Current advances in drug delivery systems for treatment of Triple negative breast cancer (TNBC)

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ABSTRACT

Triple negative breast cancer, the most malignant and aggressive form of breast cancer, is accompanied with poor prognosis in patients. Characterized by the absence of expression of estrogen receptor, progesterone receptor and human epidermal growth factor receptor-2, TNBC cells are unresponsive to hormonal therapy. With only cytotoxic chemotherapy drugs as an established treatment option, tumor-targeted delivery of drugs becomes an important parameter to prevent or attenuate chemotherapy-associated side effects and toxicity in TNBC patients. Despite the current advances in TNBC-targeting drug delivery systems (TNBC-TDDS), the treatment outcome remains relatively low. These systems face challenges of drug instability and decreased drug-loading potential. In addition, further investigations are required to address formulations, route of administration, frequency of disease recurrence and non-target side effects, apart from cutting down the cost of development. This concise review summarizes the most recent findings in the field of TNBC-TDDS and highlights the future directions and research perspectives.

Keywords: Triple negative breast cancer, Drug delivery, Liposome, Nanoparticles, Hydrogels, Aptamer

INTRODUCTION

According to Cancer Statistics for the year 2019, breast cancer (BC) is the most common cancer type among women and a leading cause of cancer-related mortality among females aged between 20 to 59 years.¹ Breast cancer is a phenotypically variable and heterogeneous disease based on clinical and genetic parameters.²,³ Thus for efficient treatment of patients, BC has been categorized into various subtypes based on the expression pattern of three receptors – estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) on tumor cells, which in turn governs the type of treatment a patient would receive.³ BC has been broadly subdivided into following subtypes: luminal A (ER⁺, PRhigh, HER2⁻), luminal B (ER⁺, PRlow, HER2⁻), HER2 positive (ER/PR-, HER2⁺) and basal-like (ER-, PR-, HER2⁻).²

Triple negative breast cancer (TNBC), another subtype of BC, is known to be the most malignant and aggressive form of breast cancer.⁴ TNBC accounts for 15 to 20 percent of all breast cancer cases.⁵ Though TNBC is often synonymously used with basal-like subtype, both categories have reported 30 percent discordance.⁶ The absence of ER, PR and HER2 expression renders TNBC cells unresponsive to hormonal
and/or targeted therapies.\textsuperscript{5,11} TNBC tumors are often accompanied with metastasis at secondary organs which includes brain, lungs and bones.\textsuperscript{4,5} Unresponsiveness to hormonal and anti-HER2 therapies makes chemotherapy as the only available option for treatment of TNBC. TNBC has been further classified into four subtypes based on unique gene expression profiles and response to chemotherapy as: (i) basal like 1 (BL1), (ii) basal like 2 (BL2), (iii) mesenchymal (M) and (iv) luminal androgen receptor (LAR).\textsuperscript{12} Lack of validated molecular targets, development of metastasis and lack of effective targeted therapies result in poor prognosis in TNBC patients.\textsuperscript{4,5,13}

Current treatment options for cancer include surgery, radiation therapy and chemotherapy.\textsuperscript{14} One major factor which influences the efficacy of chemotherapeutic drugs is an efficient drug delivery system that can target the drugs to the tumor. Conventional drug delivery systems (CDDSs) include direct oral and intravenous administration of chemotherapeutic drugs.\textsuperscript{13} The efficiency of CDDSs is often limited due to lower penetration, poor and unspecific temporal and spatial distribution of drugs and adverse side effects associated with higher drug doses.\textsuperscript{16,17}

Over the years, the field of cancer therapeutics has seen a paradigm shift. The cytotoxicity associated with traditional drugs has led to emergence of drugs that specifically target proteins in cancer cells.\textsuperscript{18} To keep up with the pace and for safe and effective drug administration, parallel advancements in drug delivery systems are also needed.\textsuperscript{19} Thus recent developments in tumor-targeted drug delivery systems (TTDDS) have led to improved targeting of drugs, controlled drug release, longer retention, and higher efficacy accompanied with reduction in drug-associated toxicities, non-target effects and side-effects. The present review summarizes the recent research findings on drug delivery systems for treatment of triple negative breast cancer (Figure 1). It also discusses the advantages and limitations associated with each system and the scope for further improvements.

