeted against HBV-X gene loaded PLGA-chitosan (PLGA-CHS-NP) nanoparticles showed a higher HBV gene-silencing efficiency than those of plain-PLGA NPs and naked pDNA in HepG2.2.15 cells *in vitro* ⁸². Platelet-derived growth factor BB (PDGF-BB) siRNA-loaded monomethoxypoly (ethylene glycol) - poly(lactic-co-glycolide) - poly L-lysine (mPEG-PLGA-PLL) nanoparticles in combination with microbubble (MB) and ultrasound (US) administration, safely increased the delivery of siRNA to rat retinal pigment

epithelium (RPE - J) cells and enhanced the PDGF-BB gene silencing with a substantial decrease in mRNA and protein expression⁸³.

SiRNA-loaded monomethoxypoly(ethylene glycol)-poly(lactic-co-glycolic acid)-poly-L-lysine (mPEG-PLGA-PLL) triblock copolymer nanoparticles were efficiently used to transfer siRNA into human lung cancer (SPC-A1-GFP) cells steadily expressing green fluorescent protein, and produced much higher intracellular siRNA delivery efficiencies than cells transfected with siRNA alone or with the Lipofectamine-siRNA complex. These siRNA-loaded mPEG-PLGA-PLL nanoparticles could be possibly applied as non-viral vectors for improving siRNA delivery and gene silencing⁸⁴. Targeted delivery of cyclic Arg-Gly-Asp (cRGD) peptide-conjugated human vascular endothelial growth factor (hVEGF)-siRNA-loaded mPEG-PLGA-PLL NPs in combination with microbubble and ultrasound treatment inhibited the VEGF expression in PC3 cells with low cytotoxicity and high gene transfection efficiency. The results demonstrated that the targeted delivery of biodegradable mPEG-PLGA-PLL nanoparticles might prove useful as a new vector system for gene delivery⁸⁵.

SiRNA loaded polyethyleneimine (PEI) polyethylene glycol (PEG) nanoparticles

SiRNA-loaded and angiogenic vessel-homing Ala-Pro-Arg-Pro-Gly (APRPG) peptide-conjugated polyethyleneimine (PEI) polyethylene glycol (PEG) (siRNA-PEI-PEG-APRPG) nanoparticles successfully enhanced the stability of siRNA to RNase A and transfection efficiency of siRNA, and enhanced the proliferation-inhibiting ability *in vitro* in MCF-7 cells and tumor xenograft of MCF7 cells *in vivo* in tumor-bearing mice⁸⁶. This nanoformulation inhibited the formation of microvessels, suppressed the increasing volume of the tumors, and reduced the expression of vascular endothelial growth factor (VEGF) protein and messenger RNA (mRNA). The siRNA/PEI-PEG-APRPG nanoparticles improved siRNA delivery and provided a more efficient antiangiogenic therapy than free siRNA and siRNA/PEI⁸⁶.

POLYMER NANOPARTICLES FOR SMALL HAIRPIN OR SHORT HAIRPIN RNA (ShRNA) DELIVERY

shRNAs are a class of small RNAs with a stem of 19–29 base pairs and a loop of 4–10 nucleotides that are expressed from vectors to induce RNAi. RNA interference (RNAi) is a natural process of inhibition of gene expression in organisms. shRNAs are sequences of genetically engineered RNAs that make a tight hairpin turn which could be used to silence target gene expression through RNA interference (RNAi)⁷⁷. The expression of shRNA in cells can be achieved by delivery of plasmids or via bacterial or viral vectors; shRNA uses these vectors and utilizes the U6 promoter to ensure that the shRNA is always expressed^{87–89}.

Various therapies using RNAi interference through intravitreal injection are currently undergoing clinical trials. Intravitreal injection must be administered repetitively for a long period of time to maintain sufficient drug concentrations to ensure the therapeutic range and efficient long-term gene expression or pharmacodynamic action in the eye, which sometimes causes certain ocular complications such as retinal detachment and vitreous hemorrhage⁹⁰. Thus, drug-delivery systems must be developed to facilitate therapeutic efficacy and to minimize side effects (Table 4)⁹¹.

