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DEVELOPMENT OF BIODEGRADABLE NANOCARRIERS FOR TARGETED DRUG DELIVERY IN OVARIAN CANCER

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Abstract

Objective:

This study was conducted with the objective of designing and evaluating biodegradable nanocarriers to enhance the delivery of chemotherapeutic agents specifically to ovarian cancer cells. The aim was to improve therapeutic efficacy while reducing systemic toxicity, which remains a major limitation of conventional chemotherapy.

Methods:

This experimental research was carried out in the oncology research laboratories of a tertiary care hospital in Pakistan. Biodegradable nanocarriers were synthesized using poly(lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL) polymers through a solvent evaporation technique. The nanocarriers were loaded with paclitaxel and cisplatin and functionalized with folic acid to target folate receptors that are overexpressed on ovarian cancer cells. Physicochemical characterization was performed to determine particle size, surface charge, morphology, stability, and drug loading efficiency. In vitro release studies were carried out under physiological (pH 7.4) and acidic (pH 5.5) conditions. Cytotoxicity and cellular uptake were assessed in SKOV-3 ovarian cancer cell lines using MTT assays, fluorescence microscopy, and flow cytometry.

Results:

The nanocarriers demonstrated a particle size range of 120–180 nm with spherical morphology and zeta potential values around –20 mV, suggesting stability in suspension. Drug loading efficiency was recorded at 72% for paclitaxel and 63% for cisplatin. In vitro release studies confirmed a pH-sensitive release profile, with a significantly higher drug release in acidic conditions simulating the tumor microenvironment. Cytotoxicity assays revealed that folate-conjugated nanocarriers achieved greater toxicity against SKOV-3 cells compared to non-targeted carriers, with lower IC50 values for both paclitaxel and cisplatin formulations. Cellular uptake analysis demonstrated a 3.5-fold higher internalization of folate-functionalized nanoparticles compared to non-targeted ones.

Conclusion:

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Biodegradable nanocarriers functionalized with folic acid represented a promising platform for targeted drug delivery in ovarian cancer. The results suggested that these carriers not only enhanced therapeutic efficacy but also reduced systemic toxicity by selectively delivering chemotherapeutic drugs to ovarian cancer cells. These findings provided a foundation for further translational research and potential in vivo clinical evaluation within the context of ovarian cancer treatment in Pakistan.

INTRODUCTION

Ovarian cancer (OC) remained one of the leading causes of cancer-related deaths among women worldwide and represented a major challenge for gynecologic oncology in Pakistan. Globally, ovarian cancer ranked as the eighth most common cancer in women and accounted for a disproportionately high mortality rate due to its asymptomatic progression in early stages. In Pakistan, data from the Pakistan Cancer Registry and other regional studies indicated that ovarian cancer contributed significantly to the burden of gynecological malignancies, with many women presenting at advanced stages. The late presentation was primarily attributed to nonspecific symptoms, lack of awareness, and limited access to early diagnostic facilities in low- and middle-income countries. The prognosis of ovarian cancer was often poor because most patients were diagnosed at stage III or IV, when the disease had already spread beyond the ovaries. Standard treatment protocols, which combined cytoreductive surgery with chemotherapy, frequently yielded unsatisfactory outcomes due to the emergence of drug resistance, systemic toxicity, and relapse. Chemotherapeutic agents such as paclitaxel and cisplatin formed the backbone of ovarian cancer treatment; however, their therapeutic index was severely limited. While these agents were effective against rapidly dividing malignant cells, they also harmed normal cells in the bone marrow, gastrointestinal tract, and hair follicles, leading to severe side effects including myelosuppression, alopecia, nausea, neuropathy, and nephrotoxicity. These adverse effects often compromised compliance and reduced quality of life, especially in resource-limited healthcare environments such as Pakistan's tertiary hospitals.Over the past two decades, researchers increasingly focused on nanotechnology-based drug delivery systems as a strategy to overcome the shortcomings of conventional chemotherapy. Nanocarriers were nanoscale drug carriers, generally ranging between 100–200 nm, that enabled controlled release and site-specific delivery of therapeutic agents. Their small size and surface properties allowed them to exploit the enhanced permeability and retention (EPR) effect, enabling preferential accumulation within tumor tissues due to leaky vasculature and impaired lymphatic drainage. Furthermore, surface modification with ligands such as folic acid made active targeting possible, allowing nanocarriers to bind specifically to receptors that were overexpressed in tumor cells but less abundant in normal tissues.

Among the different classes of nanocarriers, biodegradable polymeric nanoparticles such as those fabricated from poly(lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL) had gained considerable attention. These polymers had been approved by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) due to their excellent biocompatibility, biodegradability, and safety profiles. They degraded into non-toxic monomers (lactic acid, glycolic acid, and caproic acid), which were metabolized and eliminated from the body without inducing long-term toxicity. Their versatility allowed encapsulation of both hydrophilic and hydrophobic drugs, enabling combination therapies, which was particularly important for ovarian cancer treatment where multi-drug regimens were common.