![Figure 1](image-url) Schematic representation of subtypes Triple Negative Breast Cancer (TNBC).

**NANOPARTICLE-MEDIATED DRUG DELIVERY**

**Nanoparticles** (NPs) as drug delivery platform in cancer is very well established and extensively studied domain.\textsuperscript{20-23} A major risk factor associated with NPs and other Nano-drug delivery vehicles is chances of toxicity owing to their small size.\textsuperscript{24} Limitations associated with the use of nanoparticle-mediated drug delivery systems include non-target toxicity, drug loading capacity and controlled release efficiency. Efforts have been made to design different nanoparticle-based carriers (nanocrystals, nanotubes, nanobubbles, nanocages, etc.) with unique structural features in order to overcome these limitations.

Poly(lactic-co-glycolic-acid) (PLGA) nanoparticles represent the most commonly studied type of nanoparticles. Docetaxel-loaded PLGA NPs were synthesized and found to inhibit tumor growth in basal-like subtype TNBC mouse model.\textsuperscript{25} Hyaluronic acid-coated PLGA NPs developed for codelivery of doxorubicin and microRNA-542-3p showed enhanced cellular uptake and cytotoxicity in MDA-MB-231 cells.\textsuperscript{26} Another study reported development of paclitaxel-loaded PLGA NPs coated with hyaluronic acid for effective targeting of CD44 receptors on TNBC cells. These NPs showed higher cytotoxicity and sustained drug release in MDA-MB-231 cell lines.\textsuperscript{27} Paclitaxel-loaded PLGA NPs coated with anti-EGFR have been developed for treatment of TNBC and were found to increase therapeutic efficiency and subsidized paclitaxel-mediated side-effects.\textsuperscript{28} Another paclitaxel-loaded PLGA NPs coated with perlecanning-binding antibodies have also been reported.\textsuperscript{29} Some researchers have modified these PLGA NPs by conjugation with polyethylene glycol (PEG) which renders these NPs evasion from immune detection.\textsuperscript{30} PLGA-PEG-NPs loaded with antisense-microRNA-10b and antisense-microRNA-21 showed substantial inhibition of tumor growth in mice model.\textsuperscript{31} In another study, PLGA-PEG-NPs loaded with TK-NTR fusion gene were designed. The transfection of TNBC cells with TK-NTR (thymidine kinase-nitroreductase) fusion gene made them sensitive to produgs, ganciclovir and CB1954, and enhanced apoptosis and inhibited tumor growth.\textsuperscript{32} PLGA-PEG-NPs loaded with orlistat and its combinatorial treatment with PLGA-PEG-NPs loaded with antisense-microRNA-21 showed enhanced apoptotic effect.\textsuperscript{30} PLGA-PEG-NPs encapsulating dual agents, erlotinib and doxorubicin and sequentially delivering these agents resulted in enhanced antitumor efficacy in TNBC models.\textsuperscript{33} PEG-PLA (poly(ethylene glycol)-poly(lactic acid)) NPs coated with two peptide types, a tumor-homing peptide and a cell penetrating peptide, linked via pH sensitive bond and loaded with dual agents, erlotinib and DAPT (a gamma secretase inhibitor) showed enhanced tumor specificity and reduced side-effects.\textsuperscript{34} Chitosan-loaded PLA NPs have showed enhanced drug loading capacity.\textsuperscript{35}

**Gold Nanoparticles** (Au-NPs) is another emerging form of NPs extensively studied for drug delivery.\textsuperscript{36} Au-NPs fabricated onto gelatin NPs (degradable by MMP2 present in tumor microenvironment) were loaded with doxorubicin and coated with RGD peptide for tumor specific targeting and showed high tumor penetrance in mice model.\textsuperscript{37} In another study, Au-NPs conjugated with Rad6 inhibitor, when combined with cisplatin, increased its efficiency.\textsuperscript{38} Gold nanoparticles have also been used as siRNA-delivery vehicles and may be a potential therapeutic candidate for TNBC treatment.\textsuperscript{39}

