RESEARCH PAPER

Nanoparticle-Based Drug Delivery Systems for Cancer Therapy: Formulation and In-Vitro Evaluation..

Dr. Namdeo Bhagwan Admuthe[1], Dr Monjit Saikia[2], Priyanka B N[3], Prof. Shalet Benvin[4*]

1Assistant Professor, Department of Botany, Shri Sadguru Gangageer Maharaj Science, Gautam Arts & Sanjivani Commerce College, Kopargaon, Dist. Ahmednagar, Maharashtra, India
2Associate professor, Department of Botany, Rabindranath Tagore university, dist:-Hojai, Assam, Pin 782435
3PG Scholar, Department of Computer Science and Engineering, Adichunchanagiri University, BGS INSTITUTE OF TECHNOLOGY Mandya Karnataka India 571448
4*Prof. Shalet Benvin, Assistant Professor, Department of Computer Science and Engineering, College: Adichunchanagiri BGS INSTITUTE OF TECHNOLOGY, Mandya Karnataka India, 571448, E- mail id: shaletbenvin@gmail.com

ABSTRACT

Conventional chemotherapy for cancer treatment suffers from three major problems which include systemic toxicity and poor drug absorption and its inability to target specific tumors. Researchers developed nanoparticle-based drug delivery systems (NDDS) to solve these problems. The system achieves controlled drug release and enhanced drug absorption through the EPR mechanism and delivers drugs directly to specific cells.The researchers conducted a study to develop and test PLGA-based nanoparticles that carry docetaxel (DTX) as a breast cancer treatment, which they designed to use folic acid (FA) for receptor-based active drug delivery. The researchers produced DTX-loaded PLGA nanoparticles through the nanoprecipitation method, which they analyzed to determine their particle size and zeta potential and polydispersity index (PDI) and encapsulation efficiency (EE%) and morphology through dynamic light scattering (DLS) and transmission electron microscopy (TEM) analysis. The researchers tested the drug release of the sample by using phosphate-buffered saline (PBS) at two different pH values which included 7.4 and 5.0. The researchers used the MTT assay to study cytotoxicity effects on MCF-7 breast cancer cells and L929 normal fibroblast cells, while they tracked cellular uptake through confocal microscopy with rhodamine B-labeled nanoparticles.

The optimized DTX-FA-PLGA-NPs showed a particle size of 182.4 nanometers with a standard deviation of 6.3 nanometers and a zeta potential measurement of −28.7 millivolts with a standard deviation of 1.4 millivolts and a PDI measurement of 0.18 with a standard deviation of 0.03 and an EE measurement of 87.6 with a standard deviation of 2.1 percent. The TEM analysis showed that the particles had a spherical shape and their surfaces appeared smooth. The in-vitro release profiles showed a dual phase pattern which started with an initial burst release of approximately 28 percent within four hours and continued until 85 percent of the substance was released after 72 hours with an increased release rate occurring at pH 5.0 which simulated the conditions found inside endosomes. The MTT assay showed that DTX-FA-PLGA-NPs had an IC50 value of 38.5 nM against MCF-7 cells which was significantly less than the IC50 value of free DTX which measured 112.3 nM and the IC50 value of non-targeted nanoparticles which measured 79.4 nM. The L929 cells showed no signs of cytotoxicity at all. The study demonstrated that FA-functionalized nanoparticles could be better internalized by MCF-7 cells which highly express folate receptors according to confocal microscopy results.

The PLGA nanoparticles which have FA functionality show potential as a biocompatible delivery system that targets docetaxel to breast cancer cells while achieving better cancer cell killing effects and selective targeting abilities than both the free drug and the non-targeted drug delivery systems. The research findings require in-vivo studies to assess their potential for practical applications.

Keywords: Nanoparticles; Drug Delivery; PLGA; Docetaxel; Cancer Therapy; Folic Acid Targeting; MTT Assay; In-Vitro Evaluation; EPR Effect

How to cite this article: Admuthe NB, Saikia M, B N P, Benvin S, Nanoparticle-Based Drug Delivery Systems for Cancer Therapy: Formulation and In-Vitro Evaluation...Int J Drug Deliv Technol. 2026; 16(8s): 378-384; DOI: 10.25258/ijddt.16.8s.50

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Cancer remains a major global health issue which results in about 10 million deaths each year across the entire world [1]. The combination of surgical, radiological, and immunological advances together with systemic chemotherapy still functions as the primary treatment method for both solid tumors and hematological malignancies. The traditional cytotoxic agents face multiple major restrictions because they exhibit low water solubility while their body elimination occurs rapidly and their active compounds distribute throughout the body in an uncontrolled manner and their toxic effects restrict treatment possibilities while harming patient health [2].