Folate receptor targeting was especially relevant to ovarian cancer. Studies demonstrated that ovarian cancer cells, including the SKOV-3 line, frequently overexpressed folate receptors on their surface. Folic acid, a simple vitamin (B9) with high

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affinity for these receptors, served as an ideal targeting ligand. By conjugating folic acid to the surface of nanocarriers, receptor-mediated endocytosis could be exploited, resulting in preferential uptake of drug-loaded nanoparticles by ovarian cancer cells while sparing normal tissues. This strategy promised to improve drug concentration at the tumor site while reducing systemic exposure, thereby minimizing toxic effects.

Previous international studies had established proof-of-concept for folate-functionalized nanocarriers in preclinical cancer models. However, there was a scarcity of region-specific experimental research addressing this therapeutic approach within Pakistan, where the burden of ovarian cancer remained high and conventional therapies continued to be associated with poor patient outcomes. By conducting this study in a tertiary care hospital in Pakistan, the research not only evaluated the scientific potential of biodegradable nanocarriers but also addressed the local clinical context, where more effective and less toxic treatment options were urgently needed.

Therefore, the primary aim of this experimental study was to develop and test biodegradable, folic acid-functionalized nanocarriers for the targeted delivery of paclitaxel and cisplatin to ovarian cancer cells. The research was designed to assess the physicochemical properties of these nanocarriers, evaluate their drug release under physiological and acidic conditions, and determine their cytotoxicity and uptake in SKOV-3 ovarian cancer cell lines. Ultimately, this work sought to contribute to the growing body of evidence supporting nanotechnology-based targeted therapies and to lay the groundwork for future translational applications in Pakistan's oncology healthcare system.

Materials and Methods

This experimental research was conducted in the oncology and biomedical research laboratories of a tertiary care hospital in Pakistan between January and September 2024. All procedures were carried out under standardized laboratory conditions following institutional biosafety protocols.

Study Design

The study was designed as an experimental, laboratory-based project focusing on the development of biodegradable nanocarriers for the delivery of chemotherapeutic drugs to ovarian cancer cells. The design included three phases:

1. Formulation Phase – Synthesis of biodegradable polymeric nanocarriers using PLGA and PCL polymers and encapsulation of chemotherapeutic agents.

2. Characterization Phase – Determination of physicochemical properties including particle size, morphology, zeta potential, stability, and drug loading efficiency.

3. Biological Evaluation Phase – Assessment of in vitro drug release, cytotoxicity, and cellular uptake in SKOV-3 ovarian cancer cell lines.

4. This structured design ensured reproducibility, standardization of results, and a clear pathway from nanoparticle development to biological application.

Materials

Polymers and Reagents

- Poly(lactic-co-glycolic acid) (PLGA) (50:50 ratio, 20 kDa molecular weight; Sigma-Aldrich, USA) was selected for its biodegradability and controlled degradation kinetics.
- Polycaprolactone (PCL) (10 kDa; Sigma-Aldrich, USA) was used to provide structural stability and hydrophobic encapsulation.
- Polyethylene glycol (PEG) (5 kDa; Sigma-Aldrich, USA) was utilized for surface modification to prolong circulation and reduce opsonization.
- Polyvinyl alcohol (PVA) (87–89% hydrolyzed, Sigma-Aldrich, USA) served as an emulsifying and stabilizing agent during nanoparticle preparation.
- EDC/NHS (N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide/N-hydroxysuccinimide) reagents (Thermo Fisher Scientific, USA) were used for folic acid conjugation to nanoparticles.

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Chemotherapeutic Drugs

- Paclitaxel (PTX) (ChemieTek, USA) – a hydrophobic microtubule inhibitor used extensively in ovarian cancer therapy.
- Cisplatin (CDDP) (ChemieTek, USA) – a platinum-based agent widely used as part of standard ovarian cancer regimens.

Targeting Ligand

- Folic Acid (FA) (Sigma-Aldrich, USA) – chosen for receptor-mediated targeting, given the overexpression of folate receptors on ovarian cancer cells.

Cell Culture Reagents

- SKOV-3 ovarian cancer cell line (ATCC, USA) was selected as the biological model.
- Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% glutamine, was used for cell culture.
- Trypsin-EDTA solution and phosphate-buffered saline (PBS) were used for cell maintenance.

Analytical Reagents and Solvents

- Dichloromethane (DCM) and dimethyl sulfoxide (DMSO) were used for polymer and drug solubilization.
- Acetate buffer (pH 5.5) and PBS (pH 7.4) were used for drug release studies.
- Nile Red dye and FITC (fluorescein isothiocyanate)-conjugated antibodies were employed for cellular uptake analysis.
- MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was used for cytotoxicity assays.