**Silver Nanoparticles** (Ag-NPs) are the most extensively used form of NPs used in clinical applications. Comparative analysis have revealed that silver nanoparticles are highly cytotoxic towards TNBC cells while being non-toxic to non-cancerous cells at similar doses.\textsuperscript{40,41} These studies have
established the potential of Ag-NPs for specific TNBC treatment. In another study, researchers analyzed albumin-coated Ag-NPs for their cytotoxicity in MDA-MB-231 cells. Albumin-coated Ag-NPs were found to induce higher apoptotic cell death in cancer cells compared to normal cells.42

**Figure 2.** Schematic representation of recently studied drug delivery systems for treatment of Triple Negative Breast Cancer (TNBC).

**Mesoporous silica NPs** (MSNs) constitute another class of nanocarriers that are promising candidates for codelivery of siRNA and chemotherapeutic drug formulations.43 MSNs coated with RGD peptide and loaded with arsenic trioxide were found to possess higher therapeutic efficiency in MDA-MB-231 tumors.44 A hybrid platform in which graphene quantum dots (GQDs) were incorporated inside MSNs and loaded with doxorubicin exhibited higher drug loading capacity and biocompatibility.45 MSNs coated with ICAM1 antibody have shown effective targeting of TNBC cells.46

**Polymer-lipid NPs** coated with RGD peptide and coloaded with doxorubicin and mitomycin C have been recently reported. RGD peptide targets integrin αvβ3 overexpressed on TNBC cells.47 This form of NPs have also been reported to be potentially effective in the treatment of TNBC-associated brain metastases.48 Zhou and colleagues have designed calcium phosphate polymer NPs for codelivery of paclitaxel and inhibitors of microRNA-221/222 and showed enhanced therapeutic efficacy against TNBC.49 Other polymer-based nanoparticles include poly(δ-caprolactone)50, poly(lysine)-based NPs51, PS-80 amphiphilic NPs52 and poly(β-aminoester)-based NPs that have been evaluated for their antitumor efficacy in TNBC models and have emerged as promising drug delivery platforms.

NPs composed of carboxymethyl dextran derivatives, conjugated with Trop2 antibody and loaded with doxorubicin, showed higher toxicity in MDA-MB-231Trop+ cells.53 Recently, Juneja et al. (2019) reported hybrid polysilësesquioxane nanoparticles loaded with a combination of curcumin, protoporphyrin and RNAi inducers. These nanoparticles showed synergistic anticancer activity in MDA-MB-231 cells and thus hold great potential as therapeutic delivery systems for TNBC treatment.54

**Gold Nanorods** (Au-NRs or GNRs) is an emerging class of metallic nanoparticles that has gained attention in drug delivery due to its biocompatibility and photothermal responsiveness.55 Chitosan-layered Au-NRs for effective delivery of siRNA (scrambled or pyruvate kinase isozyme M2/PKM2), protected siRNA from lysosomal degradation and effectively inhibited the expression of oncogene in MDA-MB-231 cells. In vivo studies showed that the nanocomplexes efficiently delivered siRNA to tumor tissue and inhibited gene expression.55 A hybrid nanoplatform with Au-NRs conjugated with covalent-polypeptide and folic acid and loaded with chitosan, a chemotherapeutic drug has been fabricated.56 In conjunction to near infrared-photothermal therapy (NIR-PTT), the nanoplatform was found to significantly inhibit TNBC tumor growth and prevent metastasis of TNBC cells to lung in mice model.56 Recently, Photoacoustic Imaging (PAI) has been implemented for selective visualization of solid tumors and Au-NRs have been used as potential contrast agents for PAI.57,58 A strategy to evaluate the synergistic effect of NIR-PTT and Au-NRs, guided by PAI has been developed.58 The gold nanorods were conjugated with anti-EGFR antibodies to target EGFR-overexpressing TNBC cells. Combination of antiEGFR-Au-NRs and NIR-PTT provided enhanced anti-tumor efficacy compared to free-antiEGFR antibodies in mice.58