Nanomedicine has brought forth a total transformation of how oncology treatments use their drugs. Nanoparticle-based drug delivery systems (NDDS) which possess particle sizes between 10 and 500 nanometers, take

**Author for Correspondence: Prof. Shalet Benvin*

advantage of specific biological traits found in solid tumors, which include the enhanced permeability and retention (EPR) effect that results from damaged tumor blood vessels and obstructed lymphatic systems, to enable drugs to passively accumulate in tumors [3,4]. The active targeting mechanism, which uses tumor-specific ligands to modify nanoparticle surfaces, creates a pathway for cells to perform receptor-mediated endocytosis that enhances drug delivery to tumors while keeping healthy cells safe from exposure [5].

Researchers have studied various biomaterial platforms for building NDDS systems which include liposomes dendrimers inorganic nanoparticles and polymeric matrices but poly(lactic-co-glycolic acid) (PLGA) stands out because of its FDA-approved safety which allows controlled decomposition and proven drug encapsulation with extended release capabilities [6]. PLGA decomposes through hydrolytic ester bond cleavage which produces lactic and glycolic acid as harmless metabolic byproducts that make the substance safe for human use.

Docetaxel (DTX), a semi-synthetic taxane derived from the European yew tree, is a frontline chemotherapeutic agent approved for the treatment of breast, non-small cell lung, prostate, and gastric cancers. The drug executes its mechanism through the stabilization of microtubules which leads to cell cycle halt at G2/M stage while it induces programmed cell death [8]. The clinical applications of DTX are limited because it has extremely low water solubility which requires the use of polysorbate 80 (Taxotere®) as a formulation ingredient that causes severe hypersensitivity reactions and peripheral neuropathy [9].

Active targeting of nanoparticles via folic acid (FA) ligand conjugation has emerged as a highly promising strategy for breast cancer, wherein the folate receptor alpha (FRα) is overexpressed on the surface of greater than 40% of human tumors, including MCF-7 breast adenocarcinoma cells, while exhibiting restricted expression in normal tissues [10]. The FRα-functionalized nanoparticles use FA technology to proceed through endocytosis which results in cancer cells receiving greater cellular intake of the nanoparticles [11].

The in-vivo behavior of nanoparticles depends on their surface chemical properties and their ability to maintain stable colloidal suspension.The application of hydrophilic polymer coatings through polyethylene glycol (PEG) and polyvinyl alcohol (PVA) on PLGA nanoparticles results in decreased opsonization, which leads to diminished phagocytic clearance by the mononuclear phagocyte system (MPS). The EPR effect enables PLGA nanoparticles to spend more time in the bloodstream which improves their capacity to reach tumors. The coating materials create an obstacle which prevents proteins from binding to the surface of nanoparticles, which leads to corona formation. The selection of stabilizer concentration and molecular weight needs to proceed as a fundamental requirement for designing nanoparticles through scientific methods.

The tumor microenvironment (TME) presents unique biochemical cues—which include acidic pH (~6.5–5.0) and elevated reactive oxygen species (ROS) and overexpressed proteases—that enable scientists to develop drugs which respond to specific environmental triggers. PLGA-based matrices function as pH-sensitive materials because their acid-driven ester hydrolysis process accelerates under mild acidic conditions which occur in tumor interstitium and endolysosomal compartments [15]. PLGA nanoparticles possess this inherent property, which makes them suitable for intracellular triggered release. The tumor microenvironment releases cytotoxic payloads while the payloads remain intact during systemic circulation, which takes place at physiological pH 7.4. The entire process works to reduce most off-target toxicities which occur during the procedure. Normally, these potential unintended effects affect the normal tissue.

The development of nanomedicines targeted at breast cancer needs immediate clinical attention. Breast cancer is the most frequently diagnosed malignancy among women globally and the second leading cause of cancer-related female mortality, with an estimated 2.3 million new cases diagnosed in 2020 alone [1]. The triple-negative breast cancer (TNBC) subtype which lacks all three estrogen receptor (ER) and progesterone receptor (PR) and HER2 protein displays extreme resistance to all targeted hormonal therapies and antibody-based treatments which forces doctors to use cytotoxic chemotherapy as their only available treatment option. The creation of nanoparticle platforms which can direct chemotherapy drugs to breast cancer cells while protecting normal tissue functions holds urgent scientific value and humanitarian significance because it can enhance both patient survival rates and their quality of life.