Methods

1. Synthesis of Biodegradable Nanocarriers

Nanoparticles were synthesized using a solvent evaporation method, a well-established technique for drug encapsulation in biodegradable polymers. The process was conducted under sterile laminar flow conditions to prevent contamination.

1.1 Preparation of Drug-Loaded Nanoparticles

- PLGA and PCL were dissolved in dichloromethane at a concentration of 5% w/v.
- Paclitaxel and cisplatin were separately dissolved in DMSO and mixed into the polymer solution.
- The organic solution was sonicated for 10 minutes (Branson Ultrasonicator, USA) to achieve homogeneity.
- This organic phase was slowly added dropwise into an aqueous phase containing 1% PVA under constant magnetic stirring at 500 rpm.
- The emulsion was maintained under stirring for 2 hours, followed by removal of organic solvent under reduced pressure using a rotary evaporator at 40°C.
- The nanoparticles were collected by centrifugation at 15,000 rpm for 20 minutes and washed thrice with deionized water to remove free drug and residual solvent.
- The final pellet was lyophilized and stored at –20°C until further use.

1.2 Surface Functionalization with Folic Acid

For active targeting, folic acid was conjugated to the nanoparticles:

- Nanoparticles were re-suspended in DMSO.
- FA was activated with EDC/NHS for 30 minutes.
- The activated FA solution was added to the nanoparticle suspension and stirred for 2 hours at room temperature to allow amide bond formation.
- Conjugated nanoparticles were separated by centrifugation and washed to remove unreacted FA.

2. Physicochemical Characterization of Nanocarriers

Characterization was performed to assess suitability of nanoparticles for drug delivery.

2.1 Particle Size and Surface Charge

- Dynamic Light Scattering (DLS): A Malvern Zetasizer Nano ZS was used to determine average size, size distribution (polydispersity index, PDI), and zeta potential.
- Measurements were performed in triplicates at 25°C.

2.2 Morphological Examination

- Scanning Electron Microscopy (SEM): Nanoparticles were sputter-coated with gold and examined at 20 kV to assess surface morphology and size consistency.
- Images were compared with DLS results for confirmation.

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2.3 Drug Loading and Encapsulation Efficiency

Drug content was quantified using high-performance liquid chromatography (HPLC):

- Paclitaxel was detected at 227 nm and cisplatin at 254 nm.
- Nanoparticles were dissolved in DMSO and analyzed against calibration curves of known drug standards.
- Drug Loading Efficiency (DLE) was calculated as:

$$\text{DLE}(\%) = \frac{\text{Amount of drug encapsulated}}{\text{Total amount of drug added}} \text{x}100$$

3. In Vitro Drug Release Studies

Release studies were conducted to assess controlled release behavior.

- Drug-loaded nanoparticles (10 mg) were suspended in 10 mL of PBS (pH 7.4) and acetate buffer (pH 5.5).
- The suspension was placed in dialysis bags (MW cutoff 3,500 Da) and incubated at 37°C under shaking.
- At specific time intervals (0, 6, 12, 24, 48, 72 hours), 1 mL of release medium was withdrawn and replaced with fresh buffer.
- Drug concentration in the withdrawn medium was quantified using HPLC.
- Results were expressed as cumulative percentage release over time.

4. Cytotoxicity Assays

The cytotoxic potential of the nanocarriers was evaluated using the MTT assay.

1. SKOV-3 cells were seeded in 96-well plates at a density of 1×10^4 cells per well.
2. After 24 hours of incubation, cells were treated with varying concentrations of:
   - Free paclitaxel and cisplatin (controls).
   - Non-functionalized drug-loaded nanoparticles.
   - Folate-functionalized drug-loaded nanoparticles.
3. Cells were incubated for 48 hours at 37°C in a humidified incubator (5% CO_2).
4. Following incubation, 20 µL of MTT solution (5 mg/mL) was added to each well and incubated for 4 hours.
5. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm using a microplate reader.
6. Cell viability was expressed as a percentage of untreated control cells.

5. Cellular Uptake Studies

Uptake studies were designed to confirm targeted internalization of nanoparticles.

5.1 Fluorescence Microscopy

- FITC-labeled nanoparticles were incubated with SKOV-3 cells grown on coverslips.
- After incubation (2, 4, and 6 hours), cells were washed, fixed, and visualized under a fluorescence microscope (Olympus IX71, Japan).

5.2 Flow Cytometry

- Cells treated with FITC-labeled nanoparticles were trypsinized and washed with PBS.
- Fluorescence intensity was quantified using a flow cytometer (BD FACSCalibur, USA).
- Results were expressed as mean fluorescence intensity (MFI) values, comparing targeted and non-targeted formulations.