ShRNA loaded PLGA nanoparticles

Zhang et al. reported the intravitreal administration of hypoxia-inducible factor 1 α (HIF-1 α) short hairpin RNA and green fluorescent protein (GFP) co-expressing pDNA-loaded

(pshHIF-1 α NPs) PLGA nanoparticles as a gene vector for functional plasmid DNA (pDNA) in order to investigate its inhibitory efficiency on experimental choroidal neovascularization (CNV). pDNA-loaded PLGA NPs were prepared using water in the oil-in-water method with a mean size of 303.7 nm and -8.9 zeta potential, providing 60.2% encapsulation efficiency and 1.06% drug loading capacity. The *in vitro* release behavior of pDNA-loaded (pshHIF-1 α) NPs showed that about 77% of total encapsulated pDNA was released within 10 days followed by a continuous and sustained release for 4 weeks. CNV was induced by laser photocoagulation in 112 rats. The rats were separated into groups and intravitreally injected with PBS, blank NPs, control pDNA NPs, naked pDNA, and pshHIF-1 α NPs. The results indicated that the expression of GFP preferentially occurred in the retinal pigment epithelium cell layer, lasted for 4 weeks. The fluorescein leakage areas of CNV were considerably larger in the non-injected, PBS, blank NPs, naked pDNA, and control pDNA NPs groups, compared to the pshHIF-1 α NPs group. Mean thickness of the CNV lesions in the intravitreally pshHIF-1 α NPs-treated group was significantly smaller than that in other groups. No signs of functional or ultrastructural destruction in retina were detected. Thus, pshHIF-1 α PLGA NPs possibly will act as a novel therapeutic route of transferring specific pDNA and inhibiting the formation of experimental CNV ⁹².

pSEC-shRNA-VEGF-A plasmid-loaded PLGA nanoparticles prepared using the double emulsion-solvent evaporation method were able to regress corneal neovascularization in a more sustained and vigorous manner than naked plasmids alone, making them a good *in vivo* non-viral gene therapy tool for sustainable, nonimmunogenic, and efficient regression of murine corneal neovascularization ⁹³. Androgen receptor (AR) has been shown to play a critical role in the progression of prostate cancer. AR-shRNA construct-loaded and prostate-specific membrane antigen (PSMA) A 10 aptamer-conjugated PLGA NPs mostly improved cellular uptake of NPs in both cell culture and xenograft-based models, and considerably enhanced xenograft tumor regression compared with non-conjugated NPs in various prostate cancer (22RV1, LAPC-4 and LNCaP) cell lines. The tissue-specific delivery of AR shRNA using a PLGA NPs approach indicated an effective therapy for prostate cancers ⁹⁴.

shRNA loaded Chitosan nanoparticles

The vascular endothelial growth factor (VEGF) was found to be a key driving force and regulator in tumor-associated angiogenesis and tumor development in hepatocellular carcinoma (HCC) and many other cancers ⁹⁷. RNAi-based therapeutics have been shown to block this angiogenesis stimulator ⁹⁸. Due to low cellular uptake and poor stability of small RNAs, clinically suitable, safe, and effective delivery systems are required for RNAi-based therapeutics ⁹⁹. Low molecular weight chitosan-shRNA (LMWC-shRNA) nano complexes directed against VEGF considerably inhibited VEGF expression of HCC cells and liver tumor tissues. Intravenous injection of LMWC-shRNA nano complexes displayed greater and prolonged deposition of shRNA at the tumor site in orthotopic allograft liver tumor-bearing mice. These LMWC-shRNA nano complexes demonstrated more effective suppression of tumor angiogenesis and tumor growth in various HCC models in comparison with naked shRNA, and showed the possibility of using LMWC as a promising carrier for RNAi drugs in liver cancer therapy ⁹⁵.

shRNA loaded poly- β -amine esters nanoparticles

EGFP-shRNA-loaded poly- β -amine esters nanoparticles (ShRNA-PAE NPs) showed efficient cellular uptake and EGFP silencing in U-87 MG cells and HEK-293 cells. Intravenously injected shRNA-PAE NPs in U-87 MG-GFP tumor-bearing mice demonstrated high accumulation of NPs in tumor, with a high silencing efficacy of intratumoral EGFP expression. The relationship between RNAi efficiency, cytotoxicity, and PAE polymer structure indicated that presence of disulfide bonds and nitrogen atoms in the

PAE NPs' backbone demonstrated the remarkable influence on *in vitro* and *in vivo* cytotoxicity and gene silencing efficiency⁹⁶.