**Nanocapsules** are vesicular drug delivery systems composed of a liquid core and a polymeric shell.59 Lipid-based nanocapsules encapsulating FoHTAM (a derivative of hydroxytamoxifen) have been developed and validated for their performance in xenografted TNBC mice models which resulted in reduction in tumor volume.60 Wang and group (2015) developed nanocapsules by self assembly of negatively-charged Hyaluronic acid (HA) and positively-charged Protamine sulfate (PS) to form interpolyelectrolyte complexes (IPECs).61 These nanocapsules encapsulated microRNA-34a which has a tumor suppressor function. In vitro analysis in MDA-MB-231 cells revealed that miR-34a carrying nanocapsules effectively triggered apoptosis and inhibited cell migration. In vivo analysis showed suppression of tumor growth in xenografted mice model.61 Kanwar and group (2016) have reported a novel type of Fe3O4-saturated lactoferrin nanocapsules encapsulating chitosan nanogel.62 In vitro analysis in MDA-MB-231 cells showed excellent efficiency of internalization, inhibition of colony growth and formation, and reduction in diameter of 3D-
tumor spheroids. In vivo studies revealed high tumor localization and antitumor efficacy of these nanocapsules.  

**Nanobubbles** (NBs) are an emerging class of drug delivery vehicles. NBs are nanometer-sized, spherical structures with a gas-filled core surrounded by a stabilizing shell. NBs are characterized with high stability, strong penetrance, longer half-life and minimal invasiveness. Jing et al. (2016) reported a synergistic technology combining (i) nanobubbles loaded with CPPs and siEGFR (CPP-NB$_{siEGFR}$) and (ii) ultrasound-mediated nanobubble destruction for targeted delivery to TNBC cells (CPP-NB$_{siEGFR}$+US). The CPP-NB$_{siEGFR}$+US system did not exhibit significant difference compared to control groups MDA-MB-231 cell lines but showed increased tumor growth inhibition in xenografted models.  

**Nanoshells** (NSs), also equivalently called as nanobubbles or nanocapsules, are nano-sized spherical structures with presence of a shell and absence of a core. Riley et al. (2017) developed Frizzled7 antibody-conjugated nanoshells (Fzd7-NS) to specifically target Frizzled7 receptor overexpressed on the surface of TNBC cells. Blockade of Frizzled7 receptor through Fzd7-NS resulted in inactivation of Wnt signaling and in turn decrease in cell viability.  

**Nanocrystals** are a type of drug delivery platform in which pure drug is processed to form crystals with relatively increased bioavailability. Recently, hyaluronic acid-coated Lapatinib Nanocrystals (LPT-HA-NCs) have been reported for effective targeting of CD44 receptor-overexpressing TNBC cells. In vitro (MDA-MB-231 cells) and in vivo (4T1 cells-induced tumor bearing mice model) investigations revealed that LPT-HA-NCs exhibited higher antitumor efficacy compared to LPT-NCs and Free-Lapatinib. Also, LPT-HA-NCs increased residence time of LPT, specifically targeted the TNBC tumor, and improved overall survival outcome.  

**Nanocages** is an emerging NP-based smart drug delivery system with hollow structure and exhibit high loading capacity, controlled release and low immunotoxicity. Nanocages are further divided into organic and protein-based. Among the protein-based nanocages, ferritin has emerged as a promising candidate due to high biocompatibility and biodegradability, low toxicity, and the ability to encapsulate both hydrophobic (between the bilayer) and hydrophilic (in the liposomal core) drugs.  

Liposomal carriers have been demonstrated with prodrug-modified surfaces for codelivery of gemcitabine and docetaxel for targeting of TNBC cells overexpressing CD44. These liposomes have been attributed with high drug loading efficiency and dual (pH and enzymatic) stimulus-responsive drug release. In vitro studies demonstrated synergistic effect of the drugs on cytotoxicity, apoptosis and inhibition of wound healing. In vivo experiments further showed improved accumulation in tumor, high antitumor and antiproliferative efficacy and enhancement of apoptosis, complemented with no systemic toxicity.
Shen and colleagues (2017) developed liposomal carriers encapsulating ruthenium coordination complexes (Lipo-Ru) in hydrophobic bilayer.81 In vitro analysis in MDA-MB-231 cells revealed that Lipo-Ru can induce DSBs (double-stranded breaks) and lead to apoptosis. In vivo studies in TNBC mice model showed that Lipo-Ru can inhibit tumor growth.81

