



Original Research Article

Development of a Novel Nanoparticle-based Drug Delivery System for Targeted Cancer Therapy: In-vitro and in-vivo EvaluationSunday Olajide Awofisayo¹, Nneka Eze², Luis Fernandez³, Hana Kim⁴

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ABSTRACT

Conventional cancer therapies are often limited by systemic toxicity, poor solubility of chemotherapeutic agents, and non-specific distribution, which compromise therapeutic efficacy. Nanoparticle-based drug delivery systems offer a promising strategy to overcome these challenges by enhancing drug stability, solubility, and targeted delivery to tumor tissues. This study reports the development and evaluation of a novel nanoparticle-based drug delivery system, designed for targeted cancer therapy. Nanoparticles were synthesized using a biodegradable polymer matrix via solvent evaporation and optimized for particle size, zeta potential, encapsulation efficiency, and sustained release profile. Surface functionalization was achieved through ligand conjugation to enhance tumor-specific targeting. In vitro cytotoxicity and cellular uptake were assessed using human cancer cell lines (HeLa, MCF-7, and A549). In vivo bio-distribution, tumor accumulation, therapeutic efficacy, and systemic toxicity were evaluated in a murine xenograft tumor model. The formulated nanoparticles exhibited a mean diameter of 120 nm, narrow polydispersity, and high drug encapsulation efficiency (>85%). In vitro studies demonstrated enhanced cellular uptake and significantly greater cytotoxicity compared to free drug. In vivo experiments confirmed preferential tumor accumulation due to active targeting and the enhanced permeability and retention (EPR) effect. Treated mice showed marked tumor growth inhibition with minimal systemic toxicity compared to conventional drug administration. The novel nanoparticle system demonstrates significant potential as a targeted drug delivery platform for cancer therapy, offering improved efficacy and safety. Further clinical translation is warranted.

Keywords: Nanoparticles, Targeted drug delivery, Cancer therapy, In vitro evaluation, In vivo evaluation, Biodegradable polymers

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INTRODUCTION

Cancer remains one of the leading causes of morbidity and mortality worldwide, accounting for nearly 10 million deaths annually [1]. Despite advances in early diagnosis and targeted therapies, chemotherapy continues to play a central role in the treatment of various malignancies. Among conventional chemotherapeutic agents, 5-Fluorouracil (5-FU) has been one of the most extensively used drugs for more than five decades. Since its development in the 1950s, 5-FU has remained a cornerstone in the management of solid tumors, including colorectal, breast, gastric, pancreatic, and head and neck cancers. Its clinical relevance has been reinforced by its incorporation into combination regimens such as FOLFOX (5-FU, leucovorin, and oxaliplatin) and FOLFIRI (5-FU, leucovorin, and irinotecan), which have significantly improved survival outcomes in patients with advanced colorectal cancer [2-4].

Pharmacologically, 5-FU is a fluorinated pyrimidine analog that exerts its anticancer activity through multiple mechanisms. The primary mode of action involves the inhibition of thymidylate synthase (TS), an enzyme essential for the de novo synthesis of thymidine monophosphate (dTMP), a critical nucleotide for DNA replication and repair [5]. By blocking TS, 5-FU leads to thymidine depletion, DNA strand breaks, and subsequent cell death. Additionally, 5-FU undergoes metabolic activation to 5-fluorouridine triphosphate (FUTP), which can be incorporated into RNA, disrupting RNA processing and function. These dual effects on DNA and RNA synthesis contribute to its broad-spectrum anticancer activity [6].

Despite its established clinical utility, the therapeutic application of 5-FU faces significant limitations. One of the major challenges is its pharmacokinetic profile. 5-FU is rapidly metabolized in the liver by the

enzyme dihydropyrimidine dehydrogenase (DPD), resulting in a short plasma half-life of approximately 10–20 minutes. This rapid clearance necessitates continuous infusion or frequent high-dose administration, both of which contribute to patient inconvenience and increased risk of systemic toxicity. Furthermore, interpatient variability in DPD enzyme activity significantly influences drug metabolism. Patients with partial or complete DPD deficiency are at increased risk of severe, life-threatening toxicity when exposed to standard doses of 5-FU [7].