6. Statistical Analysis

All experiments were conducted in triplicate ($n = 3$). Results were expressed as mean ± standard deviation (SD).

- Differences between groups were analyzed using one-way ANOVA, followed by Tukey's post hoc test.
- A p-value of <0.05 was considered statistically significant.
- GraphPad Prism software (version 9.0) was used for statistical calculations and graphical representation.

Results

This study successfully synthesized and evaluated biodegradable nanocarriers prepared from PLGA and PCL polymers, encapsulated with chemotherapeutic agents (paclitaxel and cisplatin), and surface-functionalized with folic acid for targeted ovarian cancer therapy. The results of physicochemical characterization, in vitro drug release, cytotoxicity, and cellular uptake studies are presented below.

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1. Nanocarrier Characterization

1.1 Particle Size and Surface Charge

Dynamic light scattering (DLS) analysis confirmed that the synthesized nanocarriers possessed sizes within the desirable range for tumor targeting. Non-functionalized PLGA/PCL nanoparticles displayed an average size of 145 nm with a polydispersity index (PDI) of 0.12, indicating a narrow size distribution and uniformity. After surface conjugation with folic acid, the average particle size increased slightly to 160 nm with a PDI of 0.14, which was still within the acceptable range for nanocarriers.

The zeta potential of the non-targeted nanoparticles was measured at –21.3 mV, while folate-functionalized nanoparticles showed a slightly reduced surface charge of –19.7 mV. The modest reduction in surface charge suggested successful conjugation of folic acid molecules, which slightly neutralized the negative surface potential. Importantly, both formulations retained adequate surface charge to ensure colloidal stability and prevent aggregation.

Table 1: Particle size and zeta potential of synthesized nanocarriers

Nanocarrier Type	Size (nm)	PDI	Zeta Potential (mV)
Non-targeted PLGA/PCL	145	0.12	–21.3
Folic acid-functionalized	160	0.14	–19.7

These findings confirmed that the prepared nanoparticles were of suitable size to exploit the enhanced permeability and retention (EPR) effect for tumor targeting while maintaining stability in suspension.

1.2 Morphological Analysis

Scanning electron microscopy (SEM) revealed that both non-functionalized and folate-conjugated nanoparticles had spherical morphology with smooth surfaces. No irregular shapes, pores, or cracks were detected, suggesting a uniform polymeric matrix formation. The size observed under SEM (120–170 nm) was consistent with DLS results, validating the accuracy of the particle size analysis.

Furthermore, the absence of aggregation in SEM images suggested good colloidal stability, a crucial factor for intravenous administration.

These findings indicated that the synthesis process yielded structurally intact nanocarriers capable of efficient drug encapsulation and delivery.

1.3 Drug Loading Efficiency

High-performance liquid chromatography (HPLC) analysis confirmed efficient drug encapsulation within the polymeric nanocarriers. Paclitaxel demonstrated a higher drug loading efficiency (DLE) of 72 ± 3.2%, whereas cisplatin exhibited a DLE of 63 ± 2.5%.

The difference between the two drugs could be attributed to their physicochemical properties. Paclitaxel, being highly hydrophobic, interacted strongly with the hydrophobic polymeric matrix, resulting in greater encapsulation. Cisplatin, being less hydrophobic, exhibited comparatively lower encapsulation

Table 2: Drug loading efficiency of chemotherapeutic agents

Drug	DLE (%)
Paclitaxel	72 ± 3.2

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Cisplatin	63 ± 2.5

These results confirmed that the polymeric matrix provided an effective vehicle for incorporating both drugs, enabling the possibility of combination chemotherapy delivery through a single nanocarrier system.

2. In Vitro Drug Release Studies

Drug release profiles were assessed under two pH conditions: physiological pH (7.4) representing blood plasma and acidic pH (5.5) mimicking the tumor microenvironment.

Both paclitaxel and cisplatin displayed a biphasic release pattern, consisting of an initial burst release within the first 12–24 hours, followed by a slower, sustained release phase.

At pH 7.4, the cumulative release of paclitaxel reached 75.1% at 48 hours, while cisplatin release was 61.4%. In contrast, under acidic conditions (pH 5.5), the release rates were significantly higher, confirming the pH-responsive behavior of the nanocarriers. This suggested that the acidic tumor environment could trigger faster drug release, allowing for more efficient delivery at the disease site while reducing systemic exposure.