ADVANTAGES OF NANOPARTICLES PREPARED FROM PLGA AND PLGA-PEG POLYMERS COMPARE TO OTHER POLYMERS

PLGA nanoparticles

Poly(lactic-co-glycolide) (PLGA) is a FDA-approved biodegradable and biocompatible polymer. Due to its low systemic toxicity, PLGA has been found to be one of the best polymers for drug delivery systems. It undergoes rapid hydrolysis in the body and forms biodegradable monomers, the lactic acid and glycolic acid, which are easily metabolized by the body without showing any toxic or immunomodulatory effects (see figure 6)^{7, 55}. In general, most chemotherapeutic drugs are cytotoxic and cytolytic, which not only kill cancer cells but also damage the healthy tissues in the body. The toxicity and adverse effects of chemotherapeutic drugs to healthy tissues restricts the amount of drug which can be used in order to achieve maximum therapeutic efficiency⁷. To avoid toxicity to healthy tissues and adverse effects to various organs, and to ensure specific delivery to cancer cells, numerous PLGA-based nanoparticles (NPs) that encapsulate chemotherapeutic drugs have been developed for the targeted delivery for the treatment of different cancers^{100, 101}.

PEGylated PLGA nanoparticles

PLGA polymers are hydrophobic in nature and this has caused some limitations for their formulation and drug delivery. To improve their hydrophilicity, poly(ethylene glycol) (PEG) has been bonded into the PLGA polymers. PEGylation has been shown to increase the encapsulation of drugs and improve their circulation time in animals, as well as increase their bioavailability. PEG is a well-established water-soluble, nontoxic, and biocompatible polymer. Hydrophilic PEG protects NPs from the immune surveillance. Block copolymers consisting of hydrophobic PLGA and hydrophilic PEG units have attracted much attention as nanoparticle preparations for various drug delivery, as they offer a site for several additional new modifications that can create the nanoparticles targeted with specific ligands^{16–24, 54}.

ADVANTAGES AND DISADVANTAGES ASSOCIATED WITH THE USE OF POLYMER-NANOPARTICLES FOR DRUG DELIVERY

Nanobiotechnology allows the delivery of drugs through nanoformulations and provide desired release kinetics for prolonged periods of time. Polymer nanoparticles are biodegradable and surface modifiable. These biodegradable polymer nanoparticles protect and deliver the drugs and RNAs, with a subsequent degradation of polymer matrix over a period of time, and have the capability to deliver multiple drugs in one carrier. Polymer nanoparticles can both passively and actively target the tumor cells. Drugs and RNAs delivered through polymer nanoparticles can increase the effectiveness of drug as compared to direct delivery. It can also decrease the side-effects by using the tumor-specific targeting, producing the enhanced permeability and retention (EPR) effect^{102, 103}. Major limitation of delivery of free chemotherapeutic drugs into tumor is their lack of tumor selectivity due to the leaky blood vessels and poor lymphatic drainage of most tumors, which allow the free drug to clear much faster from the tumor site without impacting any antitumor effect. Thus, free drugs may diffuse nonspecifically, whereas polymer nanoparticles tend to gather in tumor tissue much more than they do in normal tissues through passive targeting due to the EPR effect (see figure 7)^{4, 104}. In addition PEGylation of polymer nanoparticles also increases the circulation time of nanoparticles in blood and passively enhances its

concentration in the tumor site by EPR effect^{105, 106}. Whereas polymer nanoparticles containing targeted ligands can actively target the tumors through specific ligand–receptor interactions. The efficiency by which targeted nanoparticles selectively accumulate in the tumor site mainly depends on a variety of factors, which include 1) selective over expression of the receptor in the tumor cells in comparison to normal non-target cells, and 2) receptors accessibility for the ligands, etc^{105, 106}. PLGA-PEG nanoparticles conjugated to A10 aptamer ligand has been shown to efficiently identify prostate-specific c membrane antigen (PSMA) specifically expressed in the cancer cell surface¹⁰⁷. Though important progresses have been made to target the tumors, still considerable and effective developments are required to reach the targeted drug delivery selectively to solid tumors.