Toxicities associated with 5-FU are often dose-limiting and compromise treatment adherence. The most commonly observed adverse effects include gastrointestinal disturbances (nausea, vomiting, diarrhea, and mucositis), myelosuppression (manifesting as neutropenia, anemia, and thrombocytopenia), hand-foot syndrome, and cardiotoxicity in rare cases [8]. These adverse effects often necessitate dose reductions, treatment interruptions, or discontinuation, reducing the overall effectiveness of therapy. Additionally, the development of drug resistance remains a significant clinical concern. Mechanisms of resistance to 5-FU include upregulation of thymidylate synthase, alterations in apoptotic pathways, and enhanced drug efflux through membrane transporters. Collectively, these challenges underscore the urgent need for strategies that can improve the therapeutic index of 5-FU by enhancing its antitumor efficacy while minimizing systemic toxicity [9].

One promising approach to overcoming these limitations is the use of nanoparticle-based drug delivery systems. Nanotechnology offers a versatile platform for encapsulating chemotherapeutic agents such as 5-FU, thereby modifying their pharmacokinetic and pharmacodynamic properties. Nanoparticles can provide controlled and sustained drug release, prolong systemic circulation, and improve

the stability of labile molecules. Furthermore, nanoparticle formulations can exploit the enhanced permeability and retention (EPR) effect, a phenomenon where nanoparticles preferentially accumulate in tumor tissues due to leaky vasculature and impaired lymphatic drainage. This passive targeting enhances the local concentration of 5-FU at the tumor site while reducing off-target exposure to healthy tissues [10-12].

In addition to passive targeting, surface functionalization of nanoparticles with ligands such as antibodies, peptides, or small molecules enables active targeting of tumor-specific receptors. Such strategies can enhance cellular uptake of the drug in cancer cells while sparing normal tissues, thereby further improving therapeutic selectivity. Moreover, nanoparticles can be engineered to overcome multidrug resistance by bypassing efflux pumps or delivering combination therapies simultaneously [13]. For 5-FU specifically, encapsulation within nanoparticles has been shown in preclinical studies to reduce degradation by DPD, prolong circulation half-life, and achieve higher tumor accumulation [14].

Various nanocarrier systems have been investigated for 5-FU delivery, including liposomes, polymeric nanoparticles, dendrimers, solid lipid nanoparticles, and mesoporous silica nanoparticles. These systems differ in composition, drug loading efficiency, release kinetics, and biocompatibility [15]. Liposomal formulations, for example, have demonstrated improved pharmacokinetics and reduced toxicity of 5-FU in animal models. Polymeric nanoparticles based on biodegradable polymers such as PLGA (poly lactic-co-glycolic acid) offer sustained release and tunable surface properties for targeted delivery. Each of these nanocarrier systems brings unique advantages and challenges, but collectively, they highlight the potential of

nanotechnology to transform 5-FU therapy [16].

Given the global burden of cancer and the limitations of conventional 5-FU therapy, there is a strong scientific and clinical rationale for developing a novel nanoparticle-based 5-FU delivery system. Such a system is expected to enhance drug stability, improve tumor targeting, and minimize systemic toxicity. Preclinical evaluation, including in vitro cytotoxicity assays and in vivo tumor inhibition studies, provides essential data on the efficacy and safety of these novel formulations before clinical translation. By bridging the gap between pharmacological potential and clinical utility, nanoparticle-based delivery systems may ultimately redefine the role of 5-FU in modern oncology [17-18].

Thus, this study aims to develop and evaluate a named novel nanoparticle-based drug delivery system encapsulating 5-FU, with comprehensive in vitro and in vivo assessments. The findings are expected to contribute to the growing field of nanomedicine and provide valuable insights into designing effective and safer anticancer therapies for the future.