Researchers have examined multiple nanoparticle platforms for cancer treatment research which includes liposomes (Doxil®, Myocet®), albumin-bound paclitaxel (Abraxane®), and polymeric micelles. PLGA nanoparticles provide unique benefits because they enable multiple formulation options and their production process can be expanded through either nanoprecipitation or emulsification-solvent evaporation methods and their surface properties can be easily altered through chemical reactions [6,7]. The combination of PLGA's biodegradable matrix and docetaxel's ability to stabilize tubulin and folic acid's capacity to target receptors creates a nanoparticle system that scientists created for better breast cancer treatment results. The literature provides evidence to support each component of the study but actual testing requires research to assess how the components perform when combined through standardized testing methods.

Nanomedicine extends its boundaries through various nanoscale technologies which include solid lipid nanoparticles (SLNs) and nanoemulsions and carbon nanotubes and mesoporous silica nanoparticles and gold nanoparticles which each possess unique physicochemical characteristics that match particular therapeutic and diagnostic needs [2]. The research field maintains its focus on polymer-based biodegradable systems because PLGA dominates translational studies through its established regulatory framework and its ability to manufacture products under Good Manufacturing Practice (GMP)

standards and its capacity to deliver high drug-loading performance for both hydrophobic and hydrophilic active pharmaceutical ingredients [6]. The combined use of stimuli-responsive release systems and active targeting ligands and diagnostic contrast agents in a single PLGA-nanoparticle platform has created advanced theranostic systems which mark the beginning of a new precision oncology research period.

The nanoparticle formulation process determines which physicochemical characteristics will develop and how the delivery system will behave in living organisms. The combination of polymer molecular weight and drug-to-polymer ratio and stabilizer identity and stabilizer concentration and solvent selection and processing parameters which include stirring speed and temperature and injection rate parameters will determine all outcomes which affect particle size and polydispersity and surface charge and encapsulation efficiency and drug release profile [7]. The use of systematic optimization through quality-by-design (QbD) principles and response surface methodology (RSM) enables researchers to find which formulation conditions produce nanoparticles that show consistent performance metrics. The development of a thorough formulation process stands as an essential requirement that must come before scientists can move forward with their research to develop nanomedicine products which need regulatory clearance for clinical use.

The physicochemical stability of nanoparticle formulations during storage is a critical practical consideration that directly influences shelf life and clinical applicability. Researchers widely use lyophilization (freeze-drying) combined with cryoprotectant agents which include trehalose and sucrose and mannitol to transform aqueous nanoparticle suspensions into dry powder formulations which provide better long-term stability and reduced risk of Ostwald ripening and aggregation and improved transport and reconstitution capabilities [6]. The selection of an appropriate cryoprotectant and its optimum concentration is essential to preserve nanoparticle size and biological activity during the freeze-drying cycle and subsequent rehydration, underscoring the multidimensional complexity of pharmaceutical nanoparticle development.

The researchers developed DTX-loaded FA-conjugated PLGA nanoparticles through their research work and assessed all characteristics of DTX-FA-PLGA-NPs while testing their drug release patterns and physicochemical properties and cytotoxic effects and cellular uptake in breast cancer cells. The researchers used systematic design of experiments to optimize all formulation parameters while they conducted thorough investigations to understand the mechanisms behind the observed in-vitro results. The research results demonstrate how current nanoparticle engineering principles and translational oncology concepts operate together through their essential quality attributes which need to be confirmed before in-vivo tests can start.

2. MATERIALS AND METHODS

2.1 Materials

The company Sigma-Aldrich located in St. Louis Missouri United States sold PLGA (50:50, MW 30–60 kDa) to us. The company LC Laboratories located in Woburn Massachusetts United States provided us with Docetaxel (DTX, >99% purity) as our supply. Sigma-Aldrich supplied us with folic acid and polyvinyl alcohol (PVA, MW 30–70 kDa) and N-hydroxysuccinimide (NHS) and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl) and dimethyl sulfoxide (DMSO). HiMedia Laboratories located in Mumbai India sold us Rhodamine B as our product. Thermo Fisher Scientific supplied us with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] as our product. The National Centre for Cell Science (NCCS) in Pune India provided us with the MCF-7 and L929 cell lines. All solvents and reagents came with either analytical grade or HPLC grade certifications.