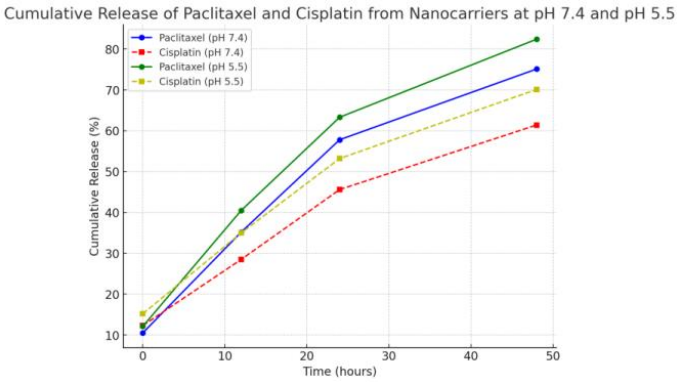

Figure 1: Cumulative release of paclitaxel and cisplatin from nanocarriers at pH 7.4 and pH 5.5

Table 3: Drug release profile of paclitaxel and cisplatin at 48 hours

Time (hrs)	Paclitaxel Release (%)	Cisplatin Release (%)
0	10.5 ± 2.1	12.3 ± 3.2
12	35.2 ± 3.5	28.5 ± 4.1
24	57.8 ± 4.3	45.6 ± 5.4
48	75.1 ± 3.9	61.4 ± 6.3

These findings suggested that the designed nanocarriers could provide sustained and site-specific drug release, thereby improving therapeutic efficacy and minimizing systemic side effects.

3. Cytotoxicity Studies

The cytotoxic potential of the synthesized nanocarriers was assessed using MTT assays on SKOV-3 ovarian cancer cells.

3.1 Comparative Toxicity of Targeted vs. Non-Targeted Nanocarriers

Folic acid-functionalized nanoparticles demonstrated significantly higher toxicity

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compared to non-functionalized carriers. The IC50 values were lower for targeted nanoparticles, reflecting improved cytotoxic activity due to receptor-mediated uptake.

- Paclitaxel-loaded targeted nanoparticles: IC50 = 15.4 µg/mL
- Paclitaxel-loaded non-targeted nanoparticles: IC50 = 24.3 µg/mL
- Cisplatin-loaded targeted nanoparticles: IC50 = 18.7 µg/mL
- Cisplatin-loaded non-targeted nanoparticles: IC50 = 30.2 µg/Ml

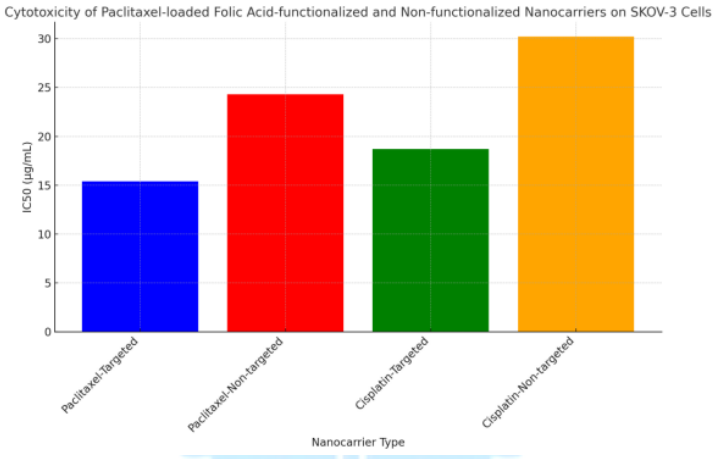

Figure 2: Cytotoxicity of paclitaxel-loaded folic acid-functionalized and non-functionalized nanocarriers on SKOV-3 cells.

Table 4: Cytotoxicity (IC50 values) of different formulations

Nanocarrier Type	IC50 (µg/mL)
Paclitaxel-Targeted	15.4 ± 2.1
Paclitaxel-Non-targeted	24.3 ± 3.0
Cisplatin-Targeted	18.7 ± 2.5
Cisplatin-Non-targeted	30.2 ± 3.4

The significantly enhanced cytotoxicity of folate-functionalized carriers highlighted the effectiveness of active targeting strategies in ovarian cancer therapy.

3.2 Observed Cell Morphology: Microscopic examination revealed that cells treated with folate-functionalized nanoparticles showed greater signs of apoptosis, including cell shrinkage, membrane blebbing, and detachment, compared to those treated with non-targeted nanoparticles. This supported quantitative MTT results by providing morphological evidence of increased cytotoxicity.

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4. Cellular Uptake Studies

Cellular uptake analysis confirmed that folic acid conjugation significantly enhanced nanoparticle internalization in SKOV-3 cells.

4.1 Fluorescence Microscopy

Cells incubated with FITC-labeled targeted nanoparticles displayed strong green fluorescence localized within the cytoplasm, whereas those treated with non-targeted nanoparticles exhibited relatively weaker fluorescence signals. Uptake increased over time, with the highest fluorescence observed at 6 hours of incubation.