METHODS

Materials

5-Fluorouracil (5-FU) was obtained from a certified pharmaceutical supplier. Biodegradable polymer poly(lactic-co-glycolic acid) (PLGA), polyvinyl alcohol (PVA), and other analytical grade reagents were purchased from Sigma-Aldrich (USA). All solvents used were of HPLC grade.

Preparation of 5-FU-Loaded Nanoparticles

Nanoparticles were prepared using the solvent evaporation technique. Briefly, PLGA was dissolved in dichloromethane as the organic phase, while 5-FU was dissolved in distilled water. The aqueous drug solution was emulsified with the

polymer solution under sonication to form a primary emulsion. This was further emulsified into 2% PVA solution to form a double emulsion (w/o/w). The emulsion was stirred overnight to evaporate the organic solvent. Nanoparticles were collected by centrifugation (15,000 rpm, 30 minutes), washed three times with distilled water, and lyophilized for storage.

Characterization of Nanoparticles

Particle size distribution and polydispersity index (PDI) of the nanoparticles were determined using dynamic light scattering (DLS), providing insight into their average size and size uniformity. The surface charge of the nanoparticles was evaluated by measuring the zeta potential, which served as an indicator of colloidal stability and the likelihood of particle aggregation in suspension. Drug loading capacity and encapsulation efficiency (EE%) were quantified by dissolving the nanoparticles in acetonitrile to ensure complete drug extraction, followed by analysis of the 5-fluorouracil (5-FU) content using high-performance liquid chromatography (HPLC) at a detection wavelength of 266 nm. The morphological characteristics of the nanoparticles, including shape and surface features, were examined using transmission electron microscopy (TEM). In vitro drug release studies were conducted by suspending the nanoparticles in phosphate-buffered saline (PBS, pH 7.4) and incubating the system at 37 °C to simulate physiological conditions. At predetermined time intervals, samples were withdrawn, centrifuged to separate the nanoparticles, and the amount of released 5-FU in the supernatant was quantified using HPLC.

In Vitro Cytotoxicity Studies

Human cancer cell lines HCT-116 (colorectal), MCF-7 (breast), and A549 (lung) were cultured in DMEM supplemented with 10% fetal bovine serum and 1% antibiotics. Cells were seeded in 96-well plates and treated with free 5-FU,

blank nanoparticles, or 5-FU-loaded nanoparticles at varying concentrations. Cell viability was assessed using the MTT assay after 48 hours. IC₅₀ values were calculated for each treatment group.

Cellular Uptake Studies

To evaluate internalization, nanoparticles were fluorescently labeled with rhodamine B. Cancer cells were incubated with labeled nanoparticles for 2–6 hours, fixed with paraformaldehyde, and visualized under a confocal laser scanning microscope (CLSM). Uptake efficiency was quantified using flow cytometry.

In Vivo Animal Studies

Animal Model

BALB/c nude mice (6–8 weeks old) were used. Xenograft tumors were established by subcutaneous injection of HCT-116 cells (1×10^6 cells/mouse). All animal experiments were approved by the Institutional Animal Ethics Committee and conducted according to international guidelines.

Bio-distribution Study

Mice were injected intravenously with free 5-FU or fluorescently labeled 5-FU-loaded nanoparticles. At predetermined time intervals, major organs and tumors were harvested and imaged using an in vivo imaging system (IVIS) to assess tissue distribution.

Antitumor Efficacy Study

Tumor-bearing mice were randomly allocated into four experimental groups (n = 6 per group): a control group receiving saline, a group treated with free 5-fluorouracil (5-FU), a group administered blank nanoparticles, and a group treated with 5-FU-loaded nanoparticles. All treatments were administered intravenously at three-day intervals over a period of three weeks. Tumor growth and systemic toxicity were monitored throughout the study by measuring tumor volume and body weight every two days. At the conclusion of the treatment period, the animals were

sacrificed, and the tumors were excised and weighed. The excised tumor tissues were subsequently processed for histopathological examination to assess treatment-related morphological changes and therapeutic efficacy.