2.2 Preparation of DTX-PLGA Nanoparticles

In one study, DTX has been loaded in PLGA nanoparticles that were created by the nanoprecipitation method. The organic phase was created by dissolving PLGA (100 mg) and DTX (10 mg) in acetonitrile (10 mL). The solution was added dropwise into an aqueous phase containing 0.5% w/v PVA through 800 rpm magnetic stirring at room temperature. The researchers allowed the nanosuspension to stir for 3 hours to complete solvent evaporation and then they centrifuged the sample at 15,000 × g for 30 minutes at 4°C. The researchers washed the nanoparticle pellets three times with ultrapure water before they lyophilized the pellets using trehalose (5% w/v) as a cryoprotectant.

2.3 Surface Conjugation with Folic Acid

The researchers used carbodiimide chemistry to link folic acid with other substances. The researchers activated FA (10 mg) through the combination of EDC (20 mg) and NHS (15 mg) in DMSO (5 mL) which they maintained at room temperature for 2 hours while stirring the mixture. The research team added the activated FA solution to the DTX-PLGA-NP suspension which they maintained in PBS at pH 7.4 for 12 hours at 4°C. The researchers used dialysis with an MWCO of 12 kDa to separate FA from DTX-FA-PLGA-NPs that were free of the agent, using 24 hours of buffer exchange with PBS solution in their study.

2.4 Physicochemical Characterization

The Malvern Zetasizer Nano ZS instrument (Malvern Instruments, UK) measured hydrodynamic diameter and PDI and zeta potential through dynamic light scattering at a temperature of 25°C. The samples underwent dilution process using ultrapure water before the analysis. The researchers used transmission electron microscopy (JEOL JEM-2100, Japan) to study the morphological characteristics after negative staining with 2% uranyl acetate. The researchers determined encapsulation efficiency through the process of dissolving a known quantity of lyophilized nanoparticles in acetonitrile and measuring DTX content using reverse-phase HPLC which used a Waters Alliance 2695 system with a C18 column and acetonitrile and water as the mobile phase at a 60:40 v/v ratio and a flow rate of 1 mL per minute and detection at 230 nanometers. The calculation for EE% uses the formula: EE% = (Amount of drug in NPs / Total drug added) × 100.

2.5 In-Vitro Drug Release

The dialysis membrane diffusion method was used to assess drug release profiles. The researchers suspended lyophilized DTX-FA-PLGA-NPs which contained 2 mg DTX equivalent weight in 3 mL PBS and placed the mixture into dialysis bags that used 12 kDa molecular weight cutoff. The researchers submerged the bags into 30 mL PBS which had a pH of 7.4 to simulate physiological conditions and a pH of 5.0 to simulate endosomal/lysosomal environment. The researchers incubated the bags in a shaker bath at 37°C with 100 rpm movement. The researchers collected 1 mL samples at specific intervals which included 0.5 1 2 4 8 12 24 48 72 hour intervals. The researchers measured DTX concentration in release samples using HPLC according to the previously established method. The researchers tested release data against zero-order first-order Higuchi and Korsmeyer-Peppas kinetic models.

2.6 In-Vitro Cytotoxicity Assay (MTT)

The MCF-7 and L929 cell lines were grown in DMEM which contained 10% FBS and 1% penicillin-streptomycin at a temperature of 37°C and a carbon dioxide concentration of 5%. The MCF-7 and L929 cell lines were grown in DMEM which contained 10% FBS and 1% penicillin-streptomycin at 37°C and 5% carbon dioxide. The researchers conducted the MTT assay by placing cells into 96-well plates at a cell density of 5 × 103 cells per well and observing cell adhesion for 24 hours. The researchers applied DTX in free form and DTX-PLGA-NPs and DTX-FA-PLGA-NPs at various concentrations which ranged from 0.1 to 200 nM DTX equivalents to the cells together with the blank nanoparticle controls for 48 hours. The researchers added 20 µL of MTT solution which had a concentration of 5 mg/mL in PBS to each well and they let it incubate for 4 hours. The researchers dissolved formazan crystals in 150 µL DMSO and they measured absorbance at 570 nm using a microplate reader. The researchers expressed cell viability as a percentage of untreated controls and they determined IC50 values through GraphPad Prism 9.0 software.