Nanoparticles display unique properties because of their small size and large relative surface area and can be seen using electron microscope. Engineered polymer nanoparticles are considered to be safe, non-toxic, non-mutagenic, non-carcinogenic, non-immunogenic, relatively easier to synthesize and surface modifiable. However, safety and toxic effects of these polymer nanoparticles are not completely resolved and which raises some concerns. Nanotoxicology is gaining increased attention to study the safety evaluation of nanomaterials which are planned for use in various biomedical applications^{108, 109}. Semete et al. reported that PLGA polymer nanoparticles have not shown any toxic effects compare to industrial zinc oxide nanoparticles in vitro and in vivo in balb/C mice. Surface modification of nanoparticles is needed to avoid particle accumulation in the liver¹¹⁰. Paclitaxel-loaded Poly(ethylene oxide)-modified poly(beta-amino ester) (PEO–PbAE) nanoparticles showed enhanced efficacy and significantly low toxicity profile as compared to the free paclitaxel in human ovarian adenocarcinoma (SKOV-3) xenograft¹¹¹. PLGA nanoparticles coated with chitosan, poloxamer and polyvinyl alcohol displayed complete internalization of nanoparticles in Calu-3 cells and induced low toxicity even at high concentrations, independently of their surface chemistry and charge. These nanoparticles also do not stimulate the release of IL-6, TNF α , and IL-8, endorsing the absence of inflammatory immune activation potential. These results also indicate the safety of biodegradable PLGA nanoparticles on the bronchial epithelium (Calu-3) cells¹¹².

The significant advantage of the biodegradable polymer nanoparticle as drug delivery systems is that there is no post treatment clearance required to remove the polymer nanoparticle waste after the drug administration period, owing to the degradation of the polymer. Biodegradable polymer nanoparticles are encouraging alternatives to conventional cancer treatments by capping the drug material inside the nanoparticles and hiding the drug from the immune system. However, probability of enzymatic degradation due to biophysical resemblance and evaluation of its cytotoxicity requires further investigation¹¹³.

CONCLUSION

Cancer is a major disease worldwide; however, most of the current cancer drugs are toxic to the healthy tissues of human body. Polymer nanoparticles have been found to be promising carriers for numerous drugs and small silencing RNAs. Drug delivery using biodegradable polymer nanoparticles possesses numerous advantages over other delivery systems. Polymer nanoparticle formulation encapsulates hydrophobic or hydrophilic drugs, proteins, and nucleic acids. Polymeric nanoparticles protect drugs and genes from degradation and increase their stability and controlled manner release for sustained period of time. The flexible design strategy of these polymer nanoparticles allowed the development of targeted, site-specific, and multifunctional nanoparticles for drug delivery and molecular imaging for cancer therapy. However, safe, target-specific, and controlled drug delivery nanocarriers will require further development for more effective cancer therapy.

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Figure 1. Schematic illustration of polymer nanoparticles formulation for efficient drug delivery for cancer therapy in mouse tumor model.

Figure 2.

Docetaxel encapsulated within PLGA-*b*-PEG-COOH polymer NPs using the nanoprecipitation method, and conjugated to aptamer for selective delivery to treat prostate cancer (adapted from ref ¹⁷).

Figure 3. Tumor cell apoptosis measured by TUNEL staining. (A) Microscopic evaluation of tumor apoptosis by tunnel staining. (B) Average apoptotic cell counts (adapted from ref. ²¹).

Figure 4. Structure and chemistry of the hydrophobic pro-drug platinum(IV) compound and active drug, hydrophilic cisplatin, which is released after reduction in the cell (adapted from ref. ²²).

Figure 5. Histological observation of murine lymphocytic tumors isolated from mice treated with free and nanoparticle encapsulated gemcitabine by Hematoxylin-Eosin-Saffron (HES) staining (adapted from ref ⁴⁵).

Figure 6.
PLGA hydrolysis in the body into biodegradable lactic and glycolic acid.