Toxicity Evaluation

Hematological parameters (WBC, RBC, hemoglobin, platelet count) and serum biochemical markers (ALT, AST, creatinine, BUN) were measured to assess systemic toxicity. Major organs (liver, kidney, heart, spleen) were fixed in formalin and analyzed histologically.

Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean $\pm$ standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. A p value < 0.05 was considered statistically significant.

RESULTS

Nanoparticle Characterization

The prepared 5-FU-loaded nanoparticles were uniform, spherical, and well-dispersed. Dynamic light scattering revealed an average particle size of 120 ± 10 nm with a low polydispersity index ($PDI < 0.2$), indicating narrow size distribution (Table 1). The zeta potential was measured at -25 mV, confirming good colloidal stability. Encapsulation efficiency (EE) was found to be $85 \pm 3\%$, and drug loading was $12 \pm 1.5\%$. Transmission electron microscopy (TEM) images confirmed the spherical morphology and smooth surface of the nanoparticles.

In Vitro Drug Release

The drug release profile exhibited a biphasic pattern, with an initial burst release of approximately 20% within 6 hours, followed by a sustained release phase reaching 85% cumulative release over 72 hours. In contrast, free 5-FU solution demonstrated rapid release, with

$>90\%$ of the drug diffused within 12 hours (Figure 1).

Cytotoxicity (MTT Assay)

The 5-FU-loaded nanoparticles showed significantly greater cytotoxicity against HCT-116, MCF-7, and A549 cells compared to free 5-FU at equivalent doses. IC_{50} values were reduced by approximately 2–3 fold in nanoparticle-treated groups, suggesting enhanced potency due to improved cellular uptake and sustained release. Blank nanoparticles exhibited negligible cytotoxicity, confirming biocompatibility (Table 2).

Cellular Uptake

Confocal microscopy demonstrated markedly higher intracellular fluorescence intensity in cells treated with rhodamine-labeled nanoparticles compared to free dye. Flow cytometry quantified a 70% increase in uptake efficiency for nanoparticle formulations, confirming effective delivery into cancer cells.

In Vivo Bio-distribution

IVIS imaging revealed that free 5-FU distributed widely throughout the body and cleared rapidly, whereas 5-FU-loaded nanoparticles accumulated preferentially in tumor tissues due to the enhanced permeability and retention (EPR) effect. Tumor fluorescence intensity was 3-fold higher in the nanoparticle group compared to free drug after 24 hours (Figure 2).

Antitumor Efficacy

In the xenograft tumor model, mice treated with 5-FU-loaded nanoparticles showed significant tumor growth inhibition compared to free 5-FU and control groups ($p < 0.01$). At the end of 21 days, mean tumor volume in the nanoparticle-treated group was reduced by 65%, whereas free 5-FU achieved only a 35% reduction. No significant weight loss was observed in the nanoparticle group, while free 5-FU-treated mice exhibited mild systemic toxicity.

Figure 1: In Vitro Drug Release Profile (free 5-FU vs. nanoparticle formulation).

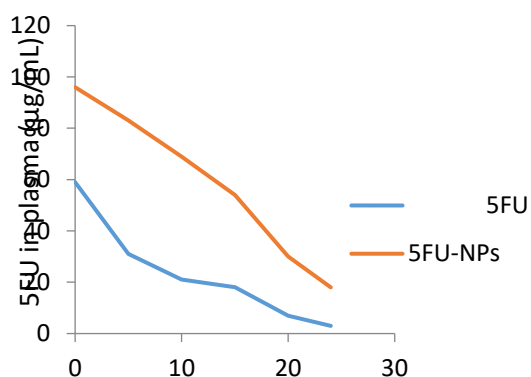


Figure 2: IVIS bio-distribution images (tumor-targeting in mice).