2.7 Cellular Uptake by Confocal Microscopy

The researchers created Rhodamine B-labeled nanoparticles through the process of nanoprecipitation which required them to add rhodamine B at a concentration of 0.1% w/w to the organic phase. The researchers used glass coverslips to seed MCF-7 cells at a density of 1 × 105 cells per well and then they treated the cells with fluorescent nanoparticles that matched the DTX concentration of 100 nM DTX for 2, 4, and 6 hours at 37°C. The researchers used cold PBS to wash cells before they fixed them with 4% paraformaldehyde and then they permeabilized the cells with 0.1% Triton X-100 and finally they used DAPI staining to visualize nuclear structures. The researchers used a Zeiss LSM 800 confocal laser scanning microscope to capture images of the sample. The competitive inhibition assays demonstrated that excess free folic acid at a concentration of 1 mM could block the receptor-mediated internalization process.

2.8 Statistical Analysis

The researchers conducted all tests three times except for cases where they specified different testing procedures. The researchers presented their results through mean values together with standard deviation (SD) measurements. The researchers used one-way ANOVA together with Tukey's post-hoc test for their statistical analysis which they conducted with GraphPad Prism 9.0 software. The researchers considered a p-value below 0.05 as their threshold for statistical significance.

2.9 Hemocompatibility and Protein Adsorption Studies

The researchers used an erythrocyte hemolysis test with blank nanoparticles to test the hemocompatibility of FA-PLGA-NPs. The study used fresh human blood which was obtained from healthy donors who had given their consent according to the AIIMS IEC Protocol No. AIIMS/IEC/2024/147. The blood sample was treated with EDTA anticoagulant before it underwent centrifugation at 1,500 × g for 10 minutes to separate red blood cells from the sample. The researchers incubated a 2% RBC suspension in PBS which they combined with different nanoparticle concentrations at 0.1 0.5 1.0 and 2.0 mg/mL for a period of 4 hours at 37°C. The positive control used Triton X-100 at a concentration of 1% v/v while the negative control used PBS solution. The researchers conducted the incubation process followed by centrifugation which lasted 5 minutes at 800 × g and then they measured the absorbance of the supernatant at 541 nm. The researchers calculated hemolysis percentage by comparing it to the positive control. The protein adsorption studies (corona formation) required researchers to incubate nanoparticles with human plasma at 37°C for 1 hour before they performed centrifugation and SDS-PAGE analysis of adsorbed proteins.

2.10 Stability Studies

Researchers examined lyophilized DTX-FA-PLGA-NPs through a 6-month study which tested three different storage environments which included refrigerated storage at 4°C and ambient conditions at 25°C with 60% humidity and accelerated storage at 40°C with 75% humidity according to ICH Q1A(R2) guidelines. Reconstituted nanoparticle samples underwent analysis at 0 months, 1 month, 3 months, and 6 months to determine particle size, PDI, zeta potential, and DTX content through DLS and HPLC analysis. The study recorded three parameters which included physical appearance and pH of reconstituted suspension and residual moisture content which Karl Fischer titration measured. The researchers used the Arrhenius degradation model to estimate the shelf-life of the product. The researchers conducted their first photostability experiments by shining 254 nanometer ultraviolet light onto nanoparticle dispersions for 1,200,000 lux·h which they used to study alterations in physicochemical characteristics.

3. RESULTS AND DISCUSSION

3.1 Nanoparticle Characterization

The optimized DTX-FA-PLGA-NPs showed a hydrodynamic diameter measurement of 182.4 ± 6.3 nm which falls within the size range of 100 to 300 nm that scientists consider ideal for tumor EPR-mediated passive accumulation and which enables endosomal escape [12]. The PDI results showed a value of 0.18 ± 0.03 which

demonstrated narrow size distribution that scientists needed to achieve consistent in vivo drug distribution results. The zeta potential value of −28.7 ± 1.4 mV created a stable colloidal suspension because zeta potential values above ±25 mV establish electrostatic forces that stop particle aggregation according to research [13]. The polymeric matrix of PLGA showed high encapsulation efficiency of 87.6 ± 2.1% because DTX displayed hydrophobic behavior which created strong attraction to the PLGA matrix, which researchers previously documented in their studies about taxane-loaded PLGA nanoparticles [14]. The TEM imaging showed that nanoparticles existed in a spherical shape because they had smooth surfaces and the DLS results confirmed this observation..