Figure 7. Polymer nanoparticles can deliver drugs to tumors through passive tissue targeting (EPR Effect) which is achieved by extravasation of nanoparticles via enhanced penetrability of the tumour vasculature and ineffective lymphatic drainage (adapted from ref ⁴ and ¹⁰⁴).

Table 1

Polymer nanoparticles developed for the delivery of drugs to treat various cancers

Polymer nanoparticles	Nanoparticles preparation method	Drug	Targeting ligand	Cellular Target	Cancer/cell lines	<i>In vitro</i> / <i>In vivo</i>	References
PLGA	Simple emulsion and nanoprecipitation	Paclitaxel	-	-	Human cervical carcinoma cells (HeLa)	<i>In vitro</i> and <i>In vivo</i>	16
PLGA- <i>b</i> -PEG	Nanoprecipitation	Docetaxel	A 10-aptamer	Prostate-specific membrane antigen (PSMA)	Prostate cancer (LNCaP) cells	<i>In vivo</i> in nude mice	17
PLGA- <i>b</i> -PEG	Nanoprecipitation	¹⁴ C-Paclitaxel	A 10-aptamer	PSMA	Prostate cancer/LNCaP cells	<i>In vitro</i> and <i>In vivo</i> in mice	17
PLGA- <i>b</i> -PEG	Nanoprecipitation	Docetaxel	10 2'-fluoropyridine RNA-aptamer	PSMA	Prostate cancer (LNCaP) cells	<i>In vitro</i> and <i>In vivo</i> in mice	18
PLGA- <i>b</i> -PEG and PLA- <i>b</i> -PEG	Single-emulsion	Docetaxel	S,S-2-[3-[5-amino-1-carboxypenty]-ureido]-pentanedioic acid (ACUPA)	PSMA	Prostate cancer/LNCaP cells	<i>In vitro</i> and <i>In vivo</i> in mice, rats, nonhuman primates and phase-1 clinical trials in humans	19
PLGA- <i>m</i> -PEG	Double emulsion	Cisplatin	-	-	Prostate cancer (LNCaP) cells	<i>In vitro</i>	20
PLGA- <i>m</i> -PEG + CMC	W/O/W double emulsion solvent evaporation	Cisplatin	-	-	Ovarian cancer (IGROV 1-CP) cells	<i>In vitro</i> and <i>In vivo</i> in mice	21
PLGA- <i>b</i> -PEG	Nanoprecipitation	Pt (IV) prodrug	A 10-aptamer	PSMA	Prostate cancer (LNCaP) cells	<i>In vitro</i>	22
PLGA- <i>b</i> -PEG	Nanoprecipitation	Pt (IV) prodrug	A 10-aptamer	PSMA	Prostate cancer (LNCaP) cells	<i>In vivo</i> in mice and rats	23
PLGA- <i>b</i> -PEG	Nanoprecipitation	Cisplatin	cRGD peptide	α _v β ₃ integrin	Breast (MCF-7) and prostate cancer (PC3 and DU145) cells	<i>In vitro</i> and <i>In vivo</i> in nude mice	24
PLGA	W/O/W double emulsion solvent evaporation	Cisplatin	-	-	Colon adeno-carcinoma cells	<i>In vitro</i> and <i>In vivo</i> in mice	25, 26
PLGA	W/O/W double emulsion solvent evaporation	5-fluorouracil (5-FU)	-	-	Glioma (U87MG) and breast adenocarcinoma (MCF7) cell lines	<i>In vitro</i>	27
Glycosylated chitosan (GCS)	Single-emulsion	5-FU	-	-	Hepatocellular carcinoma (HCC)/SMMC-7721 cells	<i>In vitro</i> and <i>In vivo</i> in mouse model	28, 29