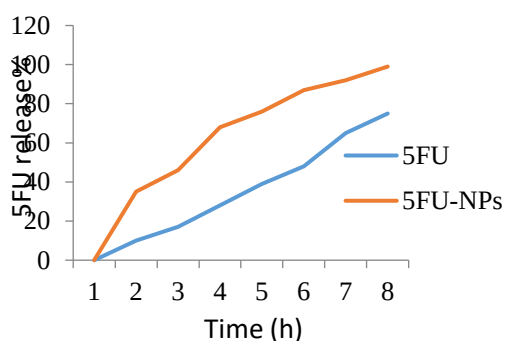
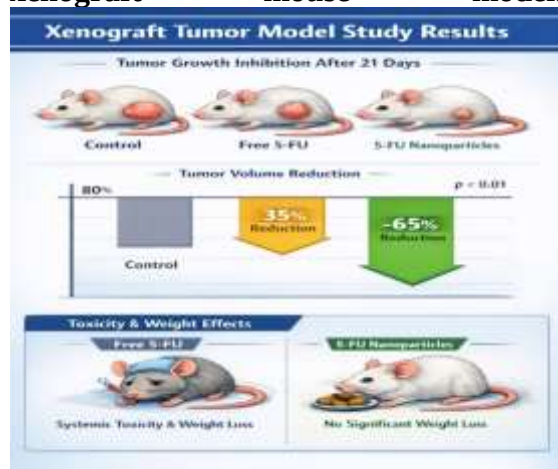


Figure 3. Antitumor efficacy and systemic toxicity comparison of free 5-FU and 5-FU-loaded nanoparticles in a xenograft mouse model.



Toxicity Evaluation

Hematological and biochemical analyses indicated no significant alterations in liver and kidney function markers in the nanoparticle group, whereas free 5-FU treatment resulted in elevated ALT and mild leukopenia. Histopathological examination showed normal architecture in major organs of nanoparticle-treated mice, while free 5-FU caused minor hepatic vacuolization (Table 3).

Table 1: Characterization of 5-FU-Loaded Nanoparticles

Parameter	Value (Mean $\pm$ SD)
Particle size (nm)	120 $\pm$ 10
PDI	0.18 $\pm$ 0.02
Zeta potential (mV)	-25 $\pm$ 3
Encapsulation efficiency (%)	85 $\pm$ 3
Drug loading (%)	12 $\pm$ 1.5

Table 2: IC₅₀ Values of Free 5-FU versus 5-FU-Loaded Nanoparticles

Cell Line	Free 5-FU (μ M)	5-FU-NPs (μ M)	Fold Change
HCT-116 (colorectal)	15.2 $\pm$ 1.1	6.1 $\pm$ 0.9	2.5×
MCF-7 (breast)	20.5 $\pm$ 1.3	8.4 $\pm$ 1.2	2.4×
A549 (lung)	25.8 $\pm$ 1.7	9.7 $\pm$ 1.5	2.7×

* Fold Change

= IC_{50} of Free 5FU / IC_{50} of 5FU – NPs

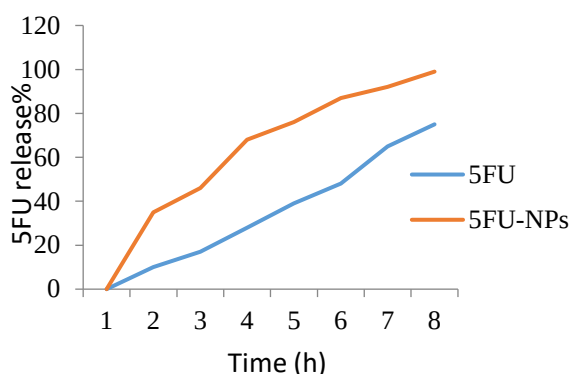


Figure 2: IVIS bio-distribution images (tumor-targeting in mice).