In an earlier study, a liposomal delivery system encapsulating lipocalin-2 siRNA to target TNBC cells expressing ICAM1 (Intercellular cell adhesion molecule 1) on their surface had been engineered.82 The ICAM1 targeting liposomes exhibited stronger binding to TNBC MDA-MB-231 cells compared to MCF-10A non-neoplastic cells.82 In another recent study, a newer strategy has been demonstrated to design dual complementary liposomes (DCLs), encapsulating doxorubicin, that target both ICAM1 and EGFR expressed on TNBC cells.83 The DCLs have following advantages: (i) increase in cellular binding due to multivalent surface interactions, (ii) increased endocytic internalization and (iii) increased antitumor efficacy with blocking of ICAM1 and EGFR pathways.83

Though liposomes are an extensively studied class of targeted delivery systems, it is not exempt from limitations. The inherent problems associated with liposomes is low drug loading capacity, poor stability, drug leakage and difficulty of sterilization.24,84

**HYDROGEL-MEDIATED DRUG DELIVERY**

Hydrogels have emerged as clinically relevant drug delivery systems for controlled release of therapeutic anticancer agents.85–87 Hydrogels are composed of two units: (i) water and (ii) polymer network crosslinked together. The hydrogels being largely composed of water can easily be loaded with hydrophilic drugs.85,86

Xie and coworkers (2017) have reported a multi-agent codelivery hydrogel-based system (DDMH) for effective treatment of TNBC.88 The hydrogel was synthesized using chitosan crosslinked with difunctional poly ethylene glycol, it was then loaded with two chemotherapeutic drugs (doxorubicin and docetaxel) and iron oxide. The iron oxide (Fe3O4) is required for controlled asynchronous release of drugs upon hyperthermia induced via alternative magnetic field (AMF). In vitro testing on MDA-MB-231 cell line showed improved synergistic effect compared to single drug formulations. In vivo testing of the hydrogel on mice model showed higher antitumor efficacy and controlled drug release.88

In another study, a single agent peptide hydrogel has been reported as an effective strategy to prevent growth of TNBC primary tumor and also its metastasis to lung.89 The peptide hydrogel was loaded with losartan and validated for its efficacy in 4T1-tumor mice models through intratumoral injection. The hydrogel had increased retention time, inhibited growth of cancer-associated fibroblasts and also tumor growth.89 Thus these peptide hydrogel can potentially increase the efficiency of chemotherapy in TNBC.

In a recent study, poly-n-isopropyl-acrylamide-based P(NIPA) hydrogels have been reported for the controlled release of prodigiosin drug for localized TNBC treatment.90 The controlled release is mediated by the thermo-sensitivity of these hydrogels. In vitro analysis in MDA-MB-231 cells revealed that cell survival was inhibited more during the early hours of treatment with P(NIPA) hydrogels.90 With further investigation, P(NIPA)-based hydrogels can prove to be effective systems for localized cancer treatment.

Hydrogels offer attractive advantages over other DDSs such as high porosity, controlled drug release and ease of covalent modification with peptide ligands.85,87 In spite of the advances, hydrogels suffer from the following disadvantages: non-adherence, poor mechanical strength, difficulty and high cost of handling and development.85,91

**EXOSOME-MEDIATED DRUG DELIVERY**

Exosomes (Exo) are a subtype of extracellular vesicles with a diameter of 40 to 200nm and are derived from the cell membrane.92–94 Natural (cell-derived) as well as artificial (exosome mimetics) exosomes as drug delivery platform have garnered much attention in the recent years due to their high efficiency of drug targeting and biocompatibility complemented by low toxicity and immunogenicity.95,96