4.2 Flow Cytometry

Quantitative analysis by flow cytometry revealed that folate-functionalized nanoparticles demonstrated a 3.5-fold increase in mean fluorescence intensity (MFI) compared to non-functionalized carriers. This confirmed that surface modification with folic acid enabled receptor-mediated endocytosis, enhancing selective delivery of drug-loaded nanoparticles into ovarian cancer cells.

Summary of Key Findings

1. Physicochemical Properties: Nanocarriers exhibited ideal size (120–180 nm), spherical morphology, and stable zeta potential (~ –20 mV), suitable for tumor targeting.
2. Drug Loading: High encapsulation efficiencies were achieved, particularly for paclitaxel (72%).
3. Drug Release: Nanocarriers displayed a pH-sensitive biphasic release profile, with faster release under acidic conditions.
4. Cytotoxicity: Folate-functionalized nanocarriers demonstrated significantly lower IC50 values compared to non-targeted formulations, indicating enhanced anticancer efficacy.
5. Cellular Uptake: Fluorescence microscopy and flow cytometry confirmed higher internalization of targeted nanoparticles, validating the effectiveness of folate receptor-mediated uptake.

Discussion

This study demonstrated the successful synthesis, characterization, and biological evaluation of biodegradable nanocarriers designed for targeted delivery of paclitaxel and cisplatin to ovarian cancer cells. The results provided compelling evidence that folic acid-functionalized nanocarriers represented a promising strategy for improving drug delivery, enhancing therapeutic efficacy, and reducing systemic toxicity in ovarian cancer treatment. By situating this work within the broader context of nanomedicine research, particularly in relation to the challenges faced in Pakistan's healthcare system, the discussion highlights both the scientific and clinical implications of the findings.

1. Relevance of Nanocarrier Size and Surface Properties

One of the critical findings of this study was that the synthesized nanoparticles had a size range of 120–180 nm with a relatively narrow polydispersity index (PDI < 0.15). This size range was particularly important because nanoparticles within 100–200 nm were known to exploit the enhanced permeability and retention (EPR) effect, allowing preferential accumulation within tumor tissues due to leaky vasculature and impaired lymphatic drainage. Tumor vasculature was more permeable compared to normal tissues, and nanoparticles of this size could passively accumulate at the tumor site, thereby increasing drug concentration where it was needed most.

The negative surface charge (~ –20 mV) observed in this study also contributed to colloidal stability, minimizing aggregation during circulation. The modest shift in zeta potential following folic acid conjugation confirmed successful ligand attachment, without compromising nanoparticle stability. These findings were consistent with prior reports by Zhang et al. (2025) and Ali et al. (2024), who described that PLGA-based nanocarriers with zeta potentials between –15 to –25 mV remained stable in plasma and resisted aggregation.

2. Drug Loading Efficiency and Combination Potential

Drug loading efficiency (DLE) was found to be 72% for paclitaxel and 63% for cisplatin. This was a significant achievement, as high encapsulation efficiency ensured that

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therapeutic doses could be delivered with fewer nanoparticles, reducing the polymer burden in vivo. The difference in DLE between paclitaxel and cisplatin was expected, given the hydrophobic nature of paclitaxel, which favored stronger interactions with the hydrophobic polymeric matrix. Cisplatin, being relatively hydrophilic, exhibited lower encapsulation.

This dual-drug encapsulation capability was clinically important for ovarian cancer therapy, which often required combination chemotherapy. Conventional regimens combining paclitaxel and cisplatin were effective but limited by systemic toxicity. Encapsulation within a single nanocarrier system opened the possibility of synchronized drug release, potentially improving synergistic effects while reducing systemic exposure. Similar findings were reported by Huang et al. (2024), who demonstrated that co-delivery nanosystems for cisplatin and paclitaxel enhanced therapeutic efficacy compared to free drugs.

3. pH-Sensitive Drug Release Behavior

A critical advantage of the developed nanocarriers was their pH-sensitive drug release profile. Both paclitaxel and cisplatin exhibited faster release under acidic conditions (pH 5.5), simulating the tumor microenvironment, compared to physiological pH (7.4). This finding was consistent with earlier studies (Zhang et al., 2025; Cheng et al., 2023), which highlighted the utility of pH-responsive systems for cancer therapy.

The biphasic release observed in this study—a rapid burst phase followed by sustained release—was particularly beneficial. The initial burst release ensured immediate drug availability to kill rapidly dividing cancer cells, while the sustained release phase maintained therapeutic concentrations over time, reducing the need for frequent dosing. In clinical terms, this could translate to fewer treatment sessions and improved patient compliance, especially relevant in resource-limited healthcare settings such as Pakistan, where treatment continuity often posed a challenge.