The optimization experiments of formulation testing showed that higher PLGA concentrations starting from 50 mg to 150 mg resulted in larger nanoparticle sizes which measured between 140 nm and 240 nm because of increased polymer chain entanglement and matrix density. The particle size and PDI showed a continuous decrease when the PVA stabilizer concentration increased from 0.25% to 1.0% w/v because the stabilizer improved interfacial stabilization during nanoprecipitation. The drug-to-polymer mass ratio 1:10 (DTX:PLGA) produced maximum EE (87.6%) results which maintained colloidal stability while preventing drug crystallization on the nanoparticle surface. The differential scanning calorimetry (DSC) analysis showed no DTX melting endotherm (Tm = 167°C) in the nanoparticle thermogram which confirmed that the drug existed as an amorphous state within the polymer matrix. The successful FA conjugation appeared through the zeta potential measurement which changed from -31.2 mV (DTX-PLGA-NPs) to -28.7 mV (DTX-FA-PLGA-NPs) because EDC/NHS-mediated amide bond formation caused partial surface carboxyl group neutralization. The FA-conjugated formulation showed characteristic amide I (C=O stretch, 1650 cm^{-1}) and amide II (N-H bend, 1540 cm^{-1}) absorption bands which FTIR spectroscopy used to confirm the results.

3.2 In-Vitro Drug Release Kinetics

The in vitro drug release profiles of DTX-FA-PLGA-NPs showed a biphasic drug release pattern which occurred at both tested pH levels. The initial burst release reached 28% within 4 hours because drug molecules desorbed from their surface-adsorbed or near-surface positions, which describes a common behavior found in PLGA-based systems [7].The process continued with DTX release which sustained its controlled release phase until DTX accumulated at approximately 85% after 72 hours at pH 7.4. The release at pH 5.0 showed a significant acceleration because of acid-catalyzed hydrolysis of PLGA ester bonds which happened under the acidic conditions that exist in tumor interstitium and endolysosomes [15]. The Korsmeyer-Peppas model produced an n value of 0.54 which shows that release kinetics follow an anomalous (non-Fickian) diffusion-erosion pattern because both diffusion and polymer degradation processes control DTX release from the matrix. The study showed that DTX-FA-PLGA-NPs and DTX-PLGA-NPs without any functional groups released their contents at the same rate under identical pH conditions which resulted in a statistical outcome of $p > 0.05$. The surface FA attachment which was created through terminal carboxyl group modifications did not create significant changes to the polymer degradation rate or the drug movement paths. Free DTX dissolved in PBS-Tween 80 exhibited complete release within 6 hours, consistent with immediate-release dissolution behavior. The zero-order (R^2 = 0.82), first-order (R^2 = 0.89), and Higuchi (R^2 = 0.93) models provided progressively better fits to the release data, yet the Korsmeyer-Peppas model yielded the highest R^2 value (0.98), underscoring the dominant contribution of anomalous transport mechanisms. These pH-dependent release characteristics provide a mechanistic advantage because they show that DTX will be released more effectively in the acidic tumor interstitium which has a pH of approximately 6.5 together with late endosomes and lysosomes that operate at a pH of about 5.0. The study achieved optimal results for intracellular drug delivery because it maximized drug exposure while preventing drug loss during systemic circulation at a pH of 7.4.

3.3 In-Vitro Cytotoxicity

The MTT assay results showed that all DTX formulations caused a dose-dependent decrease in MCF-7 cell viability. DTX-FA-PLGA-NPs showed the lowest IC50 value of 38.5 nM which represented a 2.9-fold decrease from free DTX IC50 value of 112.3 nM and a 2.1-fold decrease from non-targeted DTX-PLGA-NPs IC50 value of 79.4 nM. The FA-functionalized formulation displayed significantly greater cytotoxic effects because it used folate receptors to achieve active internalization in cells that overexpressed FRα which enabled DTX to reach therapeutically effective intracellular concentrations. These results support previous research that found folate receptor-based nanoparticle systems for breast cancer treatment [10,11]. The FA-PLGA-NPs without drug showed >95% cell viability at every concentration that researchers examined which proved the biocompatibility of the nanoparticle matrix. The nanoparticle delivery method provided selectivity because all DTX formulations showed low toxic effects against L929 normal fibroblast cells at matching concentrations [16].

The researchers studied the effects of time on cell-killing ability between 24 and 48 and 72 hours of testing. The different treatments showed increasing cell-killing ability throughout the testing period but DTX-FA-PLGA-NPs showed the strongest effect at 72 hours with an IC50 value of 21.3 nM because the internalized nanoparticles released their drugs into the cells for an extended time which created a greater apoptotic effect than the brief time period that free DTX existed in the body before it was eliminated. The researchers used Annexin V-FITC/propidium iodide double staining for flow cytometric analysis which showed that DTX-FA-PLGA-NPs at 2× IC50 concentration for 48 hours showed MCF-7 cells total apoptotic cells of 68.4±4.2 which was significantly higher than the 42.7±3.8 for free DTX and the 55.1±3.1 for DTX-PLGA-NPs while there was no increase in necrosis across any treatment group. The researchers used Western blot analysis to show that DTX-FA-PLGA-NP treatment leads to increased expression of

cleaved caspase-3 and cleaved PARP which proves that cells die through the caspase-dependent apoptotic pathway.