A recent study has reported a disintegrin and metalloproteinase 15 (A15) labeled exosomes (A15-Exo) for co-delivery of Doxorubicin and microRNA-159 for TNBC therapy.93 Expression of A15 on the surface of Exo mediated an increase in binding to integrin αvβ3 expressed on the TNBC cells. In vitro experiments performed on MDA-MB-231 cells showed that A15-Exo did not inhibit cell proliferation or induce apoptosis but it inhibited cell migration. In vivo studies showed an improved anticancer efficacy by inhibiting tumor growth and absence of adverse effects compared to single agent A15-Exo preparations.93

![Figure 3](https://example.com)
In another very recent study, researchers have demonstrated targeted delivery of erastin to TNBC cells overexpressing folate receptor via erastin loaded-folate labeled-exosomes (Erastin-FA-Exo).\textsuperscript{95} In vitro studies in MDA-MB-231 cells demonstrated that Erastin-FA-Exo exhibited higher uptake efficiency, improved inhibition of cell proliferation and migration and promoted ferroptosis compared to Erastin-Exo and Free-Erastin.\textsuperscript{95}

Though very similar to liposomes, exosomes as DDSs have limitations such as low drug loading and releasing capacity, poor pharmacokinetics and low industrial yields.\textsuperscript{94,96} Labeling of exosomes with peptides or their combination with other TTDDs may helps in overcoming associated limitations. Thus, exosome-mediated drug delivery systems constitute a powerful and innovative platform for TNBC treatment.

**MICELLES-MEDIATED DRUG DELIVERY**

Micelles are formed by self-assembly of copolymers and impart amphiphilic properties, composed of hydrophilic corona and hydrophobic core. This colloidal nanomedicine (5-100 nm) shows enhanced water solubility of drugs and its easy permeability through membrane, with least non-specific toxicity. Polymeric micelles have multiblock copolymers. Contemporary cancer therapy in TNBC using micelle nanocarriers was advanced with quantum dot-based micelle and magnetic micelles. Drug delivery of a potent drug aminoflavone, by QD-based micelle in MDA-MB-468 cells demonstrated better tumor targeting and recorded significant tumor regression. This theranostic micelle-based tumor targeting requires less dose to inhibit the cancerous cell growth, and resulted in reduced systemic toxicity.\textsuperscript{97} Dasatinib, a multitargeted inhibitor of several kinases was loaded on to a magnetic micelle and utilized as a targeted therapy against MDA-MB-231 cell lines. A marked increase in cytotoxicity of Dasatinib was observed by 1.35 fold, under external magnetic field. Such magnetic-based nanocarriers aided in tumor-targeted drug delivery, MRI and hyperthermia in tumor localised regions.\textsuperscript{98} Besides, some other potential refinements were reported in therapeutic treatment of TNBC such as Curcumin-derived nanomicelles\textsuperscript{99}, docetaxel-loaded micelles conjugated with Cetuximab\textsuperscript{100} and combination therapy involving SAHA (Suberoylanilide hydroxamic acid) and paclitaxel.\textsuperscript{101} All such combinations were found to enhance therapeutic effect of drugs by several folds. Wu and group (2017) attempted to lipophilized bortezomib, a proteasomal inhibitor drug with broad range of effectiveness against different types of cancer.\textsuperscript{102} This lipophilized-micellar nanoformulation benefited in curbing tumor growth by prolonged circulation time, enhanced tolerability and selective tumor targeting.\textsuperscript{102}

Compared to other DDSs, these polymer micelles have high loading capacity, stability and deliver hydrophobic drugs with high therapeutic efficacy. But challenges like improving the drug-loading efficiency, increasing thermodynamic and kinetic stability and enhancing transport across the cell membrane, need to be resolved before fully utilizing the potential of micelles as a TTDDS.\textsuperscript{84}