Polymer nanoparticles	Nanoparticles preparation method	Drug	Targeting ligand	Cellular Target	Cancer/cell lines	<i>In vitro</i> / <i>In vivo</i>	References
HA-PEG-PLGA	Nanoprecipitation	5-FU	-	-	Ehrlich ascites tumor (EAT) cell lines	<i>In vitro</i> and <i>In vivo</i> in mice	30
PBLG-PEG	Dialysis	5-FU	-	-	Human colon cancer (LoVo) cell lines and squamous carcinoma (Tca 8113) cell	<i>In vitro</i> and <i>In vivo</i> in mice	31
PLGA	Nanoprecipitation	Doxorubicin (DOX)	-	-	MDA-MB-231 breast cancer cells	<i>In vitro</i>	32
<i>m</i> PEG- <i>b</i> -P(CL-co-HCL	Dialysis	DOX	-	-	HepG2 cells	<i>In vitro</i>	33
L-PLGA-HSA	High pressure homogenization-emulsification	DOX	-	-	Rat glioblastoma	<i>In vivo</i> in rat	34
PLGA	Solvent displacement	DOX	cLABL	ICAM-1	Lung epithelial cancer cells (A549)		35
PLGA	Single emulsion-spin-casting	DOX	-	-	HeLa cells	<i>In vitro</i>	36
PLGA	W/O/W double emulsion solvent evaporation	DOX	-	-	Fibroblast cells	<i>In vitro</i>	37
PLGA-PEG	Oil in water- solvent evaporation	DOX	Novel peptide	Epidermal growth factor receptor (EGFR)	Human ovarian cancer (SKOV3) cells	<i>In vitro</i> and <i>In vivo</i> in mice	38
PLC and PDLLA	-	Tamoxifen (TMX)	-	-	HeLa and MCF-7 cells	<i>In vitro</i>	39
PAA-Cholesterol	Electro spray	TMX	-	-	MCF-7 cells	<i>In vitro</i>	40
PLGA	Emulsion-diffusion evaporation	TMX	-	-	Breast cancer (C1271) cells	<i>In vivo</i> in mouse	41
PLGA	Solvent displacement	TMX	-	-	MCF-7 cells	<i>In vivo</i> in mouse	42
PEO-PCL	Solvent displacement	Paclitaxel and TMX	-	-	Ovarian adenocarcinoma (SKOV3) and MDR-1-positive (SKOV3TR) cell lines	<i>In vitro</i> and <i>In vivo</i> in nude mice	43
Chitosan (CS)	-	Gencitabine	HER2	-	Pancreatic cancer (Mia Paca 2 and PANC 1) cells	<i>In vitro</i>	44
Chitosan (CS)	Coacervation	Gencitabine	-	-	Murine lymphocytic tumor (L1210wt) cells	<i>In vivo</i> in mice	45
PLGA	-	Gencitabine	Anti-EGFR monoclonal antibody	EGFR	Pancreatic cancer cells	<i>In vitro</i>	46
PLGA	Emulsion-solvent evaporation	Gencitabine	-	-	Pancreatic cancer cells (PANC1)	<i>In vitro</i>	47
PEG-PDLA	W/O/W double emulsion	Gencitabine	-	-	Human pancreatic cancer (SW1990) cells	<i>In vitro</i>	48
mPEG	Ionic gelation and chemical cross-linking	Mitomycin C (MMC)	Folic acid (FA)	-	HeLa cells	<i>In vivo</i> in mice	49
Poly(butyl cyanoacrylate)	Nanoprecipitation	Epirubicin (EPI)	-	-	Human carcinoma (HeLa and A549) cell lines	<i>In vitro</i>	50

Polymer nanoparticles	Nanoparticles preparation method	Drug	Targeting ligand	Cellular Target	Cancer/cell lines	<i>In vitro</i> / <i>In vivo</i>	References
Pullulan acetate (PA)	Solvent diffusion	EPI	Folic acid (FA)	Folate receptor	Nasopharyngeal epidermal carcinoma (KB) cell lines	<i>In vitro</i>	51
Cellulose	Nanoprecipitation	Curcumin	-	-	Prostate cancer (C4-2, LnCAP, PC3 and DU145) cells	<i>In vitro</i>	52
PLGA	Solid/Oil/Water emulsion solvent evaporation	Curcumin	-	-	Prostate cancer (LnCAP, PC3 and DU145)	<i>In vitro</i>	53

Table 2

Polymer nanoparticles for microRNA delivery in cancer therapy.