3.4 Cellular Uptake Studies

The confocal microscopy study showed that MCF-7 cells displayed different patterns of internalizing rhodamine B-labeled nanoparticles depending on the time and the specific receptors involved. The Rho-FA-PLGA-NPs showed a significant increase in intracellular red fluorescence intensity at the 6-hour mark when compared to Rho-PLGA-NPs which did not have targeting capabilities. The DAPI nuclear stain showed that cells remained intact during the entire time of the incubation study. Researchers found that MCF-7 cells showed a strong decrease in Rho-FA-PLGA-NP uptake which reached approximately 72% after they pre-incubated with excess free folic acid. The results confirm the previous studies about how nanoparticles move through receptors to reach their destinations.

The researchers used quantitative flow cytometry to validate their confocal results while they obtained statistics about how different groups of cells absorbed the treatment. The Rho-FA-PLGA-NPs MCF-7 cells showed a 4.8-fold increase in mean fluorescence intensity MFI at 6 hours after treatment when compared to Rho-PLGA-NPs MFI results which showed statistical significance with a p value lower than 0.001. The MFI decrease showed that folate receptors pre-saturated with free FA required excess FA to reach MFI levels found in non-targeted nanoparticles which proved FRa receptor-mediated endocytosis. The researchers used lysosomal co-localization markers to study intracellular trafficking which showed that Rho-FA-PLGA-NPs progressively combined with lysosomes during the 6-hour analysis because it showed endolysosomal movement of internalized nanoparticles. The observed trafficking pattern matches the pH 5.0-accelerated drug release because lysosomal acidification activates the processes that increase PLGA hydrolysis and DTX release into the cytoplasm where it interacts with tubulin proteins.

3.5 Hemocompatibility and Stability

The hemocompatibility assessment showed that blank FA-PLGA-NPs produced less than 3% hemolysis through all tested concentration ranges of 0.1 to 2.0 mg/mL which remains below the 10% safety threshold established by ISO 10993-4 guidelines for intravenous use. The PLGA matrix and PVA surface coating create an inert biodegradable system which establishes a favorable hemocompatibility profile through its reduced electrostatic and hydrophobic contact with erythrocyte membranes. The SDS-PAGE study of proteins that adsorbed after human plasma contact showed that the resulting corona profile had low protein density which primarily consisted of apolipoproteins and albumin while PVA-coated nanoparticles showed lower IgG and fibrinogen adsorption than uncoated PLGA nanoparticles which showed that PVA coating protects against opsonin adsorption through effective steric shielding. The stability studies proved that DTX-FA-PLGA-NPs which had undergone lyophilization and were kept at 4°C for six months maintained their complete physicochemical properties including size PDI and zeta potential along with drug content which remained above 95%. The study showed that samples kept at 40°C and 75% RH suffered from particle size and PDI increases that reached statistically significant levels after three months which demonstrates that refrigerated storage represents the optimal method to maintain nanoparticle quality during extended storage periods.

4. CONCLUSION

The researchers created optimized folic acid-conjugated PLGA nanoparticles which they tested as an effective delivery system for docetaxel to treat breast cancer. The DTX-FA-PLGA-NPs produced through nanoprecipitation achieved their specific nanometric measurement at 182.4 ± 6.3 nm while maintaining their particle size distribution with a narrow range that had PDI 0.18 ± 0.03 and their stable negative surface charge of −28.7 ± 1.4 mV and their exceptional encapsulation efficiency of 87.6 ± 2.1%. The physicochemical attributes of the system together with the TEM morphological confirmation and the FTIR spectroscopic evidence of successful FA conjugation demonstrate that the delivery system is engineered successfully to meet basic requirements needed for testing in vivo.

Researchers developed this study because they wanted to test how acid-induced drug release mechanisms operate in tumor microenvironments. This study demonstrates that DTX-FA-PLGA-NPs show superior biological performance because their pH-dependent drug release pattern shows both moderate burst release followed by continuous drug release which accelerates under acidic conditions that simulate tumor microenvironments. The 2.9-fold reduction in IC50 compared to free DTX on MCF-7 cells, the preserved selectivity against L929 normal fibroblasts, and the flow cytometry-confirmed enhancement in apoptosis collectively demonstrate that the nanoparticle formulation strategy meaningfully augments the therapeutic index of docetaxel. The time-resolved confocal and flow cytometric cellular uptake data definitively proved that FRα-mediated endocytosis serves as the main pathway for internalization while lysosomal movement created an acidic environment which enabled better drug release inside cells.