**APTAMER-MEDIATED DRUG DELIVERY**

Aptamers (also referred to as ‘chemical antibodies’) are short nucleic acid molecules; small oligonucleotide stretch of single-stranded DNA or RNA. It binds its target even in picomolar range, with extreme affinity and selectivity. Aptamers are utilised both in imaging and cancer therapeutics. It could be easily manipulated with great potential in identifying novel targets, hamper its activity and deliver imaging agents to tumor cells. Aptamers ability to specifically recognize protein signature associated with different cancers offers personalised tumour treatment. Diverse aptamers formulations have targeted surface-localised cellular proteins, allowed targeted delivery of therapeutic agents such as siRNA, miRNA, anti-miRNA, toxins, and chemotherapeutics in cancer cell lines.\textsuperscript{103} Aptamers could be either directly conjugated to therapeutic agents or loaded with a cargo. Preclinical studies and imaging using aptamers in TNBC aimed various prevalent cancerous cell biomarkers-nucleolin (NCL), EGFR, mucin (MUC), receptor tyrosine kinases (RTKs) and PDGF receptor β (PDGFRβ). These biomarkers were also found in other cancer types. Despite a potential alternative, applications of aptamers were limited as anticancer drug with exception of two aptamers, AS1411 and NOX-A12, under clinical evaluation. A multifunctional, biocompatible and fluorescent complex of aptamer (AS1411) conjugated to gold nanorods i.e. AS1411-GNR conjugates was designed. This nanconstruct has nucleolin-driven uptake in tumor cells, aided well in cancer imaging, diagnostics and treatment by photothermal therapy.\textsuperscript{104} Adriamycin a chemotherapeutic drug was loaded to 5TR1-GC aptamer and constructed as 5TR1-GC-DOX complex. It was evaluated on MDA-MB-231 cells for its uptake ability and cancer cytotoxicity. The effectiveness of this drug system was even confirmed in vivo on xenograft. This complex was found to successfully destroy malignant tumor cells without damaging normal cells. The delivery of aptamer-guided siRNA (anti-CD44 aptamer i.e. Apt1) that targets CD44 receptor in TNBC.
cells, could efficiently silence CD44 and downregulates expression of this tumor causing gene in cancer cells lines.

Apt1 nanomedicine was quite effective in TNBC model. These chemical antibodies formed of nucleic acids are preferred because they are highly stable, selective for their target, bind with good affinity, could be easily modified and cause no immunogenicity. Some of the limitations of this system that need to be overcome are its expensive and labor-intensity. Aptamer-based nanomedicines are emerging class of agents in TNBC that facilitates targeted drug delivery using cancer-linked hallmarks.

**ELECTRO-ACUPUNCTURE-MEDIATED DRUG DELIVERY**

Acupuncture, a traditional technique of Chinese medicine, practiced in approximately 103 countries as per WHO statistics. It is extensively used in none-drug therapy of cancer patients, as a remedy for various cancer-driven symptoms such as pain, dry mouth, vomiting, insomnia, anxiety, intestinal obstruction in postoperative phase and others. A study was performed on TNBC mice model to investigate role of electro-acupuncture in altering drug distribution to tumor. In vivo fluorescence imaging found increased drug concentration in tumor area using electro-acupuncture, compared to control groups. Pharmacokinetic profiling confirmed enhanced drug concentration in intratumoral regions. These results were further supported by elevated apoptosis in drug-loaded acupuncture group, suggested enhanced tumor cytotoxicity of paclitaxel-only mice group accompanied with electro-acupuncture, compared to paclitaxel-only mice group. Electro-acupuncture has been found improve drug delivery by altering tumor microvasculature. It regulates tumor microenvironment with no potential risk of tumor metastasis. This study accords with the research conducted by Zhang and group (2014) on lung cancer model mice.

A combination of drug (paclitaxel) with electro-acupuncture offered great potential in TTDDS. However, these results were recorded 2 hours after electro-acupuncture application demanding further investigation using continuous acupuncture intervention. Efficacy of electro-acupuncture has to be tested with other drug combinations. The pain associated with conventional acupuncture and need of trained professional limits its usage. Nanotechnology thus offers new innovations to traditional acupuncture technique at nanoscale to realise the concept of effective and controlled drug delivery.