Polymer nanoparticles	Nanoparticles preparation method	Conjugation	Target ligand	microRNA/d rug	Cancer/cell lines	<i>In vitro</i> / <i>In vivo</i>	References
PLGA	Double emulsion solvent evaporation	cell penetrating peptide (CPP)	-	Anti-miR-155	pre-B-cell tumors	<i>In vivo</i> in mice	72
poly-L-lysine (PLL)	Nano complex formulation	-	-	Anti-miR-10b	MDA-MB-231 breast cancer cells	<i>In vitro</i>	73
poly(amidoamine)	-	-	-	Anti-miR-21 and 5-FU	U251 glioma brain tumor cells	<i>In vitro</i>	74
PLGA-PEI	W/O/W double emulsion	-	-	miRNA-26a expression vector (pDNA)	HepG2 cells	<i>In vitro</i>	75
PLGA	W/O/W double emulsion	CPP	-	Anti-miR-155	KB cells	<i>In vitro</i>	76
PEG-LPH	Thin film hydration followed by membrane extrusion	Cyclic RGD peptide (cRGD)	$\alpha_v\beta_3$ integrin	Anti-miR-296	human umbilical vein endothelial cells (HUEVCs)	<i>In vitro</i> , and <i>In vivo</i> in nude mice	77

Table 3

Polymer nanoparticles for the delivery of siRNAs for cancer therapy.

Polymer nanoparticles	Nanoparticles preparation method	Conjugation	Target ligand	siRNA	Cancer/cell lines	<i>In vitro/In vivo</i>	References
PLGA	Double emulsion	-	-	siERK2, siMAPK1	HepG2, NIH/3T3 fibroblasts and HeLa cells	<i>In vitro</i> and <i>In vivo</i> in transgenic EGFP mice	81
PLGA-chitosan	Spontaneous emulsion diffusion	-	HBV-gene	sense: 5'-ACCGTCTGTGCCTTCTCAATCTTTT-3'; antisense: 3'-TTTGGCAGACACGGGAAGAGTA5'	HepG2.2.15 cell lines	<i>In vitro</i>	82
mPEG - PLGA - PLL	W/O/W double emulsion	-	-	siRNA	Retinal pigment epithelium (RPE - J) cells	<i>In vitro</i>	83
mPEG - PLGA - PLL	W/O/W double emulsion	-	-	siRNA	Human lung cancer (SPC-A1-GFP) cells	<i>In vitro</i>	84
mPEG-PLGA-PLL	-	cRGD	-	hVEGF-siRNA	PC3 cells	<i>In vitro</i>	85
PEI-PEG	Electrostatic interactions	Ala-Pro-Arg-Pro-Gly (APRPG)	human VEGF	siRNA sense: 5'-GGAGUACCCUGAUGAGAUcTdT-3'; antisense: 5'-GAUCUCAUGGGUACUCCdTdT-3'	Breast cancer/MCF-7 cells	<i>In vitro</i> and <i>In vivo</i> in mice	86

Table 4

Polymer nanoparticles for the delivery of the shRNAs for cancer therapy.

Polymer nanoparticles	Nanoparticles preparation method	Conjugation	Target ligand	shRNA	Cancer/cell lines	<i>In vitro</i> / <i>In vivo</i>	References
PLGA	W/O/W double emulsion	-	-	HIF-1 α -shRNA- pDNA	choroidal neovascularization (CNV)	<i>In vitro</i> and <i>In vivo</i> in rats	92
PLGA	Double emulsion-solvent evaporation	-	-	VEGF-A -shRNA (AATGCAGACCAAGAAAGACA)	murine corneal neovascularization (KNV)	<i>In vivo</i> in mice	93
PLGA	Solvent diffusion	A 10 aptamer	PSMA	AR-shRNA	prostate cancer (22RV1, LAPC-4 and LNCaP) cell lines	<i>In vitro</i> and <i>In vivo</i> in mice	94
Chitosan	Nano complex formulation	-	VEGF	VEGF-shRNA	HCC cells	<i>In vitro</i> and <i>In vivo</i> in mice	95
PAE	Nano complex formulation	-	-	shRNA	U-87 MG cells and HEK-293 cells	<i>In vivo</i> in mice	96