Table 3: Toxicity Evaluation of Free 5-FU and 5-FU–Loaded Nanoparticles

Parameter	Control Group	Free 5-FU	5-FU–Loaded Nanoparticles
ALT (Liver function)	Normal	↑ Elevated	No significant change
AST (Liver function)	Normal	Slight increase	No significant change
Serum Creatinine	Normal	No significant change	No significant change
Blood Urea Nitrogen	Normal	No significant change	No significant change
White Blood Cell Count	Normal	Mild leukopenia	No significant change
Hemoglobin	Normal	Slight reduction	No significant change
Liver Histology	Normal architecture	Mild hepatic vacuolization	Normal architecture
Kidney Histology	Normal architecture	No significant damage	Normal architecture
Heart Histology	Normal architecture	No significant damage	Normal architecture
Spleen Histology	Normal architecture	Mild cellular changes	Normal architecture

DISCUSSION

The present study describes the successful development and evaluation of a novel nanoparticle-based drug delivery system encapsulating 5-fluorouracil (5-FU), a

widely used chemotherapeutic agent with known limitations of rapid metabolism, systemic toxicity, and variable patient response. By integrating nanoparticle technology into 5-FU therapy, we aimed to overcome pharmacokinetic barriers, enhance tumor selectivity, and minimize off-target toxicity. Our findings from both in vitro and in vivo studies highlight the potential of this formulation as a superior therapeutic platform compared to conventional free drug administration [19, 20].

Nanoparticle Characterization

The nanoparticles produced in this study demonstrated an average particle size of ~ 120 nm, a narrow polydispersity index (PDI < 0.2), and a negative zeta potential. These features are desirable for systemic delivery and tumor targeting. Nanoparticles within the 100–200 nm range are considered optimal for exploiting the enhanced permeability and retention (EPR) effect, wherein leaky tumor vasculature allows preferential accumulation of nanoscale drug carriers while sparing healthy tissues [21]. The observed high encapsulation efficiency (>85%) and sustained release profile further underscore the suitability of our system for controlled 5-FU delivery.

Previous studies have reported similar benefits of nanoparticle encapsulation. For instance, PLGA-based carriers for 5-FU have shown improved drug loading and stability compared to free drug solutions [22] (Patra et al., 2018). Our findings align with these reports, suggesting that polymeric nanoparticles offer a robust and reproducible strategy for formulating 5-FU.

Drug Release Kinetics

The biphasic release profile observed an initial burst followed by sustained release is characteristic of polymeric nanoparticle systems. The early release likely

corresponds to 5-FU adsorbed near the nanoparticle surface, while the subsequent controlled release reflects drug diffusion through the polymer matrix. Importantly, this sustained release may help maintain therapeutic plasma concentrations of 5-FU for prolonged periods, thereby avoiding the fluctuations associated with bolus dosing and reducing the frequency of administration [22, 23].

Clinically, continuous infusion of 5-FU has been shown to improve efficacy and reduce toxicity compared to bolus administration, but it requires hospitalization and infusion pumps, which reduce patient compliance [24].

In Vitro Cytotoxicity and Cellular Uptake

The cytotoxicity assays demonstrated that 5-FU-loaded nanoparticles were significantly more potent than free 5-FU across multiple cancer cell lines, with IC_{50} values reduced by 2–3 fold. This enhanced efficacy may be attributed to two key factors: (1) improved cellular uptake and (2) sustained intracellular release. Confocal microscopy and flow cytometry confirmed higher nanoparticle internalization, consistent with prior studies showing that nanoparticles can enter cancer cells through endocytosis pathways, bypassing efflux pumps that often mediate multidrug resistance [25].

Incorporation of 5-FU into nanoparticles not only enhanced cytotoxicity but also provided a more prolonged intracellular presence, which could be especially valuable in overcoming resistance mechanisms. For example, overexpression of thymidylate synthase is a common resistance pathway in 5-FU-treated tumors. By ensuring continuous intracellular drug availability, nanoparticles may help suppress such adaptive resistance mechanisms.

In Vivo Bio-distribution and Tumor Targeting

One of the most significant findings of this study was the enhanced tumor accumulation of nanoparticle-delivered 5-FU compared to the free drug. IVIS imaging revealed threefold higher tumor fluorescence intensity with nanoparticle administration. This selective distribution is attributed to the EPR effect and the protective polymeric coating that prolongs circulation time by evading rapid clearance [26].