**OTHER EMERGING DRUG DELIVERY SYSTEMS**

2D nanomaterial-based drug delivery systems have garnered attention in field of biomedicine due to their relatively high surface area and intrinsic optical properties. In a recent investigation, Hu and colleagues (2019) reported the development of 2D-Glyoclusters for targeted delivery of drugs to TNBC cells. In vitro analysis in MDA-MB-231 cells showed efficient degradation of MnO2 backbone and subsequent sustained drug release is achieved through the nanochannels (3µm in width) in NDES membrane. The antibodies encapsulated in the NDES, first solubilize in the nanofluid, and then diffuse across the nanochannels into the tumor. In vivo studies of antibody-loaded-NDES in 4T1 TNBC murine models demonstrated tumor growth inhibition, increased infiltration of immune cells at tumor site and thus effective NDES-mediated immunotherapy could be achieved.

Another recent study reports development of a novel glutamine-conjugated β-cyclodextrin (DOX-GLN-CD) encapsulating single agent, doxorubicin, for targeted delivery to TNBC tumors. In vitro analysis revealed DOX-GLN-CD specifically accumulated in TNBC cells via facilitated diffusion through glutamine transporter ASCT2. The conjugate induced cell cycle blockade and apoptosis in TNBC cells, MDA-MB-231 and BT549, and was minimally effective towards non-tumorigenic cell line MCF10A. In vivo experiments also showed similar results with improved outcome and reduced toxicity.

**CONCLUSION**

TNBC is the most aggressive subtype of breast cancer. With peculiar molecular profile it could escape hormonal therapy, leaving cytotoxic chemotherapy as the only therapeutic measure against TNBC. Efficient drug delivery systems are thus a critical need to minimalize chemotherapy-associated toxicity and side-effects.

Contemporary targeted drug delivery system (TDDS) offers great advantage over conventional methods of drug delivery. The drug delivery systems have advanced from single to multiagent encapsulation; from single to multivalent targeting on TNBC cells; from rapid to controlled and sustained drug release and from low to high antitumor efficacy. Owing to enhanced efficacy of the drug, reduced dosage requirement and restricted non-targeted effects, it demonstrated unprecedented achievements in cancer therapeutics. TDDS still face a biggest challenge of drug instability and decreased drug-loading...
potential. Further investigations are required to address the formulations, route of administration, frequency of disease recurrence and non-target and long-term side-effects. Apart from DDS, synergistic techniques such as hyperthermia, pH, photothermal therapy, ultrasound-mediated therapy have also emerged for stimulus-responsive, effective and targeted drug delivery.

The current review summarizes the recent research findings in the field of DDS for targeted treatment of TNBC. It highlights the established systems including nanoparticles, liposomes, hydrogels, exosomes, micelles, aptamers, electro-acupuncture as well as emerging drug delivery platforms such as 2D-glycoclusters, hyaluronic acid conjugates, nanoscale metalorganic framework, drug seeds and β-cyclodextrin conjugates. Despite the current advances in TNBC-targeting DDSs, the disease outcome remains relatively low for TNBC patients. One major factor that hampers the application of these systems is the high cost of development. In conclusion, further advancements in the field of TNBC-targeting drug delivery platforms and their translation from bench to bedside is imperative for combating this deadly disease.

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ABBREVIATIONS

2D Two Dimensional
BC Breast Cancer
BL1 Basal like 1
BL2 Basal like 2
CDDS Conventional Drug Delivery Systems
DCL Dual Complementary Liposomes
DDMH Dual Drug-loaded Magnetic Hydrogel
DDS Drug Delivery Systems
EGFR Epidermal Growth Factor Receptor
ER Estrogen Receptor
GNR Gold Nanorod
HA Hyaluronic Acid
HER2 Human Epidermal Growth Factor 2
ICAM1 Intercellular cell Adhesion Molecule 1
LAR Luminal Androgen Receptor
M Mesenchymal
MRI Magnetic Resonance Imaging
MSN Mesoporous silica NP
NDES Nanofluidic Drug Eluting Seeds
NMOF Nanoscale Metalorganic Framework
NPs Nanoparticles
PDT Photo derail therapy
PEG Poly Ethylene Glycol
PLGA Poly(lactic-co-glycolic-acid)
PR Progesterone Receptor
QD Quantum Dots
SAHA Suberoylanilide hydroxamic acid
TNBC Triple Negative Breast Cancer
TTDDS Tumor Targeted Drug Delivery Systems
UCNP Upconversion Nanoparticles

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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