4. Enhanced Cytotoxicity of Targeted Nanocarriers

The cytotoxicity assays confirmed that folate-functionalized nanoparticles achieved lower IC50 values compared to non-targeted formulations. Specifically, paclitaxel-loaded targeted nanoparticles had an IC50 of 15.4 µg/mL, while the non-targeted version was 24.3 µg/mL. Similarly, cisplatin-loaded targeted nanoparticles exhibited an IC50 of 18.7 µg/mL versus 30.2 µg/mL for non-targeted. These results indicated that targeted delivery significantly enhanced drug potency against ovarian cancer cells.

This improvement could be explained by receptor-mediated endocytosis. SKOV-3 ovarian cancer cells are known to overexpress folate receptors, which facilitated the selective internalization of folate-conjugated nanoparticles. Similar outcomes were reported by Ahmed et al. (2023), who observed enhanced anticancer efficacy when folic acid-functionalized carriers were used for co-encapsulation of paclitaxel and cisplatin. The current findings validated that receptor-targeting strategies could maximize drug delivery efficiency, reducing the required dose while minimizing toxicity to healthy tissues.

5. Cellular Uptake and Receptor-Mediated Targeting

Fluorescence microscopy and flow cytometry both confirmed enhanced uptake of folate-conjugated nanoparticles compared to non-targeted formulations. Flow cytometry demonstrated a 3.5-fold increase in fluorescence intensity, confirming the role of folate receptors in nanoparticle internalization. These results aligned with previous work by Khan et al. (2022), who demonstrated that folic acid-functionalized nanoparticles exhibited higher internalization in ovarian cancer cells than non-targeted particles.

The time-dependent uptake pattern observed in this study suggested that nanoparticles continued to accumulate in cells over prolonged exposure, a desirable property for sustained therapy. This finding further supported the role of surface modification in ensuring targeted and efficient drug delivery.

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6. Clinical Implications for Ovarian Cancer Therapy in Pakistan

The relevance of this research extended beyond laboratory validation to potential clinical translation, particularly in Pakistan's healthcare context. Ovarian cancer in Pakistan is often diagnosed at advanced stages due to a lack of routine screening, cultural barriers, and limited awareness. As a result, patients are more likely to require aggressive chemotherapy, which is associated with severe systemic toxicity.

The development of biodegradable, targeted nanocarriers offered several clinical benefits:

I. Reduced Toxicity: By concentrating drugs at the tumor site, systemic exposure could be minimized, reducing adverse effects such as nephrotoxicity (cisplatin) and neuropathy (paclitaxel).

II. Improved Compliance: Sustained release of drugs could reduce dosing frequency, addressing compliance issues common in patients with limited access to tertiary care facilities.

III. Overcoming Resistance: Combination delivery of paclitaxel and cisplatin could potentially mitigate drug resistance mechanisms, a common problem in relapsed ovarian cancer.

IV. Affordability and Accessibility: While nanomedicine technologies were often considered expensive, local development of biodegradable nanocarriers using readily available polymers could reduce costs in the long term, making such therapies feasible in the Pakistani healthcare setting.

7. Comparison with Global Literature

The findings of this study were consistent with international reports emphasizing the potential of folate-functionalized nanocarriers in cancer therapy. For instance:

1. Shetty et al. (2021) highlighted folate-functionalized nanocarriers as one of the most promising strategies for targeted drug delivery across multiple cancer types.
2. Ali et al. (2024) demonstrated that targeted nanocarrier systems enhanced therapeutic outcomes in ovarian cancer by improving drug accumulation at tumor sites.
3. Donadoni et al. (2022) modeled folate-functionalized TiO_2 nanoparticles and confirmed superior active targeting compared to passive accumulation alone.

The present study contributed to this growing body of evidence by demonstrating similar outcomes in a tertiary hospital-based laboratory in Pakistan, emphasizing its relevance in low- and middle-income countries.

8. Study Limitations

While the study demonstrated promising results, certain limitations were acknowledged:

- In Vitro Design: The findings were based on SKOV-3 ovarian cancer cell lines. Although these models are well-established, they cannot fully replicate the complexity of tumor physiology in vivo.
- Lack of Animal Studies: The absence of preclinical in vivo testing limited the ability to assess pharmacokinetics, biodistribution, and systemic safety profiles.
- Single Targeting Ligand: Only folic acid was used as a targeting ligand. While effective, ovarian cancer is heterogeneous, and multi-ligand systems could potentially improve targeting efficiency.
- Limited Stability Testing: Long-term stability studies under physiological conditions were not conducted. Such studies would be essential to determine shelf-life and suitability for clinical use.

9. Future Directions

Building upon the findings of this study, several avenues for future research were identified:

- In Vivo Validation: Preclinical animal models should be employed to assess biodistribution, therapeutic efficacy, and systemic safety.
- Combination Therapies: Future formulations could incorporate additional agents such as bevacizumab or PARP inhibitors to explore synergistic effects in resistant ovarian cancers.
- Multi-Ligand Functionalization: Incorporating multiple targeting ligands (e.g., folate plus transferrin or antibodies) could improve specificity and address tumor heterogeneity.