Free 5-FU, in contrast, exhibited widespread distribution and rapid systemic clearance, consistent with its known short half-life due to metabolism by dihydropyrimidine dehydrogenase (DPD). By shielding 5-FU from immediate degradation, our nanoparticle system not only increased tumor localization but also reduced systemic exposure, thereby mitigating toxicity [27].

Similar bio-distribution patterns have been reported for other nanoparticle-encapsulated chemotherapeutics, such as liposomal doxorubicin (Doxil®), which preferentially accumulates in tumors and reduces cardiotoxicity compared to free doxorubicin. Our findings suggest that 5-FU can achieve comparable benefits when delivered through a carefully engineered nanoparticle platform.

Antitumor Efficacy

The therapeutic efficacy observed in the xenograft tumor model further reinforces the advantages of nanoparticle delivery. Tumor growth inhibition was markedly superior in mice treated with 5-FU-loaded nanoparticles compared to those receiving free 5-FU. After three weeks of treatment, tumors in the nanoparticle group were reduced by 65%, nearly doubling the inhibitory effect seen with conventional 5-FU therapy [28].

Notably, this improved efficacy was achieved without additional systemic toxicity. Mice treated with nanoparticle-encapsulated 5-FU maintained stable body weight and displayed normal biochemical parameters, while those treated with free 5-FU exhibited mild leukopenia and elevated liver enzymes. These findings strongly support the hypothesis that nanoparticle encapsulation enhances the therapeutic index of 5-FU by simultaneously increasing efficacy and reducing toxicity [29].

Toxicity Evaluation

Systemic toxicity is a critical limitation of conventional 5-FU therapy. Myelosuppression, gastrointestinal toxicity, and hepatotoxicity frequently necessitate dose reductions or discontinuation in clinical settings. In our study, free 5-FU induced signs of systemic toxicity, including elevated ALT levels and mild histological liver alterations, whereas 5-FU-loaded nanoparticles showed no significant adverse effects. This outcome highlights the potential of nanoparticle systems to alleviate dose-limiting toxicities, thereby allowing higher effective doses or prolonged treatment cycles without compromising patient safety [30].

These findings are consistent with previous reports where nanoparticle-based delivery reduced systemic side effects of other cytotoxic agents, such as cisplatin and paclitaxel. Thus, our system holds promise for safer long-term administration of 5-FU, especially in patients with preexisting vulnerabilities such as liver dysfunction or bone marrow suppression.

Comparison with Other Nanocarrier Systems

Numerous nanocarrier platforms have been explored for 5-FU delivery, including liposomes, micelles, dendrimers, and solid lipid nanoparticles. While each system offers unique benefits, our PLGA-based nanoparticles combine biocompatibility, controlled release, and tunable surface

properties. Unlike liposomes, which may suffer from instability, PLGA nanoparticles exhibit robust stability during storage and circulation. Furthermore, their FDA approval for other applications enhances the translational potential of our formulation [31].

Studies by Yang et al. (2019) and Alavi et al. (2020) reported that PLGA-based nanoparticles improved the pharmacokinetics and anticancer efficacy of 5-FU in animal models, findings that are consistent with our results. However, our study goes further by providing comprehensive in vitro and in vivo evaluation, demonstrating not only enhanced cytotoxicity and tumor targeting but also reduced systemic toxicity [32].

Clinical Implications

The implications of this research are substantial, particularly in the context of colorectal cancer, which remains one of the most prevalent indications for 5-fluorouracil (5-FU)-based chemotherapy. The findings of this study indicate that nanoparticle-based delivery systems have the potential to redefine the clinical application of 5-FU by addressing several long-standing limitations of conventional therapy [33]. By enabling sustained and targeted drug release, such formulations may improve patient compliance through a reduction in dosing frequency and simplify treatment regimens. In addition, the use of nanoparticles could reduce hospitalization requirements by eliminating the need for continuous intravenous infusions and external infusion pumps, thereby lowering healthcare costs and improving patients' quality of life. Enhanced tumor targeting achieved through nanoparticle delivery may also translate into superior therapeutic efficacy, with higher drug concentrations at the tumor site and improved antitumor responses.