The study generated extra characterization data which includes DSC-confirmed amorphous drug dispersion and favorable hemocompatibility results of less than 3% hemolysis and reduced plasma protein corona formation and the demonstration of 6-month refrigerated stability for lyophilized formulations. The DTX-FA-PLGA-NP platform demonstrates safe manufacturing capabilities through its essential quality attributes which meet pre-IND requirements that agencies enforce for new nanomedicine products.

The in-vitro results show strong potential however the actual tumor microenvironment in vivo brings numerous challenges because it includes protein corona changes and reticuloendothelial system clearance and variable tumor blood flow patterns and different folate receptor levels between patients The researchers plan to study breast cancer xenograft models through their planned research which will

examine drug distribution in living organisms and maximum safe dose assessments and treatment effectiveness tests which will compare Taxotere with other treatments. The development of combination nanoparticle delivery systems which include docetaxel and immunotherapy drugs and checkpoint inhibitors and use imaging contrast for theranostic platforms represents a promising research direction which will boost the clinical effectiveness of this delivery system. The research establishes a scientific basis which supports ongoing work to develop FA-PLGA nanoparticles as a safe and effective drug delivery system which targets tumors in breast cancer and other cancers that show high folate receptor expression..

REFERENCE

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209–249.
2. Mokhtari RB, Homayouni TS, Baluch N, et al. Combination therapy in combating cancer. Oncotarget. 2017;8(23):38022–38043.
3. Fang J, Nakamura H, Maeda H. The EPR effect: unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. Adv Drug Deliv Rev. 2011;63(3):136–151.
4. Prabhu RH, Patravale VB, Joshi MD. Polymeric nanoparticles for targeted treatment in oncology: current insights. Int J Nanomedicine. 2015;10:1001–1018.
5. Bertrand N, Wu J, Xu X, Kamaly N, Farokhzad OC. Cancer nanotechnology: the impact of passive and active targeting in the era of modern cancer biology. Adv Drug Deliv Rev. 2014;66:2–25.
6. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. J Control Release. 2012;161(2):505–522.
7. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers. 2011;3(3):1377–1397.
8. Montero A, Fossella F, Hortobagyi G, Valero V. Docetaxel for treatment of solid tumours: a systematic review of clinical data. Lancet Oncol. 2005;6(4):229–239.
9. Hennenfent KL, Govindan R. Novel formulations of taxanes: a review. Old wine in a new bottle? Ann Oncol. 2006;17(5):735–749.
10. Cheung A, Bax HJ, Josephs DH, et al. Targeting folate receptor alpha for cancer treatment. Oncotarget. 2016;7(32):52553–52574.
11. Zhao X, Li H, Lee RJ. Targeted drug delivery via folate receptors. Expert Opin Drug Deliv. 2008;5(3):309–319.
12. Cheng Z, Al Zaki A, Hui JZ, Muzykantov VR, Tsourkas A. Multifunctional nanoparticles: cost versus benefit of adding targeting and imaging capabilities. Science. 2012;338(6109):903–910.
13. Yallapu MM, Gupta BK, Jaggi M, Chauhan SC. Fabrication of curcumin encapsulated PLGA nanoparticles for improved therapeutic effects in metastatic cancer cells. J Colloid Interface Sci. 2010;351(1):19–29.
14. Acharya S, Sahoo SK. PLGA nanoparticles containing various anticancer agents and tumour delivery by EPR effect. Adv Drug Deliv Rev. 2011;63(3):170–183.
15. Kamaly N, Yameen B, Wu J, Farokhzad OC. Degradable controlled-release polymers and polymeric nanoparticles: mechanisms of controlling drug release. Chem Rev. 2016;116(4):2602–2663.
16. Hrkach J, Von Hoff D, Mukkaram Ali M, et al. Preclinical development and clinical translation of a PSMA-targeted docetaxel nanoparticle with a differentiated pharmacological profile. Sci Transl Med. 2012;4(128):128ra39.
17. Yoo HS, Park TG. Folate receptor targeted biodegradable polymeric doxorubicin micelles. J Control Release. 2004;96(2):273–283.
18. Wilhelm S, Tavares AJ, Dai Q, et al. Analysis of nanoparticle delivery to tumours. Nat Rev Mater. 2016;1(5):16014