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- Clinical Translation: Pilot-scale production and eventual clinical trials in Pakistan could determine feasibility, cost-effectiveness, and acceptance within the local healthcare system.

Conclusion

This experimental study successfully demonstrated the development and evaluation of biodegradable nanocarriers designed for the targeted delivery of chemotherapeutic drugs to ovarian cancer cells. Conducted in a tertiary hospital research facility of Pakistan, the study aimed to address one of the most significant challenges in oncology: how to improve the efficacy of conventional chemotherapy while minimizing systemic toxicity. By encapsulating paclitaxel and cisplatin within PLGA/PCL-based nanocarriers and functionalizing them with folic acid, we were able to create a delivery system that was both efficient and selective.

The synthesized nanocarriers exhibited several favorable properties. They possessed a particle size within the optimal range of 120–180 nm, spherical morphology, and stable surface charge, all of which are essential features for tumor-targeting via the enhanced permeability and retention (EPR) effect. Drug loading efficiency was high, particularly for paclitaxel (72%), which suggested that the polymeric matrix provided excellent compatibility for hydrophobic drugs. Cisplatin, though less hydrophobic, also achieved satisfactory encapsulation efficiency (63%). These results indicated that the system could support dual-drug encapsulation, an important feature for combination therapies in ovarian cancer.

The drug release profiles were particularly promising, as the nanocarriers displayed pH-responsive behavior. Both paclitaxel and cisplatin exhibited faster release under acidic conditions mimicking the tumor microenvironment compared to neutral physiological pH. This selective release pattern suggested that the system could minimize drug leakage in circulation and maximize release where it was most needed, reducing systemic exposure and toxicity.

Cytotoxicity assays using SKOV-3 ovarian cancer cells further confirmed the advantages of targeted delivery. Folic acid-functionalized nanocarriers displayed significantly enhanced cytotoxic effects compared to non-targeted versions, as reflected in lower IC50 values. This outcome was consistent with the premise that folate receptors, overexpressed on ovarian cancer cells, facilitated receptor-mediated uptake. Complementary cellular uptake studies using fluorescence microscopy and flow cytometry demonstrated that folate-conjugated nanoparticles achieved a 3.5-fold increase in internalization compared to non-functionalized controls.

Taken together, these findings strongly supported the hypothesis that biodegradable, folate-functionalized nanocarriers could enhance the delivery and therapeutic impact of chemotherapeutic agents in ovarian cancer. The results aligned with global research trends (Shetty et al., 2021; Zhang et al., 2025; Ahmed et al., 2023; Ali et al., 2024), reinforcing the broader applicability of this approach, while also contributing novel insights from a Pakistan-based tertiary care setting.

From a clinical perspective, the implications were substantial. In Pakistan, where ovarian cancer is frequently diagnosed at advanced stages, patients often undergo aggressive chemotherapy that produces debilitating side effects. By improving selectivity and reducing systemic toxicity, targeted nanocarrier systems could improve quality of life, enhance treatment compliance, and potentially reduce healthcare costs associated with managing chemotherapy-related complications. Moreover, the ability to encapsulate multiple drugs within a single carrier created opportunities to overcome drug resistance, a persistent challenge in ovarian cancer management.

However, this study also acknowledged several limitations. The findings were confined to in vitro experiments using a single cell line model, which cannot fully replicate the complexity of the tumor microenvironment in vivo. The absence of animal studies limited insights into biodistribution, clearance, and systemic safety. Additionally, only folic acid was used as a targeting ligand, whereas future strategies might benefit from dual or multi-ligand systems to address tumor heterogeneity. Stability studies under simulated physiological conditions were also limited, and extended

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evaluations would be required before clinical translation.

Despite these limitations, the results laid a strong foundation for future research. Preclinical animal models should be employed to validate therapeutic outcomes and safety profiles. Expanding the system to include additional chemotherapeutic or biologic agents could open avenues for combination regimens tailored to resistant ovarian cancers. Furthermore, local development of cost-effective nanocarrier production pipelines in Pakistan could eventually support clinical trials, making such advanced therapies accessible to patients in resource-limited settings.

In conclusion, the development of biodegradable, folate-functionalized nanocarriers represented a significant step forward in ovarian cancer therapy. By combining biocompatibility, targeted delivery, pH-sensitive release, and enhanced cytotoxicity, this approach offered a viable alternative to traditional chemotherapy. While further in vivo and clinical studies are necessary, the findings of this study highlighted the potential of nanotechnology to revolutionize cancer care in Pakistan and globally, offering hope for improved outcomes in a disease that continues to pose substantial clinical challenges.

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