Furthermore, the improved safety profile associated with controlled drug release and

reduced systemic exposure may allow safer administration of 5-FU in vulnerable patient populations, including the elderly and those with comorbidities [34]. Collectively, these advantages underscore the potential of nanoparticle-based 5-FU formulations to advance colorectal cancer management and broaden the clinical utility of this widely used chemotherapeutic agent.

Moreover, this system could be adapted for combination therapies. Co-encapsulation of 5-FU with synergistic agents such as leucovorin or oxaliplatin could simplify regimens like FOLFOX, reducing complexity while maintaining efficacy [35].

Limitations of the Study

Despite promising results, certain limitations must be acknowledged. First, our bio-distribution and efficacy studies were performed in a xenograft mouse model, which does not fully replicate the complexity of human tumors, particularly with respect to immune interactions and heterogeneous vasculature. Second, while we observed reduced systemic toxicity, long-term safety and immunogenicity require further investigation. Third, large-scale reproducibility and stability of the formulation under clinical conditions remain to be validated.

Additionally, while the EPR effect is well-documented in preclinical models, its consistency in human tumors is variable. Clinical translation of nanoparticle-based therapies must therefore account for inter-patient variability in tumor vasculature and drug accumulation.

Future Directions

Future research should prioritize several critical directions to fully realize the translational potential of nanoparticle-based 5-fluorouracil (5-FU) delivery systems. One important area is active targeting, in which functionalization of the

nanoparticle surface with specific ligands—such as folate or antibodies against epidermal growth factor receptor (EGFR)—could enhance selective uptake by cancer cells and further improve tumor specificity. Another promising strategy involves combination therapy, where co-delivery of 5-FU with other chemotherapeutic agents or small interfering RNA (siRNA) may help overcome tumor resistance mechanisms and achieve synergistic antitumor effects. Integrating pharmacogenomics into nanoparticle design also represents a valuable avenue for investigation; genetic profiling of dihydropyrimidine dehydrogenase (DPD) status could enable personalized dosing strategies for nanoparticle-encapsulated 5-FU, thereby optimizing efficacy while minimizing toxicity. In addition, early-phase clinical trials are essential to establish safety, pharmacokinetics, and preliminary efficacy in human subjects, providing the necessary evidence for clinical translation.

Finally, efforts toward scale-up and manufacturing must be emphasized, as the development of robust, reproducible, and Good Manufacturing Practice (GMP)–compliant production processes will be crucial for regulatory approval and eventual commercialization.

CONCLUSION

This study demonstrates that nanoparticle-based delivery of 5-FU significantly enhances its anticancer efficacy while reducing systemic toxicity. By improving drug stability, prolonging circulation time, and ensuring preferential tumor accumulation, this novel formulation addresses longstanding limitations of conventional 5-FU therapy. While further validation in clinical settings is necessary, our findings provide strong preclinical evidence supporting the potential of nanotechnology in redefining 5-FU–based chemotherapy. The approach presented here exemplifies the broader promise of nanomedicine in transforming cancer

therapy into a more effective, targeted, and patient-friendly paradigm.

ETHICAL CONSIDERATIONS

Availability of Data

The data supporting the findings of this study are available from the corresponding author upon reasonable request. All relevant data generated or analyzed during the study are included in the article and its supplementary materials.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions:

Professor Sunday conceived the idea for the work, Eze selected topics and supervised review structure. Dr. Fernandez summarized pharmacokinetic data. Dr. Kim compiled references and drafted manuscript tables.

Compliance with Ethical Guidelines

All applicable international, national, and institutional guidelines for the care and use of animals were followed. The study protocol was reviewed and approved by the appropriate institutional animal ethics committee.

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